

Licochalcone B ameliorates DSS-induced ulcerative colitis in mice through inhibiting the PI3K/AKT signaling pathway and restoration of purine metabolism and intestinal barrier integrity

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Abstract

Ulcerative colitis (UC) is a recurrent inflammatory bowel disease with limited safe long-term therapeutic options. Licochalcone B (LicB), a bioactive chalcone isolated from *licorice*, possesses evident anti-inflammatory effects, yet its comprehensive molecular mechanism against UC remains poorly defined. We established DSS-induced colitis mouse models with low-dose LicB (LicB-L), high-dose LicB (LicB-H), and sulfasalazine (SASP) positive control groups, and performed DAI scoring, histological staining, ELISA, untargeted metabolomics, molecular docking, and western blotting to clarify its efficacy and underlying pathways. LicB-H significantly decreased disease activity scores, repaired damaged colonic mucosa, and upregulated tight junction protein occludin, while dose-dependently suppressing serum IL-1 β , TNF- α , IL-6, and IFN- γ . Metabolomic profiling demonstrated LicB reversed aberrant purine metabolism and restrained overactive PI3K/AKT signaling. Molecular docking validated stable high-affinity binding (≤ -7.0 kcal/mol) between LicB and key targets BCL2, mTOR, ESR1, Src, and HSP90AA1. Collectively, LicB alleviates DSS-triggered UC by synchronously modulating PI3K/AKT inflammatory cascades and purine metabolic homeostasis, which makes it a promising candidate for UC adjuvant therapy.

Introduction

Ulcerative colitis (UC) is a non-specific intestinal inflammatory disease that primarily affects the colorectum. Its typical clinical manifestations include abdominal pain, mucous bloody stool and tenesmus¹. This disease is both prone to recurrence and hard to cure, and current clinical strategies are mainly focused on symptom relief². The prevalence of UC is rising worldwide with a younger onset trend³, which has the effect of greatly affecting patients' quality of life, resulting in heavy social burdens. Intestinal barrier damage, abnormal immune responses and gut microbiota dysbiosis are key pathological features of UC⁴⁻⁷. Conventional treatments for the disease, including the administration of salicylates, hormones and biological agents, have the disadvantages that they often cause adverse side effects and fail to halt long-term disease progression^{8,9}. Given these limitations, natural compounds have become promising alternatives. Therefore, in the present study, we aimed to explore the protective effects and underlying mechanism of the natural chalcone compound licochalcone B (LicB) against dextran sulfate sodium (DSS)-induced UC.

Glycyrrhizae Radix et Rhizoma (commonly known as *Gan Cao*), a key component of traditional Chinese medicine prescriptions, contains abundant bioactive ingredients with diverse pharmacological activities¹⁰. Notably, LicB, a natural chalcone compound isolated from *Glycyrrhizae Radix et Rhizoma*, possesses a unique α,β -unsaturated carbonyl structure that endows it with potent anti-inflammatory, antioxidant and immunomodulatory properties¹¹. It acts as a selective inhibitor of the NLR family pyrin domain containing 3 (NLRP3) inflammasome by binding to NIMA-related kinase 7 (NEK7) to disrupt the NLRP3-NEK7 interaction, thereby suppressing inflammasome activation in macrophages and affording protection against NLRP3-mediated diseases in murine models¹². LicB also inhibits nuclear factor- κ B

(NF- κ B) signaling, where the NF- κ B signaling pathway has been shown to be a key pathway driving UC-associated inflammation via its suppression of both protein kinase A (PKA) activation and lipopolysaccharide (LPS)-induced NF- κ B phosphorylation, thereby inhibiting the release of TNF- α , nitric oxide (NO) and monocyte chemoattractant protein-1 (MCP-1) in RAW264.7 cells¹³. LicB has been shown to possess a range of robust antioxidant activities, including the scavenging of ABTS (+) radicals, suppression of reactive oxygen species (ROS) generation, and attenuating oxidative damage via nuclear factor erythroid 2-related factor 2 (Nrf2) pathway activation^{14,15}. As an inhibitor of 15-lipoxygenase (15-LOX) (IC₅₀=9.67 μ M), LicB binds specific 15-LOX residues to reduce the release of TNF- α ¹⁶, while also alleviating inflammation through regulating the NF- κ B/NLRP3 signaling pathway in LPS-stimulated cells. These findings support the potential of LicB for UC treatment, targeting the disease's core pathological features of excessive inflammation and oxidative stress. However, although the anti-inflammatory and antioxidant properties of LicB have been widely reported and match the pathological characteristics of UC, the exact mechanism underlying its therapeutic action against this disease has yet to be fully elucidated.

To systematically elucidate the therapeutic effects of LicB on UC, the present study has integrated a multidimensional research strategy: First, metabolomic analysis was performed on intestinal samples from UC models prior to and after LicB intervention to identify the key metabolic perturbations that are modulated by LicB; secondly, this analysis was combined with network pharmacology to predict potential targets and pathways underlying the anti-UC activity of LicB, followed by molecular docking validation to verify the binding affinity between LicB and core predicted targets; finally, pathological staining was employed to evaluate intestinal mucosal damage repair, and western blot (WB) analysis was performed to detect the expression of proteins associated with inflammation, apoptosis and intestinal barrier function. Through this comprehensive approach, this study aimed not only to elucidate the specific molecular mechanisms via which LicB exerts its therapeutic effects on UC, but it also sought to provide experimental evidence for LicB as a promising monomeric drug candidate for UC treatment. Ultimately, the present study is expected to lay a solid theoretical foundation for future clinical research on LicB-based UC therapies, thereby offering a potential new option for the clinical management of UC.

Materials and methods

Chemicals and reagents.

LicB (CAS no. 58749-24-9) was purchased from Chroma-Biotechnology Co., Ltd., and its purity was > 98%. It was prepared by the manufacturer through ethanol reflux extraction of *Glycyrrhizae Radix et Rhizoma*, followed by silica gel column chromatography separation and purification. Dextran sulfate sodium salt (DSS) (molecular weight, 500 kDa) was obtained from Macklin Biochemical Co., Ltd., whereas sulfasalazine (SASP) was purchased from Hengcheng Co., Ltd. The Alcian Blue/Periodic Acid-Schiff (AB/PAS) and the Ki67 detection kits, the TUNEL apoptosis assay kit, the immunohistochemical detection kits [including those for detecting proliferating cell nuclear antigen, phosphorylated (p)-AKT and occludin] and the mice enzyme-linked immunosorbent assay (ELISA) kits (including those for the

detection of IL-1 β , TNF- α , IL-6 and IFN- γ) were obtained from Servicebio Technology Co., Ltd. Detailed information on the primary antibodies associated with the WB and the ELISA kits is provided in Tables I and II.

Animal experiments.

Six-week-old male C57BL/6J mice (weighing 20 ± 2 g) were purchased from Chongqing Kangge Biotechnology Co., Ltd. [license no.: SYXK (Yu) 2023-0008]. The mice were housed in a specific-pathogen-free animal laboratory with a controlled temperature of $25 \pm 5^\circ\text{C}$ and in a 12 h light/12 h dark cycle. After 1 week of adaptive feeding with free access to food and water, the mice were used for subsequent experiments. All animal experimental procedures in this study were reviewed and approved by the Laboratory Animal Ethics Committee of Chongqing Academy of Animal Sciences (ethics approval no.: XKY-20241218). All animal manipulations, anesthesia and euthanasia procedures implemented in the present study strictly complied with the ARRIVE guidelines and national laboratory animal administration regulations of China. Euthanasia operations followed the standards described in the AVMA Guidelines for the Euthanasia of Animals (2020), and prohibited anesthetics such as chloral hydrate and ether were not adopted in this work. Strict humane endpoints were predefined for this DSS-induced ulcerative colitis mouse model to reduce animal distress; any mouse showing over 20% weight loss within 48 h, persistent severe rectal bleeding, lethargy and anorexia lasting more than 24 h, hunched posture with unresponsiveness to stimuli, severe dehydration, collapse from massive bloody diarrhea, or unmanageable sustained pain during modeling would be humanely euthanized ahead of the planned experimental endpoint. A total of 40 mice were randomly divided into five groups using a random number table method ($n = 8$ mice in each group): The control group (Control), the model group (UC), the high-dose LicB (LicB-H) treatment group¹⁷, the low-dose LicB (LicB-L) treatment group, and (as a positive control group) the SASP group¹⁸. Mice in the control and UC groups were administered 0.9% sodium chloride solution via gavage once daily for 14 consecutive days, whereas those in the LicB-H, LicB-L and SASP groups received suspensions of 6.25 mg/kg LicB, 3.13 mg/kg LicB and 200 mg/kg SASP, respectively, via the same route for 14 days. From day 8 to day 14 of the experiment, all mice except those in the Control group were provided with drinking water containing 3% DSS (Dalian Meilun Biotech Co., Ltd.) to induce the UC models (Fig. 1A). During the modeling period, the body weight, fecal characteristics and occult blood status of mice in each group were observed and recorded daily. The disease activity index (DAI) of the mice was scored according to the criteria listed in Table III. For comparison, two widely used alternative intervention protocols, namely concomitant drug-DSS co-administration and post-DSS therapeutic treatment, have been well documented^{19,20}.

At the end of the experiment, all mice were anesthetized by intraperitoneal injection of urethane at a dose of 1,000 mg/kg body weight. Blood samples were collected from the retro-orbital venous plexus after anesthesia and prior to euthanasia, with a volume of approximately 0.8-1.0 ml per mouse. Subsequently, all mice were euthanized by cervical dislocation. Complete death was confirmed by the permanent disappearance of spontaneous breathing, heartbeat, and corneal reflex to ensure humane euthanasia.

Metabolomic profiling of mouse serum samples.

Sample collection and pretreatment.

Serum samples were isolated from peripheral blood of C57BL/6J mice in Control group, the UC models, and the LicB-H group, immediately snap-frozen in liquid nitrogen, and stored at -80°C prior to analysis to minimize metabolic degradation. Sample pretreatment was performed using the Metabolite Extraction Kit of Shanghai Personalbio Technology Co., Ltd., following the manufacturer's protocol with minor modifications: Pre-cooled methanol-acetonitrile-water (2:2:1, v/v/v) containing internal standard (L-2-chlorophenylalanine; 1 µg/ml) was added to frozen serum samples. The mixture was vortex-mixed for 30 sec, sonicated on ice for 10 min, and then incubated at -20°C for 1 h to precipitate the proteins. After centrifugation at 12,000 g/min for 15 min at 4°C, the supernatant was transferred to a new centrifuge tube and freeze-dried. The lyophilized powder was subsequently reconstituted with 100 µl acetonitrile-water (1:1, v/v), vortex-mixed for 30 sec, and centrifuged again at 12,000 g/min for 10 min at 4°C, before the final supernatant was filtered through a 0.22-µm organic phase filter membrane for subsequent detection.

Ultra-performance liquid chromatography-quadrupole-time-of-flight tandem mass spectrometry (UPLC-Q-TOF-MS/MS) analysis.

Metabolomic detection was performed using a UPLC-Q-TOF-MS/MS system (Waters Corporation). The UPLC conditions were as follows: Separation was performed on an ACQUITY UPLC HSS T3 column (2.1x100 mm; 1.8 µm; Waters Corporation) maintained at 40°C. The mobile phase consisted of solvent A (0.1% formic acid in water) and solvent B (acetonitrile containing 0.1% formic acid) with the following gradient elution program: 0–1 min, 5% B; 1–9 min, 5–95% B; 9–12 min, 95% B; 12–12.1 min, 95 – 5% B; and 12.1–15 min, 5% B. The flow rate was 0.4 ml/min, and the injection volume was 2 µl. The MS/MS conditions were as follows: The electrospray ionization (ESI) source was operated; the capillary voltage was set to 3.0 kV (positive) and 2.5 kV (negative), with a source temperature of 120°C; the desolvation gas temperature was 500°C; and the desolvation gas flow rate was 800 l/h. MS data were collected in full-scan mode with a mass range of m/z 50–1200, and MS/MS spectra were acquired using collision-induced dissociation (CID) with a collision energy of 20–40 eV. Quality control (QC) samples, prepared by mixing equal volumes of all test samples, were injected every six samples to monitor the system stability.

Data processing and bioinformatic analysis.

Raw MS data were converted into the mzML format using ProteoWizard software, and processed with XCMS for peak picking, retention time correction and peak alignment. Metabolite identification was performed by matching accurate mass, retention time and the MS/MS spectra against the Human Metabolome Database (HMDB), METLIN, and the in-house database of Shanghai Personalbio Technology Co., Ltd. After normalization using the internal standard, multivariate statistical analysis was performed with SIMCA-P 14.1 software. Principal component analysis was used to evaluate the overall

metabolic differences between groups, and partial least squares-discriminant analysis was subsequently applied to screen differential metabolites, with the criteria of variable importance in projection (VIP) > 1 and $P < 0.05$. Pathway enrichment analysis of differential metabolites was performed using the web-based tool MetaboAnalyst 5.0, with the level of significance defined by $P < 0.05$ and false discovery rate (FDR) < 0.25.

Network pharmacology analysis of the potential targets and molecular mechanisms of LicB on UC.

The SwissTargetPrediction (<http://www.swisstargetprediction.ch/>) and TargetNet (<http://targetnet.scbdd.com/calcnnet/index/>) databases were used to predict the potential targets of LicB. The UC-associated targets were collected from the GeneCards (<https://www.genecards.org/>), OMIM (<https://www.omim.org/>), and TTD (<https://db.idrblab.net/ttd/>) databases. Overlapping targets were identified using the Venny 2.1.0 tool, and visualized with a Venn diagram. These overlapping targets were subsequently imported into the STRING database (<https://string-db.org/>; species: *Homo sapiens*; confidence score > 0.4) to construct a protein-protein interaction (PPI) network. Cytoscape 3.9.1 software was then utilized to analyze the topological structure and screen for hub genes. The MCC algorithm in CytoHubba plug-in of Cytoscape software was used to screen the top 10 hub genes from the PPI network. A search of the online resource Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways (top 20 pathways) for the overlapping targets was performed using the DAVID database (<https://david.ncifcrf.gov/>), with a screening criterion of $FDR < 0.05^{21-23}$. The results were visualized using the ggplot2 package in R 4.2.1 software. Finally, to systematically explore the potential molecular mechanisms via which LicB intervenes in UC, information on LicB, the hub genes and key pathways was integrated to construct and visualize a “LicB-Target-Pathway” network using Cytoscape 3.9.1 software.

Histological staining and immunohistochemical (IHC) analysis.

Hematoxylin-eosin (H&E) staining.

Colon tissues were fixed in 4% paraformaldehyde, paraffin-embedded, and sectioned into 5- μ m slices. The paraffin sections were dewaxed, rehydrated, stained with hematoxylin, differentiated, dyed blue and subsequently eosin-stained. After dehydration and clearing, the sections were mounted with neutral balsam. Finally, the extent of inflammatory cell infiltration and tissue damage was evaluated using light microscopy.

AB-PAS staining.

Dewaxed 5- μ m sections were stained with 1% AB, rinsed, oxidized, and incubated with Schiff reagent (Servicebio Technology Co., Ltd.). After hematoxylin counterstaining, the sections were dehydrated, cleared, and mounted. Goblet cell function and mucus layer integrity were subsequently assessed using light microscopy.

IHC analysis for occludin detection.

Paraffin sections were dewaxed and rehydrated, and then subjected to antigen retrieval by boiling in 0.01 M citrate buffer, followed by cooling. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide (H₂O₂) in PBS, followed by three washes in PBS; subsequently, non-specific binding was blocked with 5% BSA in PBS, and the sections were then blotted dry. Afterwards, sections were incubated with anti-occludin primary antibody (1:200 dilution; Wuhan Servicebio Technology Co., Ltd.) overnight at 4°C in a humidified chamber, washed with PBS, and incubated with HRP-conjugated secondary antibody (1:500). After washing with PBS, signals were visualized with 3,3'-diaminobenzidine (DAB) (1:100), and the sections were counterstained with hematoxylin and then dehydrated, cleared and mounted. For the quantification, occludin-positive cell percentages were calculated using Image-Pro Plus 6.0 software.

Ki67 immunofluorescence staining.

For Ki67 immunofluorescence staining, sections were dewaxed and rehydrated, prior to the antigen retrieval step and blocking with 5% BSA. The sections were then blotted dry prior to incubation with anti-Ki67 primary antibodies (1:150 dilution; Wuhan Servicebio Technology Co., Ltd.) overnight at 4°C in a humidified chamber. The sections were then washed with PBS and incubated with fluorescent secondary antibodies. After washing with PBS, nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI), and the sections were then mounted with anti-fade medium. Confocal microscopy was used to observe the cells, and the percentages of Ki67-positive cells relative to the total DAPI-positive cells were calculated using Image-Pro Plus 6.0 software.

WB analysis.

To investigate changes in the expression levels of the PI3K/AKT signaling pathway- and apoptosis-associated proteins, total proteins were extracted from colon tissues using RIPA lysis buffer containing 1% phenylmethylsulfonyl fluoride and 1% phosphatase inhibitor cocktail (Wuhan Servicebio Technology Co., Ltd.). The protein concentration was determined using a BCA Protein Assay Kit (Wuhan Servicebio Technology Co., Ltd.), and 30 µg protein was loaded per lane for 10% SDS-PAGE electrophoresis at 120 V. After separation, the proteins were transferred onto 0.45-µm PVDF membranes at 300 mA for 90 min. The membranes were subsequently blocked with 5% non-fat milk in TBST buffer (containing 10 mM Tris-HCl, 150 mM NaCl and 0.1% Tween-20 at pH 7.4) for 1 h at room temperature to block non-specific binding sites. Primary antibodies (specific details provided in Table II) were diluted according to the manufacturers' recommendations, and incubated with the membranes overnight at 4°C with gentle shaking. After three washes with TBST (5 min each wash), the membranes were incubated with HRP-conjugated secondary antibodies (1:5,000 dilution in TBST containing 5% non-fat milk) for 1 h at room temperature. After a further three washes with TBST, protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Wuhan Servicebio Technology Co., Ltd.), and the bands were imaged using a gel-imaging system (Bio-Rad Laboratories, Inc.). Band intensities were quantified using densitometric analysis using ImageJ software (v1.53e), and normalization was performed against the internal reference compound, GAPDH ²⁴.

Serum cytokine evaluation.

ELISA was used to detect the serum levels of IL-1 β , TNF- α , IL-6 and IFN- γ . Blood samples were centrifuged at 3,000 x g for 10 min at 4°C to separate serum, which was stored at -80°C prior to analysis. Assays were performed following the manufacturers' instructions (Wuhan Servicebio Technology Co., Ltd.). Briefly, 100 μ l standard solutions (diluted to 0, 15.625, 31.25, 62.5, 125, 250, 500 or 1,000 pg/ml) or diluted serum samples (1:2) were added to each well of the pre-coated microplate, and incubated at 37°C for 90 min. After washing 5 times with wash buffer, 100 μ l biotinylated detection antibody was added, and the microplates were incubated further at 37°C for 60 min. After a further 5 washes, 100 μ l HRP-conjugated streptavidin was added, and the microplates were incubated at 37°C for 30 min. After washing, 100 μ l substrate solution was added, and the plates were incubated at 37°C for 15 min in the dark. The reaction was stopped by adding 50 μ l stop solution, and the absorbance was measured at 450 nm within 15 min using a microplate reader (BioTek; Agilent Technologies, Inc.). Cytokine concentrations were calculated from the standard curve.

Molecular docking analysis between LicB and hub targets.

Based on the previous core target prediction results, molecular docking analysis was performed between LicB and the top five ranked targets. The docking was carried out using the open-source program AutoDock Vina 1.2.0 (<https://autodock.scripps.edu>). The 3D molecular structure file of LicB was downloaded from PubChem (<https://pubchem.ncbi.nlm.nih.gov>), whereas protein structures were obtained from the Protein Data Bank (www.pdb.org) for precise docking studies. The docking grid box was centered on the active pocket of each target protein, with dimensions set to 20x20x20 Å and a grid spacing of 0.375 Å, ensuring complete coverage of potential binding sites. The exhaustiveness of the docking run was set to 32, and the binding affinity between LicB and each target was evaluated according to the binding energy (kcal/mol), where a lower binding energy of the target protein indicated a more stable protein-ligand complex. An interaction between the target protein and the drug molecule was considered to have high affinity when the binding energy was ≤ -7 kcal/mol²⁵.

Statistical analysis.

All experimental data are presented as the mean $\pm$ standard deviation. Statistical analysis was performed using GraphPad Prism 10.0 (Dotmatics). The Shapiro-Wilk test and Levene's test were used to verify data normality and homogeneity of variance, respectively. One-way ANOVA was applied for intergroup comparisons when the data met statistical assumptions, whereas the Kruskal-Wallis test was used for non-normal data. Multiple comparisons were performed using a modified Dunnett's test following ANOVA, or Dunn's test following Kruskal-Wallis analysis. Pearson correlation analysis was performed for correlation assessment. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

LicB improves DAI scores in DSS-induced UC mice.

From day 8 onwards, 3% DSS solution was provided to mice for free access in all four groups to induce the UC model, and significant intergroup differences were observed in the DAI scores of each group. Specifically, the Control group maintained a DAI score of 0 throughout the period, whereas the UC model group showed a gradual increase in its DAI score to 4–6 points as the modeling progressed. By contrast, the scores of all the treatment groups were lower compared with that of the model group, with the order being LicB-H group (1–2 points) < SASP group (2–3 points) < LicB-L group (3–4 points). The rate of increase in DAI scores in the treatment groups was slower compared with that in the model group. The LicB-H group only exhibited mild symptoms of UC, whereas the symptom relief effects in the LicB-L and SASP groups were sequentially weaker. Taken together, these results suggested that LicB-H exerted more pronounced effects on DSS-induced UC compared with LicB-L, and that this could effectively reduce disease activity (Fig. 1B).

Identification of core targets and key signaling pathways of LicB in UC.

A total of 5,944 UC-associated disease targets were retrieved from the GeneCards, OMIM and TTD databases, whereas 172 potential drug targets of LicB were predicted using the SwissTargetPrediction and TargetNet databases, resulting in 105 common overlapping targets (Fig. 2). These overlapping targets were imported into the STRING database to construct a PPI network. Using the MCC algorithm, the top 10 hub targets, including the proteins BCL-2, heat shock protein HSP 90-alpha (HSP90AA1), estrogen receptor 1 (ESR1), mTOR and Src, were identified. Subsequent molecular docking experiments were focused on the first five key targets among these. Gene Ontology (GO) functional enrichment analysis revealed that the core targets were mainly involved in biological processes (BP) such as “regulation of apoptosis”, “positive regulation of cell population proliferation” and “signal transduction”, and that they were localized to cellular components (CC) including the cytoplasm, nucleus and plasma membrane, and possessed molecular functions (MF) such as “ATP binding” and “kinase activity”. The KEGG pathway enrichment analysis revealed that these targets were significantly enriched in signaling pathways closely associated with inflammation and immunity, including the PI3K-AKT, MAPK and hypoxia-inducible factor-1 (HIF-1) signaling pathways, suggesting that LicB may exert its therapeutic effects on UC by regulating these pathways. Additionally, the “LicB-target-pathway” network constructed using the Cytoscape software showed that the PI3K-AKT signaling pathway had higher connectivity and occupied core positions in the network compared with the others; therefore, the PI3K-AKT signaling pathway was selected for further validation in the subsequent experiments.

Metabolomic analysis of therapeutic effect of LicB on UC.

To explore metabolic alterations in UC and the regulatory role of LicB-H, differential metabolites were screened using $VIP > 1$ and $P < 0.05$. In total, 225 differential metabolites were identified between the Control and the UC groups, 153 between the UC and the LicB-H groups, and 36 core differential metabolites were identified via their intersection (Table SI). KEGG pathway enrichment analysis ($P < 0.05$; $FDR < 0.25$) revealed that the most altered pathways comparing the Control and the UC groups included

purine metabolism, ether lipid metabolism and others, with purine metabolism being already abnormally changed. Re-enrichment of the 36 core metabolites confirmed that purine metabolism was associated with the most prominent changes. Further validation revealed key purine metabolites were significantly disrupted in UC, although the disruptions were notably ameliorated by LicB-H treatment, confirming that purine metabolism is the pathway that is most significantly altered by LicB-H; therefore, restoring its homeostasis may be the key therapeutic mechanism for LicB in UC (Fig. 3).

Docking affinity findings.

To obtain structural biology evidence supporting the direct role of LicB in modulating the core pathological processes of UC, molecular docking analysis was performed between LicB and five key proteins that are involved in regulating critical pathways, including inflammatory responses, mucosal repair and apoptosis. Using a binding energy cutoff of ≤ -7.0 kcal/mol, the results revealed that LicB exhibited stable binding modes and high affinity for these five targets (Fig. 4); namely, BCL-2 (-9.327 kcal/mol), mTOR (-9.071 kcal/mol), ESR1 (-8.662 kcal/mol), Src (-9.0 kcal/mol) and HSP90AA1 (-7.145 kcal/mol). These findings suggested that these five proteins may be critical targets mediating the effects of LicB on the core pathological processes of UC.

LicB treatment leads to increases in the concentrations of serum cytokines in UC-modeled mice.

To evaluate the therapeutic effect of LicB on UC, the serum levels of IL-1 β , TNF- α , IL-6, and IFN- γ in each group were detected by ELISA. Compared with the Control group, the levels of all the detected pro-inflammatory cytokines were significantly increased in the DSS-induced UC model group ($P < 0.05$), demonstrating the presence of severe intestinal inflammation. LicB treatment led to a clear reversal of these abnormal changes; relative to the UC model group, cytokine levels were decreased by 15–25% in the LicB-L group ($P < 0.05$) and by 25–35% in the LicB-H group ($P < 0.05$). Additionally, the cytokine levels in the LicB-H group were found to be close to those in the SASP group (the positive control group). Collectively, these results suggested that LicB alleviates DSS-induced UC via suppressing the release of pro-inflammatory cytokines, and treatment with LicB-H produces a more potent anti-inflammatory effect (Fig. 5).

LicB treatment ameliorates pathological damage in UC-modeled mice.

H&E staining revealed severe mucosal ulceration, crypt destruction and dense inflammatory cell infiltration in the UC group, whereas the Control group exhibited intact mucosal architecture. After administering the respective treatments, the LicB-H and SASP groups showed significantly attenuated levels of inflammation, with reduced neutrophil accumulation and partial crypt regeneration, whereas LicB-L treatment displayed moderate levels of improvement (Fig. 6A). The AB/PAS staining experiments revealed blue punctate areas, representing AB-positive acidic mucins. Overall, the normal Control group showed uniform and dense staining, indicating normal mucosal barrier function. By contrast, the UC group exhibited marked destruction of the mucus layer, with impaired mucosal barrier function. Following intervention with LicB, however, the secretion of mucus in mouse colon tissues was increased,

a well-formed mucus layer was observed around the crypts, and the mucosal barrier function was restored (Fig. 6B). These results suggested that the administration of LicB markedly improves the symptoms of DSS-induced UC. According to the occludin IHC analysis, the UC group displayed a marked reduction in occludin expression, reflecting disrupted intestinal barrier function. Administration of LicB-H led to a significant upregulation of occludin expression, which approached the level of the Control group, and this effect was an improvement compared with that observed with the SASP group, with the LicB-L treatment group showing only a mild level of improvement (Fig. 6C).

LicB ameliorates colon proliferation in UC-modeled mice.

Compared with the Control group, the numbers of Ki67-positive proliferative cells in the colonic crypt base of the UC model group decreased significantly, confirming the successful establishment of the UC model with an impaired colon epithelial regeneration capability. The numbers of Ki67-positive cells in both the LicB-H group and the SASP group were restored to the level of the Control group, indicating that both treatment with either LicB-H or SASP could effectively restore the proliferation of colonic mucosal cells and enhance epithelial regeneration to maintain mucosal homeostasis. Although the LicB-L group showed some improvement in cell proliferation, its effect was weaker compared with that of the LicB-H group, demonstrating the superior efficacy of the higher dose. Collectively, these findings suggested that LicB treatment exerts a beneficial therapeutic effect on UC mice through rescuing impaired colonic epithelial proliferation (Fig. 7).

LicB improves PI3K/AKT signaling, apoptosis and intestinal barrier function in UC-modeled mice.

The results from the WB analysis showed that, compared with the Control group, the expression levels of the p-PI3K and p-AKT proteins in the mice of the DSS-induced UC group were significantly increased ($P < 0.05$). Following LicB intervention, however, the levels of these phosphorylated proteins in both the treatment groups were significantly decreased ($P < 0.05$), with the LicB-H group showing a more obvious downregulation effect. In terms of the apoptosis-associated proteins, the expression levels of the pro-apoptotic proteins Bax, p-Bad and cleaved caspase-3 in the UC group were found to be significantly higher compared with those in the Control group ($P < 0.05$). LicB intervention caused a significant inhibition of the expression of the abovementioned pro-apoptotic proteins, thereby demonstrating an anti-apoptotic effect. In terms of barrier function-associated indicators, the expression of the tight-junction protein occludin in the UC group was found to be significantly lower compared with that in the Control group ($P < 0.05$), demonstrating that the physical barrier of intestinal epithelial cells was damaged in the DSS-induced UC model. Following LicB intervention, the expression of occludin was found to be significantly upregulated compared with the UC group ($P < 0.05$), a finding that was consistent with the results of the occludin IHC analysis, therefore mutually verifying the regulatory effect of LicB on intestinal barrier function. Taken together, these results suggested that LicB is able to reverse the downregulation of occludin, implying that it may exert a potential protective effect on the impaired intestinal barrier (Fig. 8).

Discussion

The present work systematically investigated the therapeutic efficacy of LicB on DSS-induced UC mice, and explored the underlying mechanisms from multiple perspectives, including disease activity, histopathology, metabolomics, molecular docking analysis and signaling pathways. Collective data indicated LicBH exerted superior therapeutic effects than LicBL, with efficacy comparable to SASP. Consistent with prior reports on natural flavonoids alleviating UC via mucosal protection^{26,27}, this work newly uncovered LicB dual regulation of PI3K/AKT signaling and purine metabolism, providing preclinical theoretical evidence for its clinical development (Fig. 9).

In terms of disease activity and histopathology, LicB treatment markedly reduced DAI scores and relieved colonic injury. H&E staining showed LicBH mitigated ulceration, crypt loss and inflammatory infiltration; AB/PAS and occludin IHC verified restored mucus barrier integrity, consistent with Cheng et al (2023)²⁸. LicBH's capacity to upregulate occludin was equivalent to SASP. Ki67 immunofluorescence further suggested LicB rescued impaired crypt epithelial proliferation, with weaker efficacy observed in LicBL groups, offering reference for subsequent dose optimization research.

For inflammatory modulation, ELISA data showed LicB-H reduced serum pro-inflammatory cytokines by 25–35%. This anti-inflammatory property aligns with published studies on natural compounds targeting NF- κ B/MAPK^{29,30}, while we further validated direct target binding via molecular docking. Docking results revealed LicB stably bound five core proteins with binding energy ≤ -7.0 kcal/mol; mTOR and BCL2 exhibited the strongest binding affinity. Unlike most flavonoid studies merely describing anti-inflammatory phenotypes without target verification or metabolic regulation, our work combined docking and metabolomics to provide structural evidence for LicB's multi-target actions^{31,32}.

Untargeted metabolomics identified purine metabolic homeostasis as a novel functional axis of LicB-H. Among 36 differential metabolites, disordered purine pathways were most significantly reversed by LicB-H. Purine metabolic imbalance drives intestinal inflammation via pro-inflammatory intermediate accumulation and suppressed epithelial proliferation³³. This finding complements existing research that only focused on LicB's anti-inflammation and anti-apoptosis effects, and highlights metabolic reprogramming as a supplementary therapeutic pathway.

WB results confirmed LicB restrained hyperactivated PI3K/Akt and downregulated pro-apoptotic proteins. PI3K/AKT overactivation triggers inflammation, epithelial apoptosis and barrier damage in UC³⁴. Network pharmacology screened PIK3CA and PIK3CB as core hub genes with high network connectivity, consistent with Li et al. (2024) that PI3K/AKT inhibition relieves UC³⁵. Our work extended previous conclusions by linking PI3K/AKT cascade to purine metabolism, implying potential pathway crosstalk worthy of further exploration.

This research possesses three distinct strengths. First, we constructed a complete evidence chain integrating DAI scoring, histology, metabolomics, molecular docking and western blot, avoiding

limitations of single-index research. Second, purine metabolism was newly identified as LicB's regulatory target, enriching the research on natural small-molecule metabolic regulation. Third, combined docking and protein detection confirmed direct LicB-target binding, improving the mechanistic precision compared to studies only detecting pathway changes.

Several limitations remain to be acknowledged. First, we only observed correlation between PI3K/AKT and purine metabolism, without clarifying their downstream interactive molecules. Second, PI3K/AKT inhibitor control groups were absent, so we cannot confirm LicB's therapeutic effect fully relies on this pathway. Third, the single DSS inflammatory model cannot fully recapitulate complex clinical UC pathogenesis, restricting translational extrapolation.

Future research directions are proposed accordingly: Rescue experiments will clarify whether LicB modulates purine metabolism through PI3K/AKT signaling; combined LicB-PI3K/AKT intervention assays will verify pathway necessity; DSS combined gut dysbiosis models and human colonic organoids will be applied to validate translational value. Additional tight junction marker ZO-1 will also be detected to supplement mucosal barrier evidence.

Conclusions

In conclusion, LicB-H exerted superior therapeutic effects compared with LicB-L against DSS-induced UC in mice. LicB alleviated intestinal inflammatory injury through three core regulatory routes: improving hyperactivated PI3K/AKT signaling, restoring disrupted purine metabolic homeostasis, and repairing damaged intestinal mucosal barrier integrity. Despite the limitations of this work, LicB may serve as a promising candidate agent for UC intervention, and its multi-layer regulatory characteristics provide a solid theoretical basis for subsequent in-depth mechanistic research and drug development.

Declarations

CRedit authorship contribution statement

Yong He and Ke Fu: Writing-original draft, Validation, Data curation. Rong Ming: Writing-review & editing, Conceptualization. Ling-yu Wu, and Peng Chen: Writing-review & editing, Validation, Conceptualization. Ning Xu and Gang-qiang Du: Methodology, Data curation. Yong He: Funding acquisition. Zai-feng Wang and Jian-qing Li: Writing-review & editing, Methodology, Supervision.

Clinical trial number

Not applicable.

Patient consent for publication

Not applicable.

Animal Ethics Statement

All animal experimental procedures conducted in this study were reviewed and approved by the Laboratory Animal Ethics Committee of Chongqing Academy of Animal Sciences (Ethics Approval No.: XKY-20241218).

Data availability

Data will be made available on request.

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Declaration of interest statement

All authors declare that they have no conflicts of interest. This research was carried out without any financial or personal relationships that could have influenced the results.

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Tables

Tables are available in the Supplementary Files section.

Figures

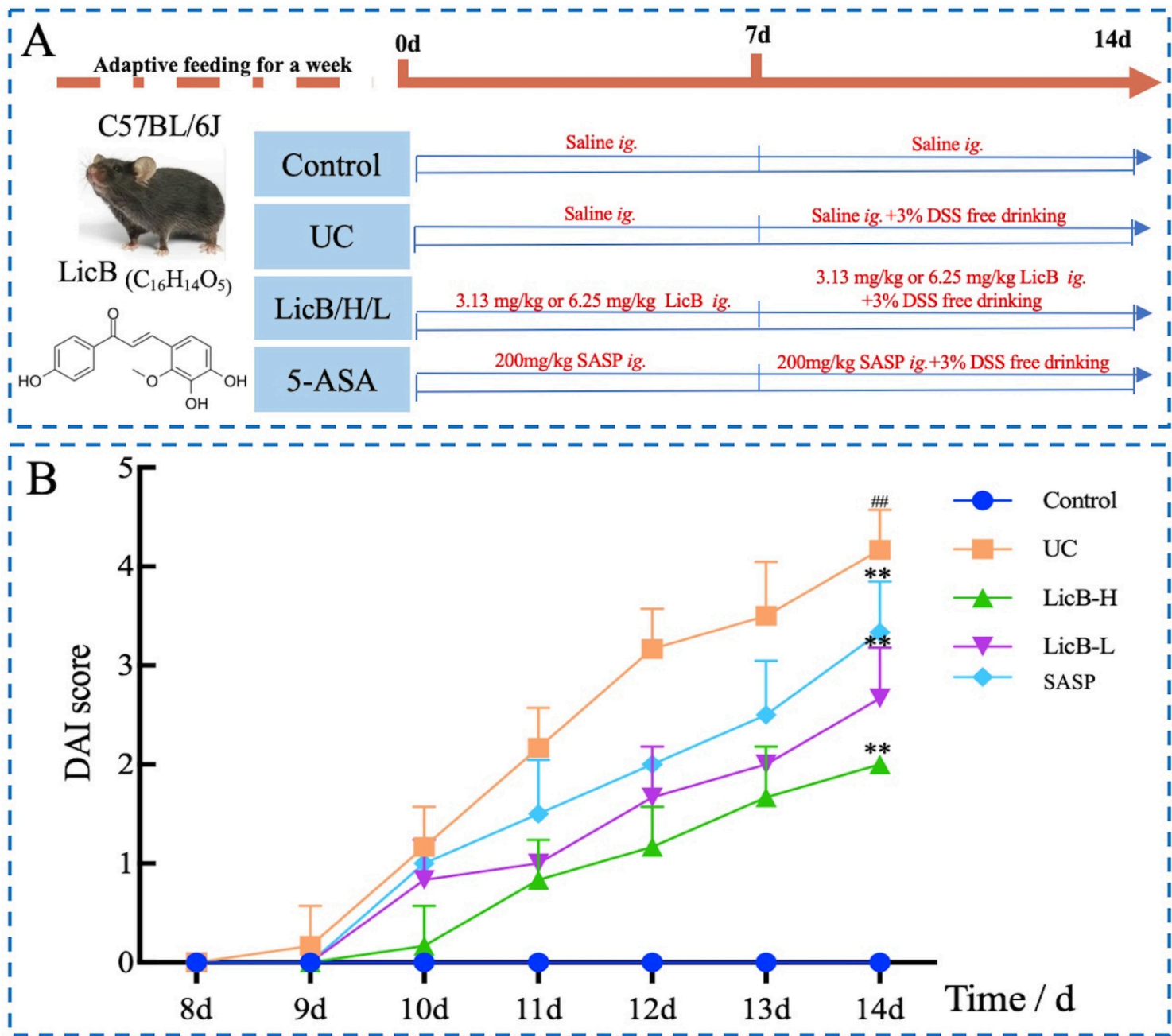


Figure 1

(B) PPI network of overlapping genes. (C) GO enrichment analysis. (D) The cluster analysis of the central 10 targets. (E) KEGG pathways enrichment analysis. (F) LicB-UC-Targets-Pathways enrichment analysis.

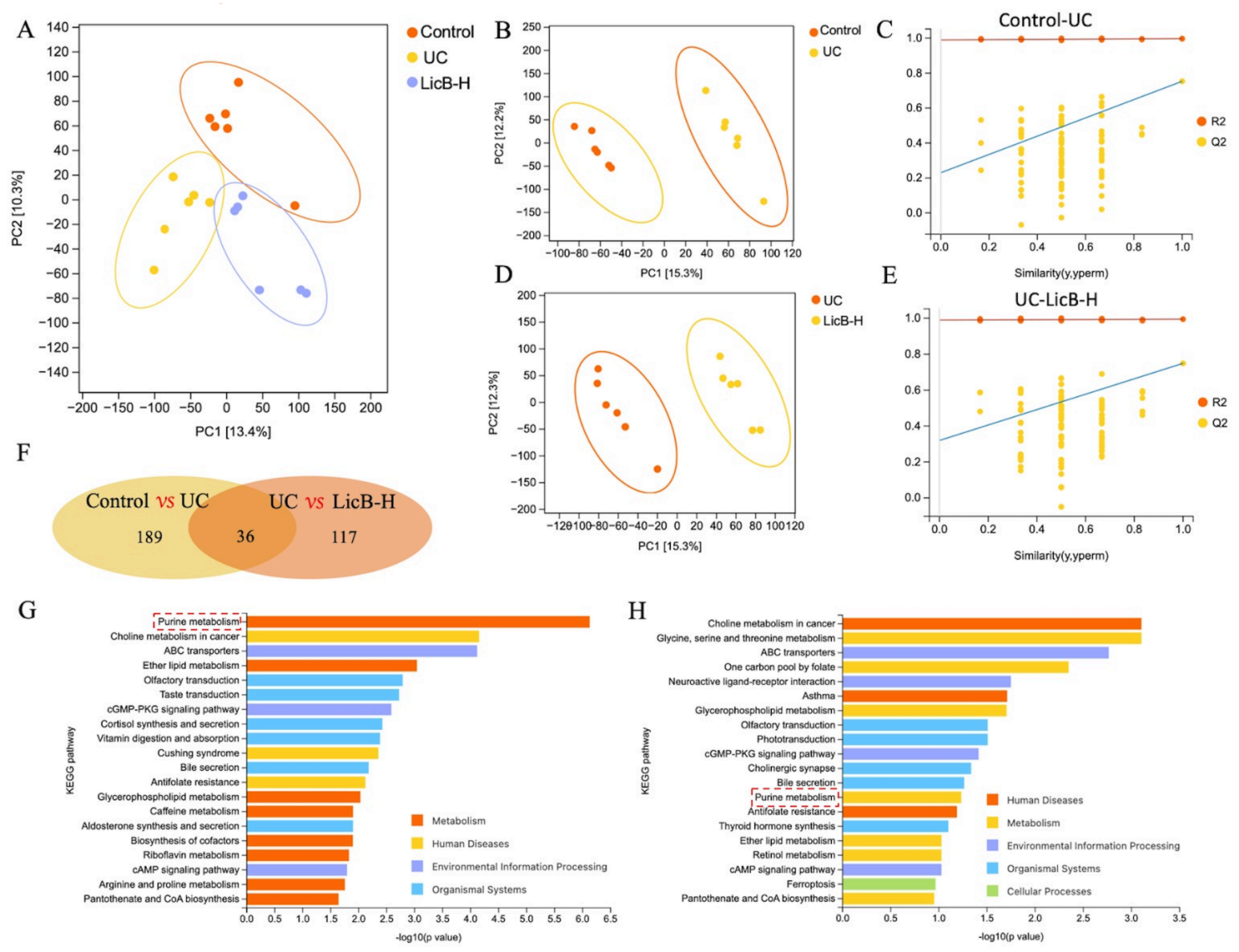
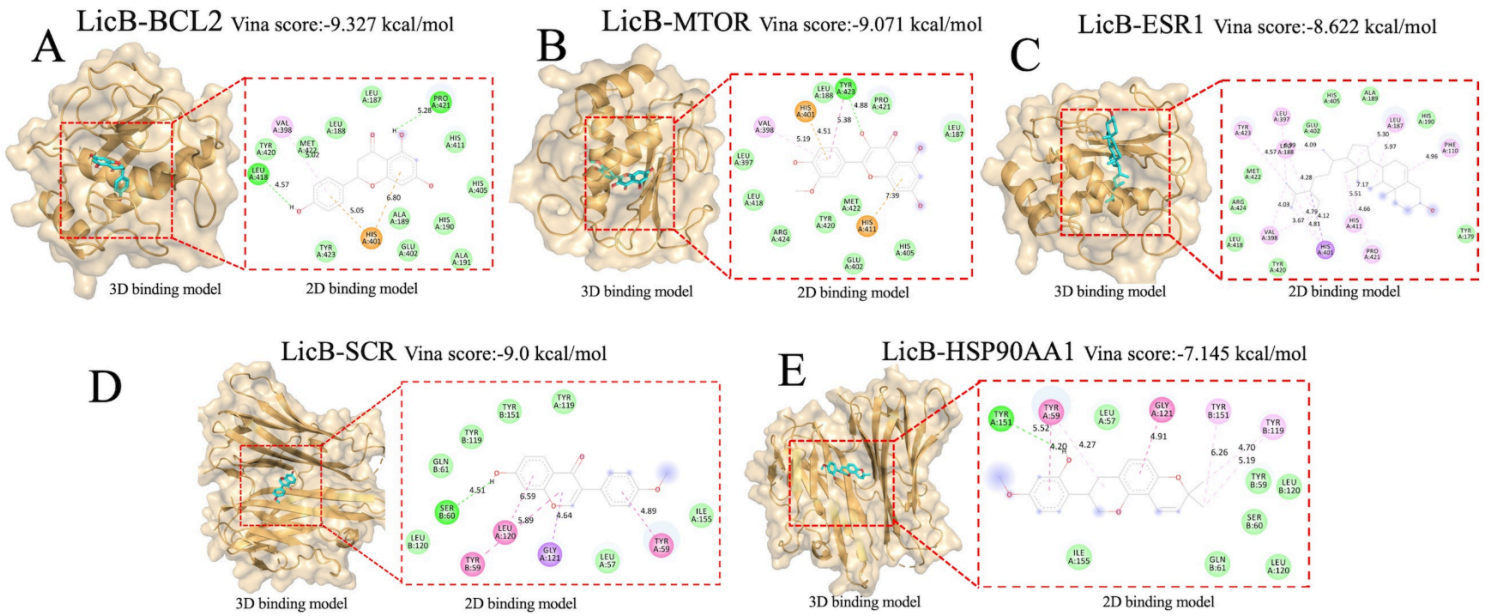


Figure 3

Untargeted metabolomic analysis reveals the effect of LicB treatment on the metabolite profiles of UC mice. (A) PCA scores plot illustrating the metabolic separation among the Control, UC, and LicB-H groups. (B–E) PLS-DA models and corresponding permutation tests were employed to distinguish metabolic profiles between the Control and UC groups (B, C), as well as between the UC and LicB-H groups (D, E). (F) Venn diagram showing the overlap of differential metabolites among the Control vs. UC and UC vs. LicB-H comparisons (differential metabolites screened by VIP > 1 and $P < 0.05$). (G, H) KEGG pathway enrichment analysis (performed using MetaboAnalyst, significance threshold: $P < 0.05$, FDR < 0.25) of differential metabolites in the Control vs. UC (G) and UC vs. LicB-H (H) groups ($n = 6$).



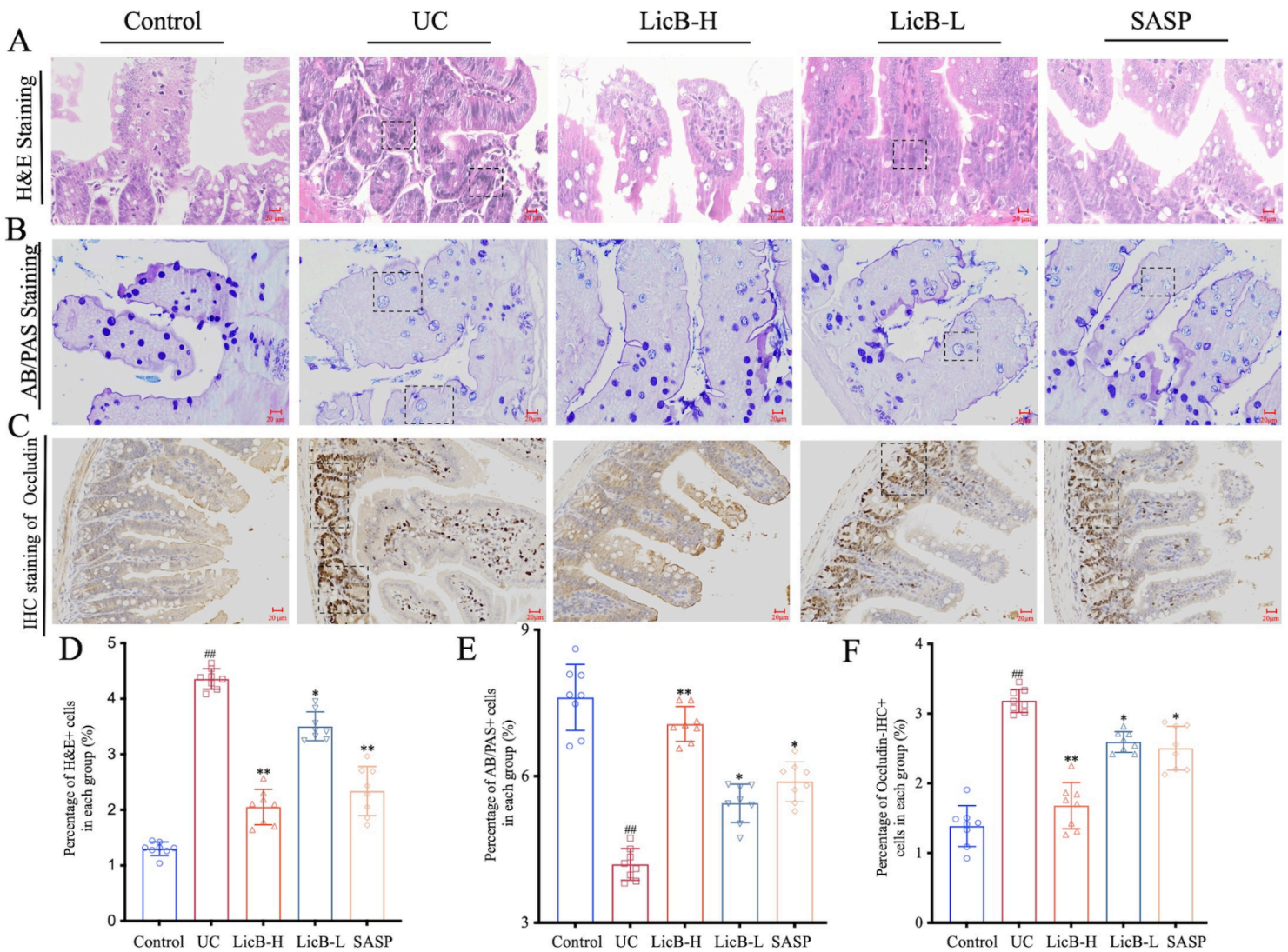


Figure 6

LicB mitigates colon mucosal damage in DSS-induced UC mice. Black box highlights pathological changes. (A, D) H&E staining (B, E) AB/PAS staining in each group, and (C, F) Immunohistochemical expression changes of Occludin protein. ^{##} $P < 0.01$ vs Control group. ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs UC group.

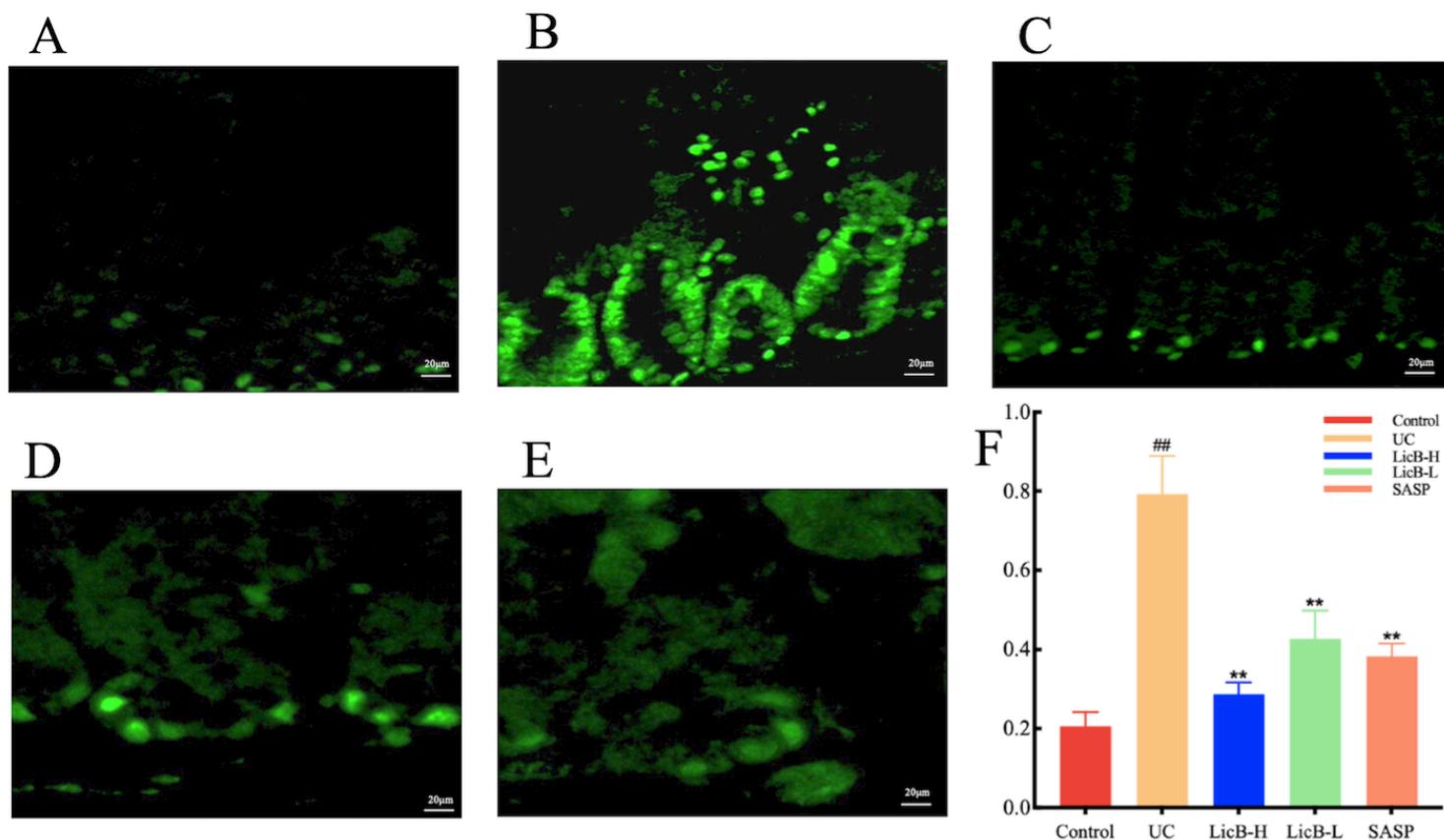


Figure 7

Immunofluorescence staining for Ki-67 in colon tissues from different groups. (A) Control group; (B) UC group; (C) LicB-H group; (D) LicB-L group; (E) SASP group. (F) Quantitative analysis of Ki-67 expression. ## $P < 0.01$ vs. Control group. ** $P < 0.01$ vs. UC group. Scale bar = 20 μm.

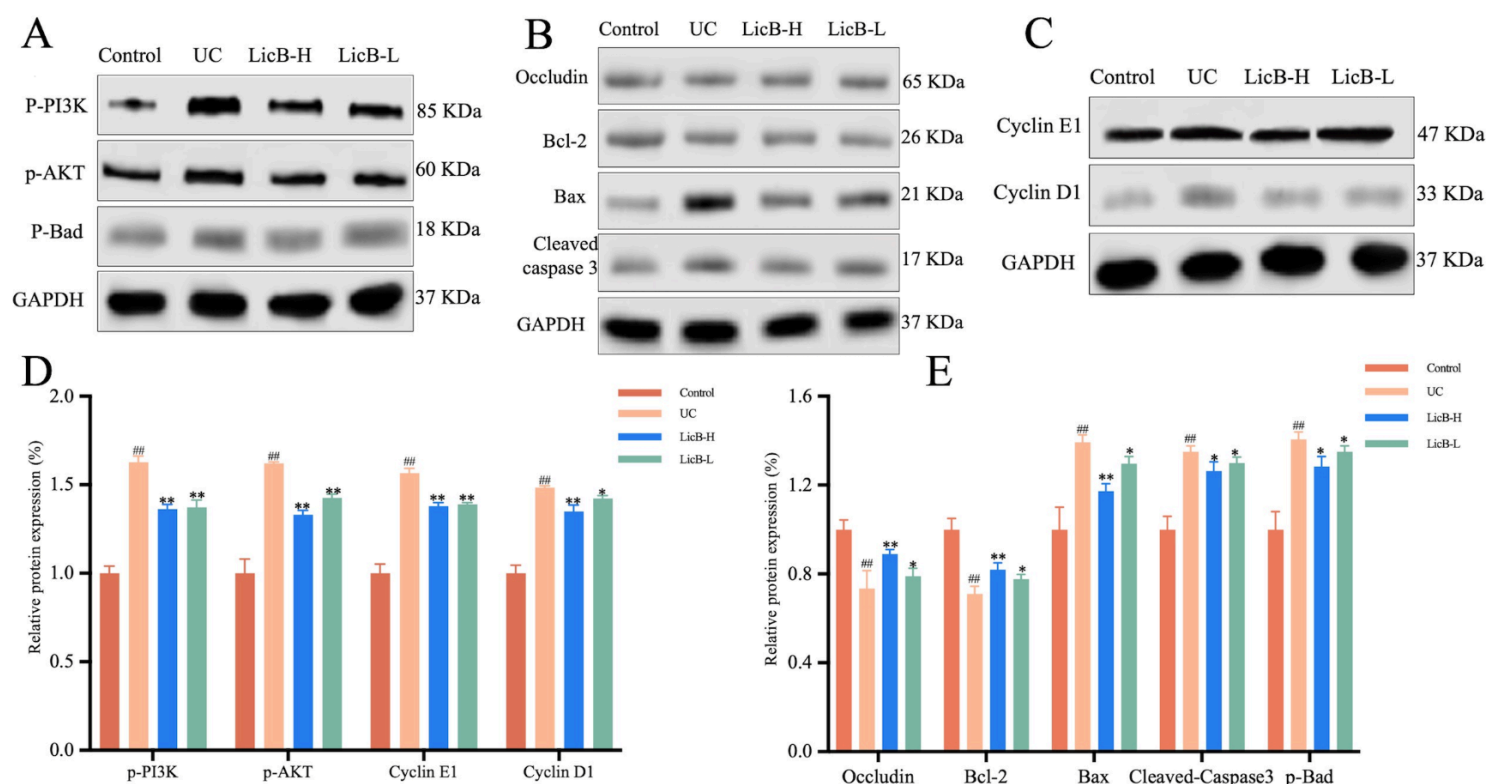


Figure 8

Effect of LicB on apoptosis factor levels and the PI3K/AKT pathway in DSS-induced UC. Note: (A-C) Cropped western blot bands for p-PI3K, p-AKT(Ser473), p-Bad, Occludin, Bax, BCL-2 and Cleaved-caspase3. Black dividing lines and blank white spaces are used to separate spliced lanes, and all uncropped full-length original blot membranes can be found in Supplementary Figure S3. (D-E) Quantitative bar charts of relative protein expression levels in each group. ^{##} $P < 0.01$ vs Control group. ^{*} $P < 0.05$, ^{**} $P < 0.01$ vs UC group.

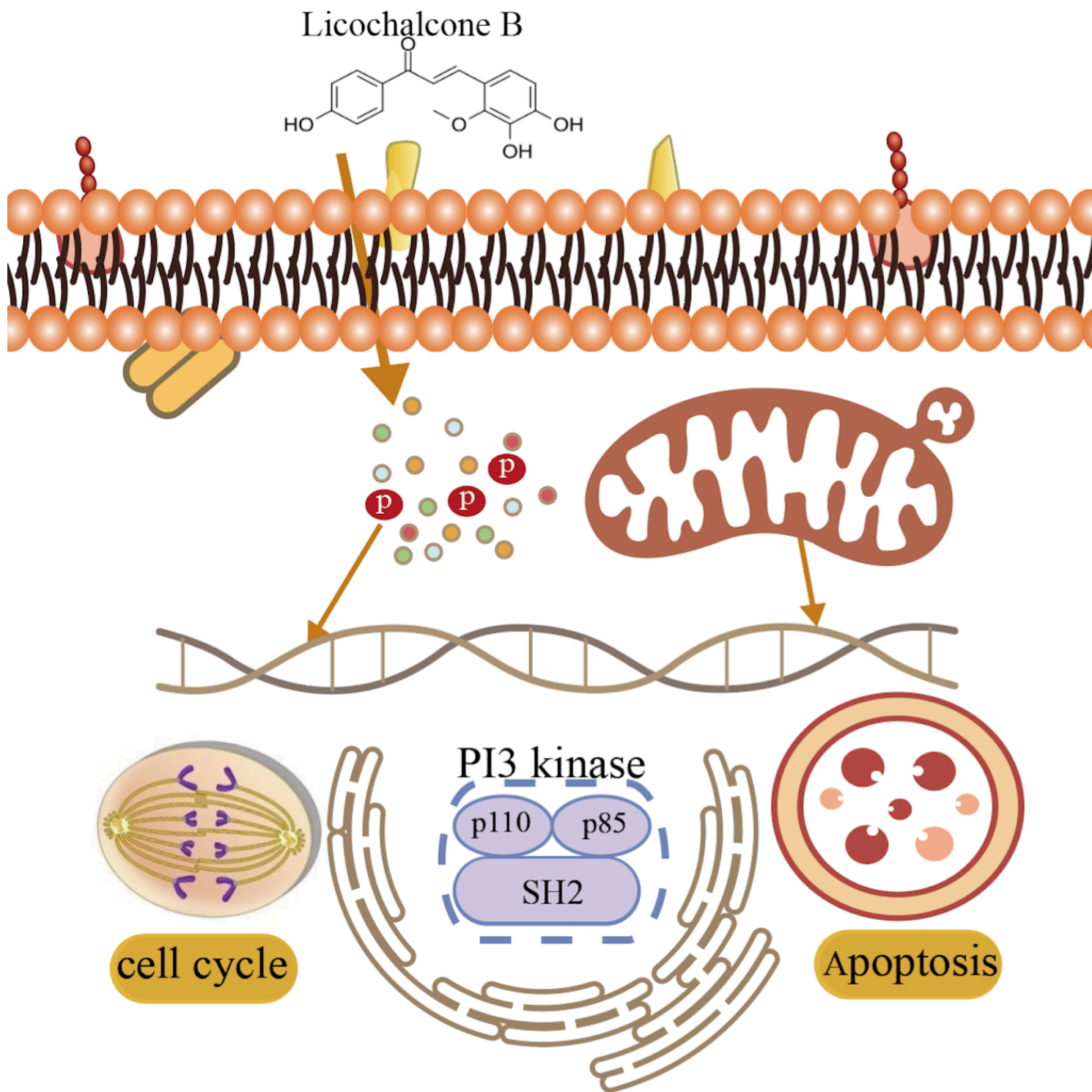


Figure 9

Mechanism schematic of licochalcone B in treating DSS-induced ulcerative colitis via PI3K/Akt suppression.

Supplementary Files

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