

The protective effects of *Panax notoginseng* in the treatment of inflammatory bowel disease: in silico and in vivo studies

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Author contributions

Wang JW, Xue HM, and Ding QR contributed to the conception of the study. Yue GH and Ding GY performed the chemical identification and in silico study. Wang JW, Ding QR, and Kim H participated in the animal experiment. Hao YG and Zhang HY contributed to supervision of this study. Wang JW and Yue GH wrote the original manuscript. Wang JW, Yue GH, and Hao YG participated in review and revision.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

PNE, *Panax notoginseng*; IBD, inflammatory bowel disease; ROS, reactive oxygen species; ISC, intestinal stem cell; EB, enteroblast cell; EC, enterocyte cell; EE, enteroendocrine cell; SDS, sodium dodecyl sulfate; DSS, dextran sulfate sodium; ESI, electrospray ionization; DEGs, differentially expressed genes; PPI, protein protein interaction; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; BP, biological process; CC, cellular component; MF, molecular function; DAPI, 4',6-diamidino-2-phenylindole; 7-AAD, 7-aminoactinomycin D; DHE, dihydroethidium; JNK, jun-Nterminal kinase; MAPK, Mitogen-activated protein kinase; JAK1, Janus kinase 1; JAK2, Janus kinase 2; JAK3, Janus kinase 3; GFP, green fluorescent protein; PBS, phosphate buffered saline.

Citation

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Abstract

Background: *Panax notoginseng* (PNE) is a prominent traditional Chinese medicine with extensive beneficial effects on the immune system. However, the precise mechanism of PNE in treating inflammatory bowel disease (IBD) remains unclear. **Methods:** We first used an extensive metabolomics approach utilizing UPLC-ESI-Q TRAP-MS/MS to identify the metabolite components of PNE aqueous extract. Moreover, the mechanism of PNE in treating IBD was investigated through in silico analysis including RNA-seq analysis, Network pharmacology and Molecular docking. Then a *Drosophila* toxin-induced intestinal inflammation model was employed to investigate further. **Results:** A total of 1,543 metabolites of PNE aqueous extract were characterized using UPLC-ESI-Q TRAP-MS/MS. In silico analyses showed that 97 IBD hub targets were targeted by 21 PNE ingredients. Kyoto Encyclopedia of Genes and Genomes results indicated that PNE may play an anti-IBD role through the Mitogen-activated protein kinase (MAPK) signaling pathway and other immune-related signaling pathways. Moreover, 11 top hits compounds of PNE show a good affinity binding to IBD targets. The experimental results demonstrated that PNE can effectively improve the survival rate of adult *Drosophila* while also inhibit the excessive proliferation and differentiation of intestinal stem cells induced by sodium dodecyl sulfate. Furthermore, PNE notably lower the epithelial cell mortality, the accumulation of reactive oxygen species and the activation of oxidative stress-associated jun-Nterminal kinase (JNK) pathway. **Conclusion:** Our data suggests that PNE aqueous extract has a significant protective impact on the intestinal homeostasis of *Drosophila*. These findings establish a basis for utilizing PNE in clinical investigations and managing IBD.

Keywords: *Panax notoginseng*; inflammatory bowel disease; *Drosophila*; intestinal homeostasis; JNK pathway

Highlights

1. *Panax notoginseng* (PNE) decreases reactive oxygen species (ROS) level and dead cells in inflammatory bowel disease (IBD) *Drosophila*.
2. PNE maintains intestinal homeostasis by regulating jun-Nterminal kinase (JNK) pathway.
3. Multiple evidence combined experimental pharmacology and in silico study.

Medical history of objective

The report of PNE in traditional Chinese medicine first appeared in literature *Shennong Bencao Jing* (ShengNong's herbal classic, unknown author, 2nd centuries C.E.). *Ben Cao Gang Mu* authored by Li Shizhen during the Ming Dynasty, PNE receives detailed exposition within this tome, elucidating its medicinal properties, indications, and methods of employment, which further enriches the medicinal history of PNE. Limited clinical studies have been conducted to evaluate the efficacy of PNE in patients with IBD. More research is needed to determine the optimal dosage, duration, and potential side effects of using *notoginseng* in the management of IBD.

Background

IBD encompasses both Crohn's disease and ulcerative colitis. The irregular immune response of the gastrointestinal immune system, along with the malfunction of the intestinal mucosal barrier towards intestinal bacteria can lead to the occurrence of this disease [1]. IBD is currently difficult to entirely cure, and almost all treatments only alleviate symptoms [2, 3]. Therefore, there is an urgent need to develop effective and safe drugs to treat IBD. The disturbance of intestinal homeostasis may contribute to the progress of IBD [4]. Recently, *Drosophila* has been demonstrated to be a powerful model for studying intestinal inflammation and intestinal homeostasis [5, 6]. The *Drosophila* intestinal immunity depends initially on the dynamic balance between the generation of antimicrobial peptides and ROS [7]. The antimicrobial peptides play a crucial role in the inductive defense mechanism. ROS produced by the mechanism of dual oxidases (DUOX), which can resist the infection of most microorganisms and pathogens [5]. Furthermore, ROS can stimulate intestinal stem cell (ISC) proliferation [6]. Also, excessive ROS can destroy intestinal epithelial cells and intestinal homeostasis. Adult *Drosophila* midgut epithelium harbors ISCs. ISCs divide asymmetrically to produce a fresh ISC and an enteroblast cell (EB). The EBs subsequently mature into enterocyte cells (ECs) and enteroendocrine cells (EEs). Moreover, one ISC can be divided into two identical ISCs or two identical EBs in symmetric division [8]. Additionally, EEs can be formed through the differentiation of EE progenitor cells derived from ISCs [9]. To maintain intestinal homeostasis, stem cell division increases in reaction to pathogens and harmful substances, like sodium dodecyl sulfate (SDS), dextran sulfate sodium (DSS), and paraquat [10]. Numerous pathways are implicated in controlling the division and differentiation of ISCs. Knocking down the epidermal growth factor receptor or Wingless within stem/progenitor cells leads to progressive loss of cells [11, 12]. Moreover, JNK pathway also played a key role in managing intestinal homeostasis in response to injury [13]. The coordination of these pathways maintains intestinal homeostasis, however, over activation can result in an excessive buildup of intestinal cells, ultimately leading to the formation of tumors [11–13].

PNE (also known as Sanchi), the dried roots and rhizomes of *Panax notoginseng* (Burk.) F. H. Chen, a famous traditional Chinese medicine [14]. PNE exerts a wide range of positive impacts on various systems, including hematological, immune system, cardiovascular system, central nervous and endocrine system [15–19]. Many studies have shown that PNE can treat or reduce various toxic reactions [19, 20]. In addition, PNE possesses antioxidant, antitumor, and

anti-inflammatory effects [21, 22]. Nevertheless, there is currently limited research on the exact mechanism of how PNE treats IBD, especially on a molecular level.

Metabolomics plays a crucial role in understanding biological phenotypes and their underlying mechanisms. It provides a more intuitive approach to comprehending biological processes and is commonly employed in various fields such as disease research, food safety, and phytopharmacology [23]. The potential pharmacological value of PNE could be revealed using high-throughput metabolomics technology. In drug design, the failure of lead compounds often stems from poor pharmacokinetics and toxicity. Oral administration of drugs can be achieved through ADMET measurement [24]. It is widely acknowledged that the evaluation of the absorption, distribution, metabolism, excretion, and toxicity of chemicals should be conducted early in the development process.

In this study, we investigate the mechanism of PNE in treating IBD. The metabolomics, bioinformatics, network pharmacology and molecular docking methods were used to analyze the components of PNE and IBD targets. Moreover, the *Drosophila* gut damage model induced by toxic chemicals was used to investigate the mechanism of PNE in the maintenance of intestinal homeostasis. These studies will provide a theoretical basis for the application of PNE in clinical research and treatment of IBD.

Materials and methods**Preparation of PNE and extraction**

PNE was provided by Renmintongtai Pharmaceutical Corporation (Harbin, China) and authenticated by Xiuhua Wang, the professor of botany at Northeast Forestry University (Harbin, China). The plant was checked with <http://www.theplantlist.org>. Twenty grams of chopped PNE was soaked in 200 mL of deionized water for 12 h at room temperature and then boiled for 3 h before filtering. Next, the filter residue was heated in 200 mL of deionized water for 3 h and filtered. The above filtrate was mixed and concentrated to 100 mL to obtain 20% PNE (0.2 g/mL). PNE was stored at -20°C .

UPLC-ESI-Q TRAP-MS/MS

Add an equal volume of 70% methanol to 100 μL of PNE water extract, rotate every 15 min, centrifuge (12,000 rpm, 3 min), absorb the supernatant, and filter the sample at 0.22 μm /particle size. Store in syringe vials for the next analysis. Sample extracts were analyzed by UPLC-ESI-MS/MS platform and mass spectrometry (ExionLCTM AD, Shanghai, China). UPLC conditions were the same as described above [25]. Injection set 2 μL . Mass spectrometry analysis was determined by esi-triple quadrupole linear ion trap QTRAP-MS. The working parameters of the electrospray ionization (ESI) source are as mentioned as previously described [26]. Software Analyst 1.6.3 and MultiaQuant software were used to perform qualitative and quantitative analysis of metabolites based on MWDB (Wuhan Metware Biotechnology Co., Ltd., Wuhan, China).

Network pharmacology

Screening of effective ingredients and target in PNE. The SMILE of PNE was obtained from Pubchem, and the drug Pharmacokinetics parameters were analyzed using ADEMT Lab2.0 [24]. Therapeutic Target prediction was performed using SwissTargetPrediction (Probability > 0.1) and PharmMapper [27, 28].

Acquisition for IBD target. The keywords "IBD" or "inflammatory bowel disease" were entered into Genecards (Degree > 20) and DisGeNet to obtain proven IBD targets for the subsequent studies [29, 30]. Then, we screened differentially expressed genes (DEGs) using microarray sequencing of IBD from the GSE75214 of GEO database (<https://www.ncbi.nlm.nih.gov/geo/>) to ensure the integrity of the research data. The R project and the limma R package were employed to screen DEGs with the criteria of absolute value of fold change ≥ 2 and P value ≤ 0.05 . uniprot (www.uniprot.org) was used to unify genes symbol. Finally, all potential target genes of IBD were obtained for further analysis after removing duplicates.

Construction of protein protein interaction (PPI) and target net. The predictive targets were analyzed by using STRING database (*Homo sapiens*, interaction threshold 0.7) [31]. Cytoscape (ver.3.9.2) was employed for network analysis according to multiple degree parameters. Finally, the therapeutic targets were mapped with PNE compounds to construct target network relationships in Cytoscape.

Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. GO analysis is a valuable tool for understanding the biological functions, pathways, and locations of gene enrichment within cells. The KEGG database, which offers up-to-date gene function annotations, enables systematic analysis for gene function. By integrating GO and KEGG enrichment analyses, researchers can acquire functional information on a vast number of genes at a macroscopic level and identify potential drug signaling pathways. To obtain comprehensive data on four terms: biological process (BP), cellular component (CC), molecular function (MF), and KEGG pathways, enrichment analyses of them were performed using the DAVID database [32]. An online tool (<https://www.bioinformatics.com.cn>) was used for visualization.

Molecular docking

The three-dimensional crystal structure of protein receptor were collected from PDB (<https://www.rcsb.org/>) database. The sdf files of drug ligands were downloaded from PubChem. Then, the receptors and ligands were imported into Pymol for preparation. Molecular docking based on AutoDockVina was used to evaluate binding energy [33]. The binding mode was set to 10 to obtain the optimal pose. CB-Dock2 was used for searching cavities and visualization [34].

Drosophila husbandry and treatments

Drosophila were cultured on standard cornmeal-yeast medium under 25 °C and 65% humidity with a 12 h light/12 h dark cycle. The stocks used in this study were: w¹¹¹⁸, Puc-lacZ (a downstream target of the JNK pathway) (Bloomington Stock Center, Bloomington, IN, USA), Su(H)GBE-lacZ (mark EBs) (Tsinghua Fly Center, Beijing, China), esg-Gal4 UAS-GFP (label progenitors: ISCs + EBs) and NP1-Gal4 UAS-GFP (EC specific driver) kindly provided by Rongwen Xi from Tsinghua Institute of Multidisciplinary Biomedical Research [35]. Tub-Gal80ts/CyO; D1-Gal4 UAS-GFP/TM6B (ISC specific driver) was obtained from Bruno Lemaître from Swiss Federal Institute of Technology Lausanne [36]. For *Drosophila* intestinal inflammation model, 3–5-day-old *Drosophila* adults were starved for 2 h and then transferred into vials containing 5 layers of filter paper with 400 µL of 5% sucrose solution containing 0.6% SDS (Sigma, Wilmington, DE, USA), or 4% DSS (Sigma, Wilmington, DE, USA) or SDS/DSS supplemented with 5% or 10% PNE solution. The filter papers and solution were changed once every 24 h. For the following immunostaining, 7-aminoactinomycin D (7-AAD) and dihydroethidium (DHE) staining, adult females aged 3–5 days were fed with 5% sucrose, 0.6% SDS or 0.6% SDS supplemented with 10% PNE for 72 h.

Survival analysis

The 3 to 5 days old adults (fifteen males and fifteen females cultured on standard medium) were selected for survival analysis of *Drosophila* intestinal inflammation model, and the survival condition was recorded every day. The survival rate was evaluated by the Kaplan-Meier approach. The log-rank test was used for the computation of results between the exposure group and two dosing groups. The proportional hazard model was used to estimate the death hazard ratio between each dosing group and the exposure group. All of these analyses were conducted using GraphPad Prism (ver. 8.0). The experiments were repeated three times.

Immunostaining

Midguts were immunostained as previously described [37]. In brief, 15–20 midguts of adult females were dissected in pre-cold phosphate buffered saline (PBS) and fixed in 4% paraformaldehyde for 30 min, then blocked in 5% goat serum for 30 min before staining with

primary antibodies overnight at 4 °C. The samples were stained with secondary antibodies for 2 h and Hoechst for 10 min at room temperature. The samples were mounted with 70% glycerol. Primary antibodies used in this study were rabbit anti-Green fluorescent protein (GFP) (1:200, Thermo Fisher Scientific, Waltham, MA, USA), rabbit anti-PH3 (1:200, Millipore, Burlington, MA, USA, mitotic stem cells marker), mouse anti-β-gal (1:200, Promega, Madison, WI, USA), mouse anti-Prospero (1:200, DSHB, Iowa City, IA, USA, EE cells marker) and secondary antibodies Alexa Flour 488 anti-rabbit, Alexa Flour 488 anti-mouse and Alexa Flour 594 anti-mouse (all 1:200; Thermo Fisher Scientific, Waltham, MA, USA). Every experiment was repeated at least three times independently.

7-AAD and DHE staining

Dead cells and ROS level were detected by 7-AAD and DHE staining, respectively. The dissected 10–15 guts in pre-cold PBS, were then incubated in 7-AAD (5 µg/mL in PBS, Thermo Fisher Scientific, Waltham, MA, USA) and DHE (5 µm/mL in PBS, Thermo Fisher Scientific, Waltham, MA, USA) for 30 min. The guts were fixed in 4% paraformaldehyde for 5 min and stained with 4',6-diamidino-2-phenylindole (DAPI) for 10 min. Each experiment was independently repeated at least three times.

Statistical analysis

Images were analyzed with ImageJ and statistical significance was determined by student's-t test and One-way ANOVA in GraphPad Prism (ver 6.0). *P* values greater than 0.05 indicated no significance; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

Results

Chemical compositions of PNE

The chromatograms were analyzed by TIC/XIC and MRM (Supplementary Figure S1), in the MRM detection chromatogram, each color of the spectral peak represents a different metabolite. Using the UPLC-ESI-Q TRAP-MS/MS detection and Metware database, 1,543 metabolites at three levels were detected (Supplementary Table S1). The first level has the highest confidence, with nearly 100% accuracy in detecting metabolites. In PNE, 476 ingredients at first level were identified, belonging to 11 types. Among these types, organic acids, vitamins, amino acids, nucleotides, alcohol compounds, and lipids were identified as plant primary metabolites. Phenolic acids, alkaloids, terpenoids, lignans, coumarins, and flavonoids were identified as secondary metabolites with high biological activity. In this study, only secondary metabolites at level 1 were selected for downstream analysis, totaling 295 compounds.

Network pharmacology

The putative targets of PNE. The ADME-related properties of 295 compounds were evaluated using the online tool ADMETlab 2.0. Next, the compounds are screened by filters based on several similar drug rules from Lipinski, GSK, and Golden Triangle. A total of 36 potential drug-like compounds were screened (Supplementary Table S2). Additionally, 350 drug targets were obtained from SwissTargetPrediction and PharmMapper database.

IBD related targets. There are 948 and 440 IBD-related targets that met the screening criteria obtained from GeneCards and DisGeNet, respectively. A total of 95 DEGs were identified as dysregulated genes in IBD using the “limma” package. These DEGs were visualized by the heatmap (Figure 1A) and volcano plot (Figure 1B). In the Venn plots, 60 up-regulated genes and 35 down-regulated genes were screened (Figure 1B). Upon pooling the IBD targets from these sources, a total of 1,304 IBD-related targets were acquired after removing duplicates, as illustrated in Figure 1C.

The therapeutic targets of PNE for IBD and PPI analysis. To identify potential PNE therapeutic targets in IBD, a total of 350 drug targets and 1,304 IBD-related targets were matched. Then 97 common targets (Figure 2A) were obtained to construct the PPI network (Figure 2B). And the larger node represents the stronger protein interactions.

21 potential entities (PNEs) and the corresponding 97 targets were imported into the Cytoscape to construct a drug-ingredient-target network for treating IBD with PNE (Figure 2C).

Gene enrichment analysis. Through GO and KEGG analysis, the regulatory mechanism of PNE in IBD was further studied. GO results include 13 BPs, 8 CCs and 13 MFs, and drawing the GO function enrichment map according to the $P < 0.05$ (Figure 3A). The BP was mainly involved in the immunity, innate immunity and apoptosis; the CC mostly took part in membrane, and cytoplasm; the MF participated primarily in the kinase and transferase.

The KEGG pathway analysis included 121 terms, and the several pathways associated with inflammation including Mitogen-activated protein kinase (MAPK), Ras, JAK-STAT, tumor necrosis factor, and Toll-like receptor pathway were identified (Figure 3B). The result indicated that PNE could treat IBD patients by regulating multiple signaling pathways. Among these predictive pathways, MAPK pathway is important in inflammation-related signal transduction

which associated with inflammation and cellular stress. As shown in Figure 3C, JNK pathway further incorporates the p53 pathway, Toll-like pathway, tumor necrosis factor pathway, and extracellular signal-regulated kinase (ERK) 5 pathway, which can be served as an IBD indicator more comprehensively. Besides, the MAPK-JNK pathway is already known which played a crucial role in the pathogenesis of IBD [1]. The abnormal activation of this pathway can lead to dysregulation of the inflammation response and imbalance of intestinal homeostasis [1]. However, whether the MAPK-JNK pathway is a target for PNE treatment of IBD needs further experimental evidence to confirm.

Computational validation of ingredients-targets interactions

To verify the reliability of the network pharmacology results, AutoDockVina was used to evaluate the binding energy between PNE compounds and targets. In general, the lower binding free energy indicates a stronger binding ability. Based on the PPI and target

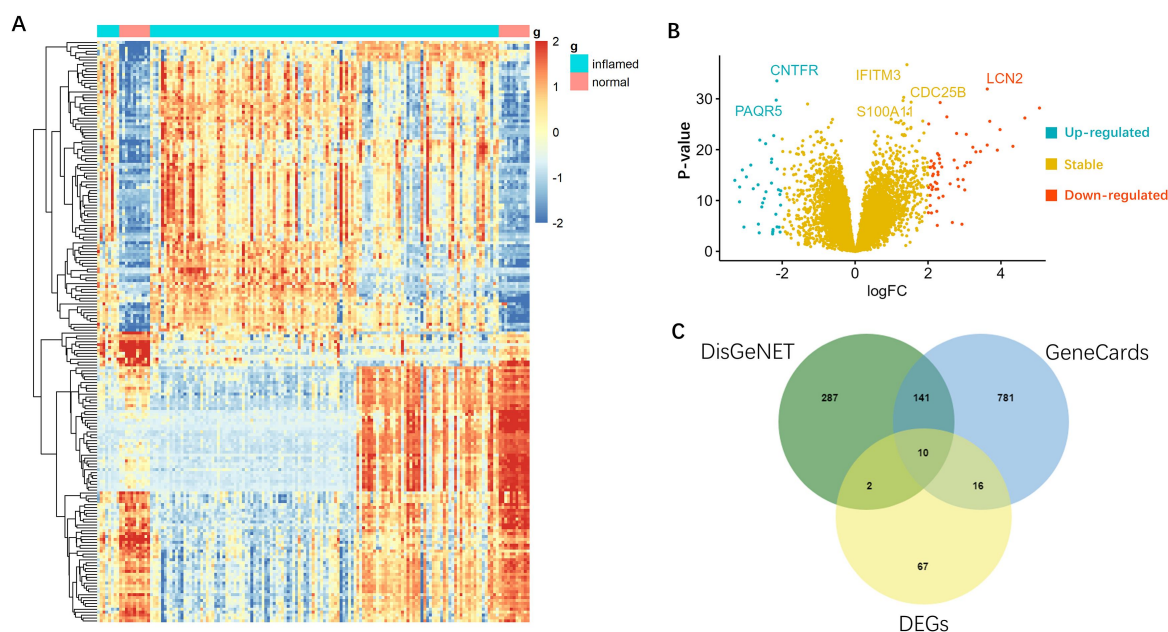


Figure 1 IBD related targets. (A, B) DEGs were displayed as heatmap (A) and volcano plot (B). (C) Venn diagram of 1,304 IBD-related targets. DEGs, differentially expressed genes.

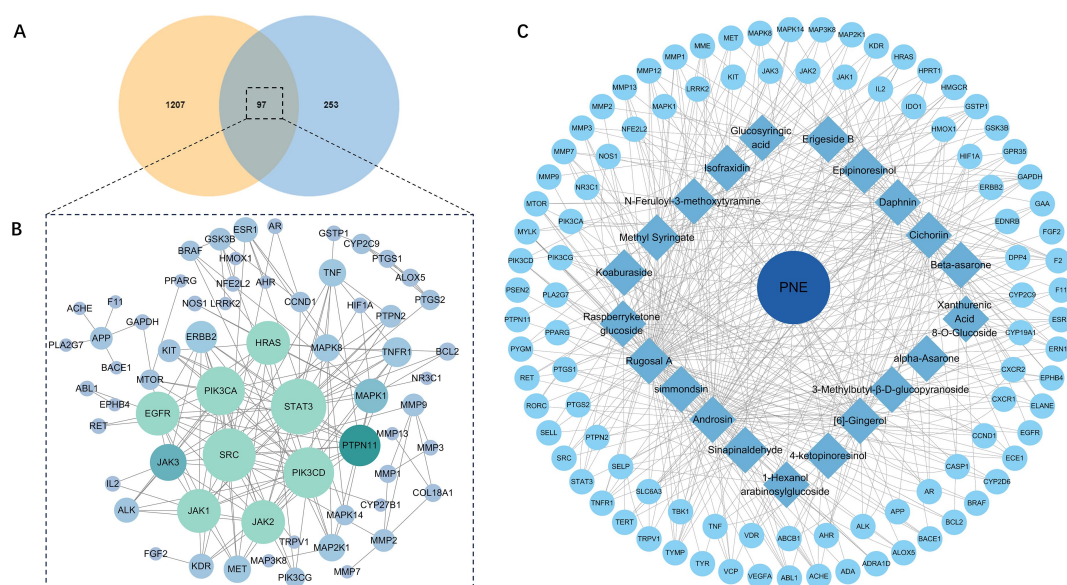
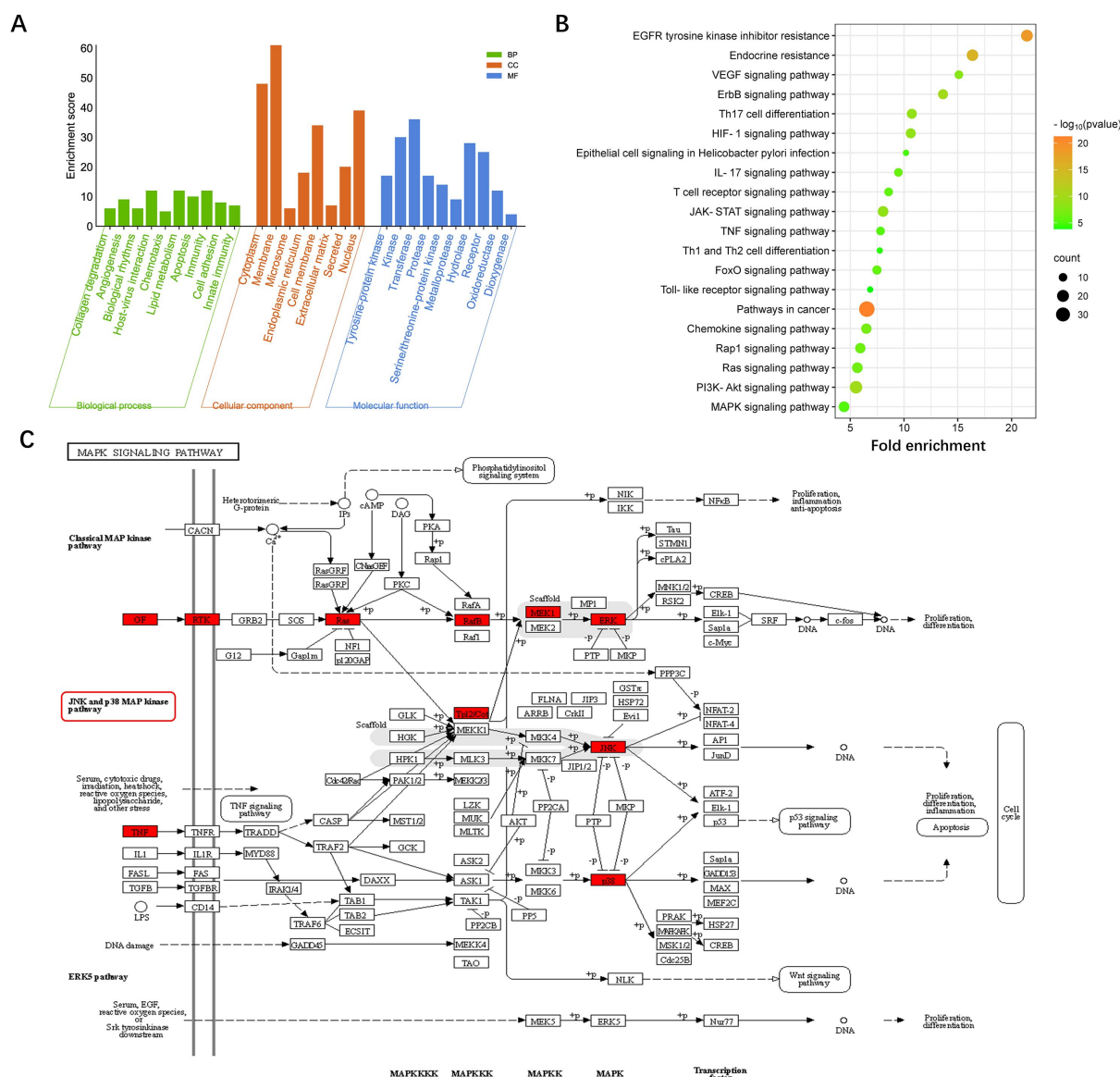


Figure 2 The therapeutic targets of PNE for IBD and target network. (A) Common targets of PNE and IBD. (B) PPI targets network. (C) PNE-ingredients-targets network. The rhombic portion for compounds, peripheral rounded portion for targets. PNE, *Panax notoginseng*.



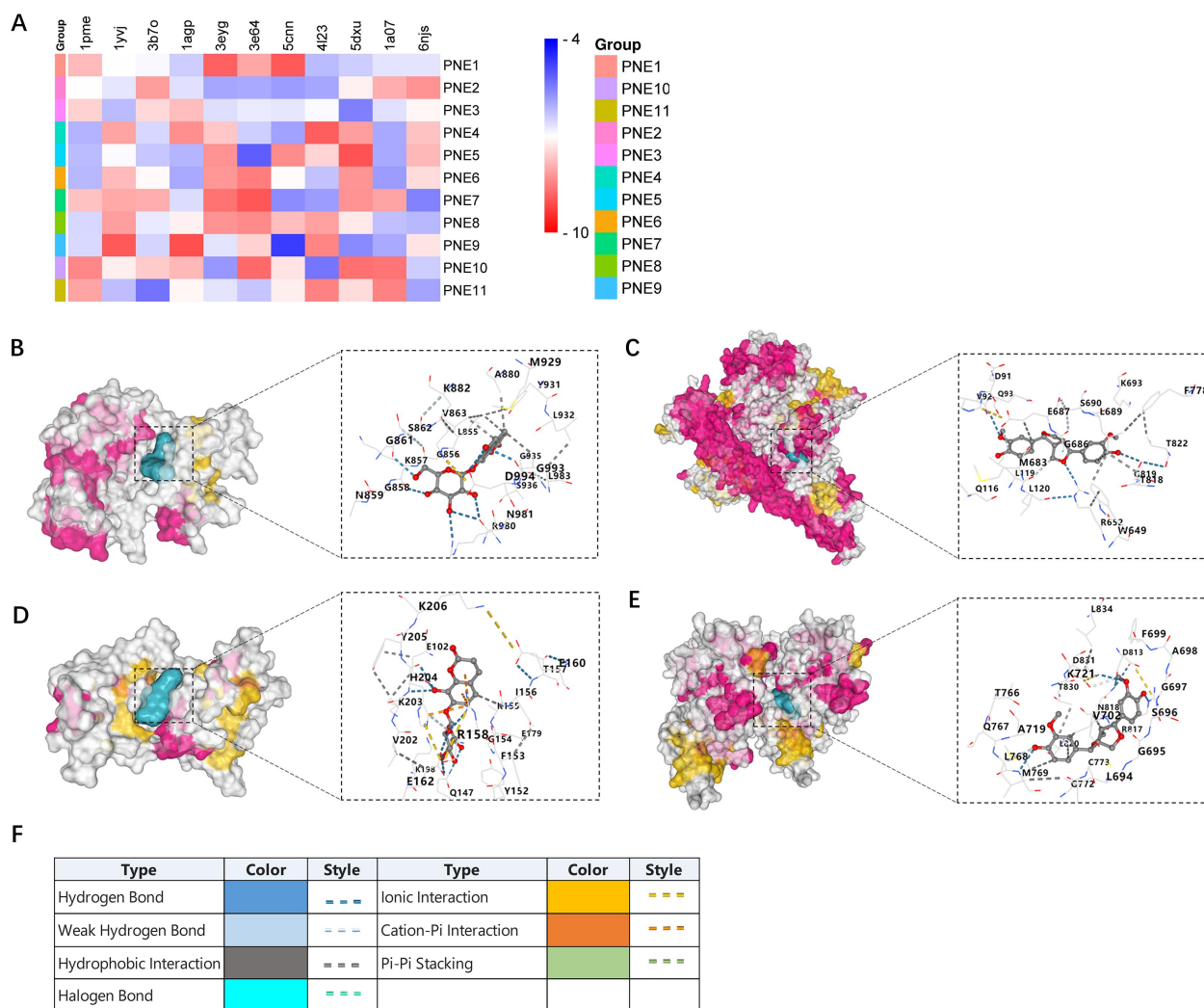


Figure 4 Docking complexes of hub targets with PNE compounds. (A) Distribution of all the dockings score visualized as a heatmap. (B) 3e54 with PNE5; (C) 1a07 with PNE4; (D) 5dxu with PNE9; (E) 5cnn with PNE9; (F) Chemical interaction items between proteins and small molecules. PNE, *Panax notoginseng*.

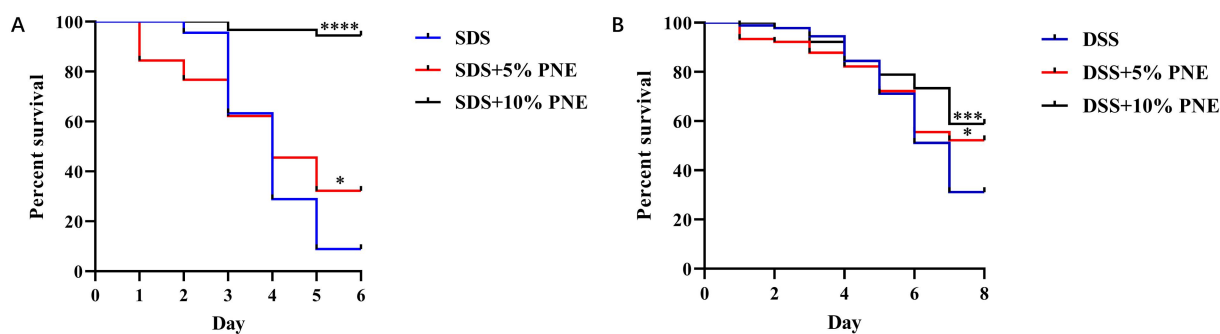


Figure 5 Survival rates of the control and PNE feeding groups treated with SDS and DSS. Both 5% and 10% PNE supplementation significantly improved the survival rates of *Drosophila* compared to 0.6% SDS (A) or 4% DSS (B) group at 25 °C for 4 to 7 days. Survival curves were generated from three separate experiments (n = 90). **P* < 0.05, ****P* < 0.001, *****P* < 0.0001. PNE, *Panax notoginseng*; SDS, sodium dodecyl sulfate; DSS, dextran sulfate sodium.

The midgut epithelium homeostasis is the core of the health of *Drosophila*, and inflammatory factors can induce the proliferation of ISCs for them, resulting in a big increase in the number of progenitor cells [39, 40]. However, the accumulation of progenitor cells due to overproliferation of intestinal stem cells will destroy the epithelium homeostasis [41]. Next, we sought to determine whether PNE has a protective effect on the intestinal of *Drosophila*. The transgenic strain esg-Gal4 UAS-GFP can specifically label progenitor cells (ISCs + EBs)

esg-GFP⁺ cells in the control. Meanwhile, the esg-GFP⁺ cells were greater increased and clustered in the SDS group (Figure 6A, 6B, 6E). PNE supplementation showed a significant decrease in GFP⁺ cells (Figure 6C, 6E). In addition, the esg-GFP⁺ cells were not altered in midguts fed PNE compared to the control (Figure 6D, 6E), indicating PNE is not toxic to *Drosophila*. These results suggest that PNE could rescue the aberrant proliferation of progenitor cells.

To further support this finding, the anti-PH3 antibody was

employed to label mitotic stem cells. Consistent with previous studies, PH3⁺ cells rised in whole midguts under SDS stress (Figure 7) [42]. PNE supplementation dramatically reduced the increase in PH3⁺ cells in SDS-treated *Drosophila* (Figure 7). Therefore, these results indicate that PNE alleviates the excessive proliferation of progenitor cells by SDS in *Drosophila*.

PNE can rescue the abnormal differentiation of progenitor cells induced by SDS

To investigate whether the number of progenitor cell enlargement was caused by ISCs or EBs, we used Delta-GFP and Su(H)-lacZ to mark ISC and EB, respectively. The number of Delta-GFP⁺ and Su(H)-lacZ⁺ cells were increased by 105.8% and 83.1% in the SDS group, respectively (Figure 8A, 8B, 8E, 8F). This result demonstrated that SDS increases

the differentiation of ISCs and EBs. Moreover, PNE supplementation restored the ISCs and EBs back to levels comparable to those in the sucrose group (Figure 8A, 8B, 8E, 8F). These results showed that PNE inhibits the excessive differentiation of ISCs and EBs induced by SDS.

EES and ECs are terminally differentiated cells based on the cell lineage [43, 44]. We analyzed the number of ECs with the EC-specific markers NP1-GFP and an antibody Prospero (pros) to label EEs. The number of ECs was unchanged after SDS ingestion compared with control (Figure 8C, 8G); the pros⁺ cells were significantly decreased in the SDS-treated midguts but increased after PNE supplementation, similar to the level in the sucrose group (Figure 8D, 8H). Taken together, these results illustrated that PNE inhibited the excessive differentiation of ISC and EB induced by SDS and promoted differentiation to EEs.

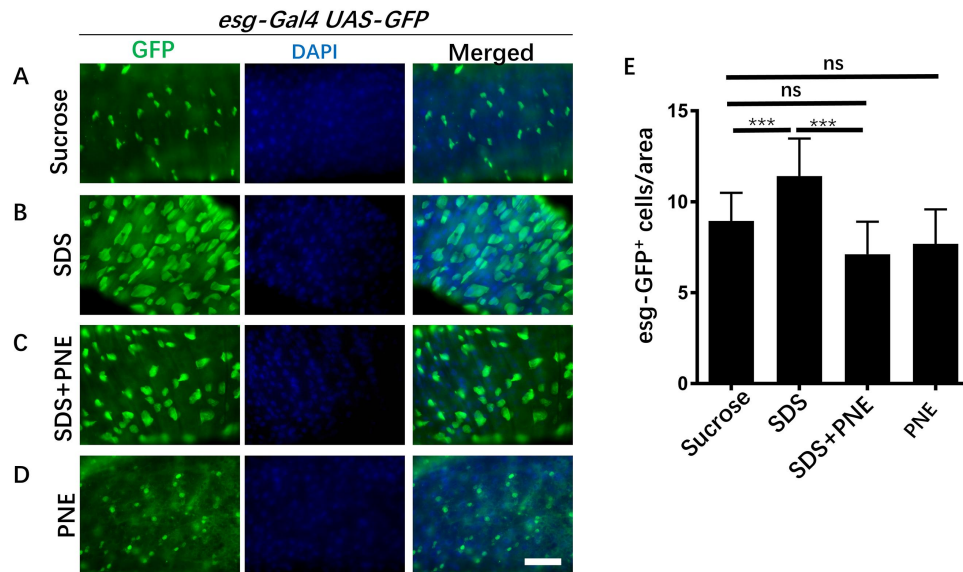


Figure 6 PNE inhibits the excessive proliferation of progenitor cells in adult midgut induced by SDS. Immunofluorescence images of the dissected midguts of *esg-GFP* in adult females. *Esg*⁺ cells were marked with GFP in green and DAPI in blue. *Esg-GFP*⁺ cells were clustered after treatment with SDS compared with sucrose (A, B). The increased *esg-GFP*⁺ cells were dramatically restored by 10% PNE supplementation in SDS (C). PNE supplementation did not affect the *esg-GFP*⁺ cells (D). Quantification of the number of *esg-GFP*⁺ cells (E). These data represented at least three independent experiments (n = 30). ***P < 0.001. Scale bars represent 50 μ m. ns, no significance. PNE, *Panax notoginseng*; SDS, sodium dodecyl sulfate; GFP, green fluorescent protein; DAPI, 4',6-diamidino-2-phenylindole.

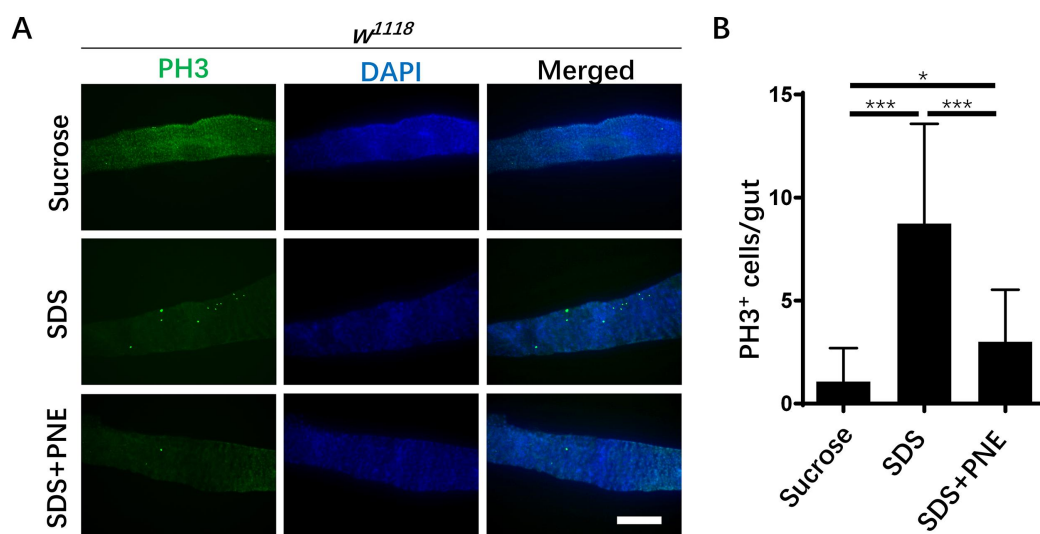


Figure 7 PNE can effectively alleviate injury-induced ISC proliferation. (A) The number of dividing cells of adult females' midguts of *w¹¹¹⁸* was counted using anti-PH3 antibodies (red). The number of PH3⁺ cells was increased following SDS treatment, and 10% PNE supplementation dramatically recovered the PH3⁺ cells. (B) Quantification of PH3⁺ cells (n = 30). *P < 0.05, ***P < 0.001. Scale bars represent 200 μ m. PNE, *Panax notoginseng*; SDS, sodium dodecyl sulfate; DAPI, 4',6-diamidino-2-phenylindole.

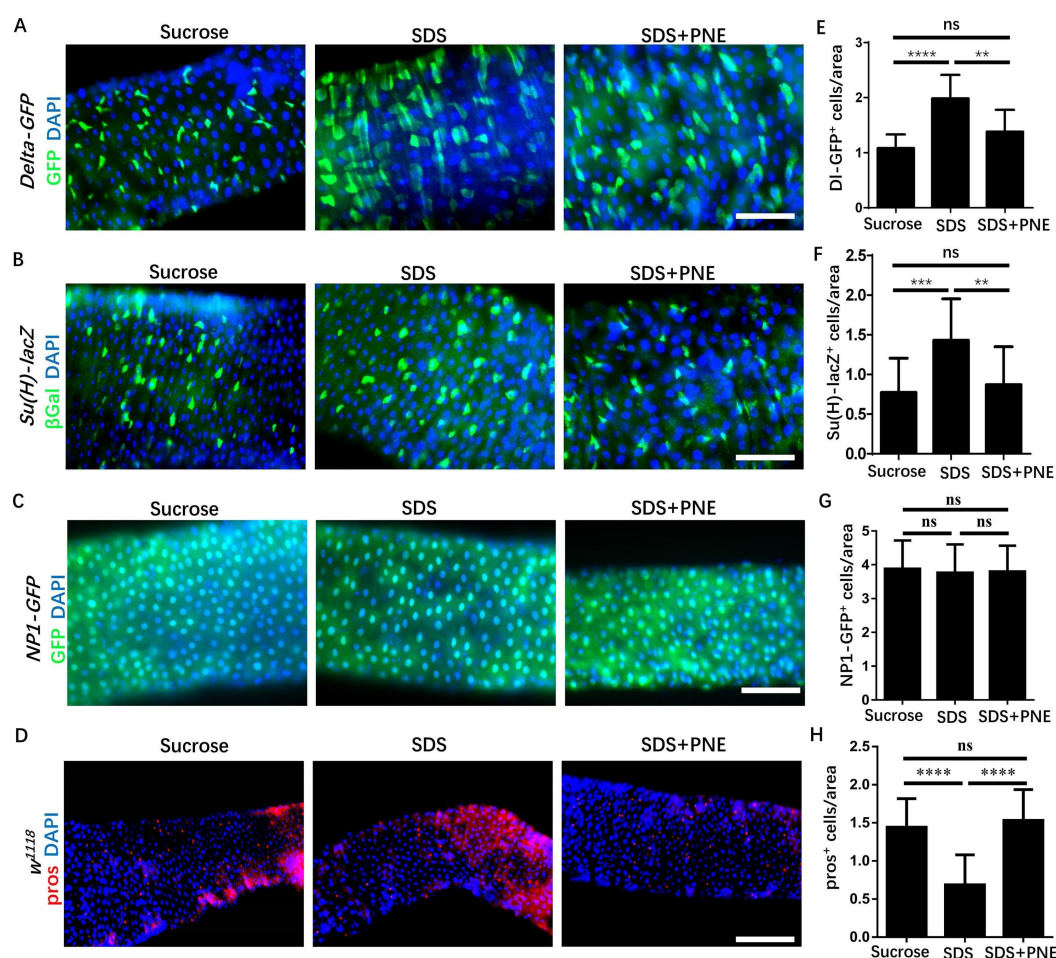


Figure 8 PNE restricts ISC proliferation and differentiation to EE. (A) Immunofluorescence images of the midguts expressing the Delta > GFP. DI-GFP⁺ cells were marked with GFP (green) and DAPI (blue). The DI-GFP⁺ ISCs were increased after treatment with SDS, and supplementation of 10% PNE significantly rescue the DI-GFP⁺ cells. (B) The number of Su(H)-lacZ⁺ EBs were detected to show a significant decreased in *Drosophila* fed SDS plus 10% PNE compared with *Drosophila* fed SDS and were not significantly different compared with *Drosophila* fed sucrose. (C, D) ECs and EEs were detected with Myo1A > GFP reporter and pros antibodies. ECs were not significantly different in all conditions (C). After 10% PNE supplementation in SDS treatment, the number of EE cells returned to the control level compared with SDS (D). (E, F) Quantification of the number of DI-GFP⁺ cells (E) and Su(H)-lacZ⁺ cells (F). (G, H) Quantification of the number of ECs (G) and EEs (H) (n = 30). **P < 0.01, ***P < 0.001, ****P < 0.0001. Scale bars represent 50 μ m. ns, no significance; PNE, *Panax notoginseng*; SDS, sodium dodecyl sulfate; GFP, green fluorescent protein; DAPI, 4',6-diamidino-2-phenylindole.

PNE protects the adult gut against SDS-induced epithelial cell death

Previous studies showed SDS can cause apoptosis or cell death in the midgut of *Drosophila* [39, 45]. We used 7-AAD staining to examine the protective effect of PNE following SDS damage. As expected, SDS triggered significant cell death; however, the number of dead cells significantly decreased after feeding with PNE (Figure 9A, 9D). Therefore, PNE can significantly inhibit the massive death of intestinal epithelial cells caused by SDS. It is known that SDS can induce ROS production, and excessive accumulation of ROS can damage cell function [46]. Therefore, we next used DHE to detect the ROS level in the intestinal tract. ROS levels were significantly increased induced by SDS (Figure 9B), as expected. However, PNE supplementation relieved the damage and restored ROS levels back to those of midguts in the control group (Figure 9B, 9E). Our findings suggested that PNE can protect *Drosophila* midgut from SDS-induced tissue damage.

PNE alleviates SDS-induced intestinal damage through JNK pathway

Previous studies have shown that the JNK pathway regulates ISC self-renewal and reactions to stress, infection, and intestinal damage [11, 41]. JNK pathway activation causes ISC expansion in aging

Drosophila [42, 47]. In addition, the accumulation of ROS activates JNK signaling, consequently promoting ISC proliferation [48]. To determine whether SDS treatment activated the JNK pathway in the intestine, we used the Puc-lacZ reporter to detect this pathway. Puc is a JNK target and represents the level of JNK pathway [49]. Puc-lacZ expression was markedly elevated in the SDS-treated posterior midguts relative to controls; after PNE feeding, activated JNK levels were markedly reduced (Figure 9C, 9F). Together, these results showed that PNE can protect the intestinal epithelium from gut damage by inhibiting oxidative stress related JNK pathway.

Discussion

The global incidence of IBD is increasing [39]. The pathogenesis of IBD is yet unknown, which is caused by the interaction of multiple genetic and environmental variables [50]. The clinical features of IBD are diverse and absent specificity, such as diarrhea and bloody stool, etc. [51, 52]. Many preclinical models of IBD have been established to research the pathophysiology and treatment. SDS and DSS have been used frequently to induce IBD in rodent studies [53, 54]. Several studies confirmed that oxidative stress is largely implicated in the pathophysiology of IBD [55]. Extensive levels of ROS can cause oxidative stress, which causes intestinal disorders and

overproliferation of ISCs [56]. In addition, the abnormalities of several signaling pathways such as P38 MAPK, MAPK-JNK, PI3K/Akt, NF-kappaB signaling pathways are crucial in the inflammatory process and pathogenesis of IBDs [1]. SDS and DSS can also induce intestinal inflammation in *Drosophila*, and their phenotypes are similar to those of mammalian intestinal inflammation models, including intestinal epithelial cell injury, large production of ROS, and activation of stress-related pathways such as MAPK, JNK, NF-kappaB, etc. [5, 6]. Restoration of intestinal homeostasis is required for the gut to resist chronic inflammation and maintain immune responses. Moreover, excessive activation of the intestinal immunity in turn can cause the imbalance of intestinal homeostasis and the epithelium damage, leading to local IBD, dysplasias and cancers [57–60]. Therefore, the restoration of intestinal homeostasis is crucial for the health of the organism.

Anti-inflammatory drugs and biologics are the main treatments for IBD at the moment, but all medications just reduce symptoms and have a high risk of recurrence rate and a high cost of care [61–64]. In the treatment of IBD, traditional Chinese medicine offers unique benefits owing to the use of various natural compounds and herbal extractions. These alternatives are favored for their low toxicity, minimal side effects, and high tolerability [65]. Therefore, it is imperative to conduct further research on natural products that demonstrate promising effects in managing IBD. The multiple compounds contained in PNE have been confirmed to have anti-inflammatory and antioxidant effects [66–68]. Additionally, investigations have indicated that PNE can effectively impede the growth of colorectal cancer cells, alleviate tumor growth in colorectal cancer model mice induced by DSS, and reduce the expression of inflammatory factors [69, 70]. However, its specific active ingredients and mechanisms for the treatment of IBD remain unclear. Here, we found that PNE can alleviate the survival rate reduction caused by SDS in *Drosophila*. The excessive ROS induced by SDS can cause intestinal epithelial cell apoptosis and excessive proliferation and differentiation of ISCs through activating the JNK signaling pathway in *Drosophila* [71]. PNE supplementation could reduce the high levels of ROS, the number of excessive dead cells and the excessive proliferation and differentiation of ISCs induced by SDS. PNE maintains the intestinal homeostasis by inhibiting the activation of JNK signaling pathway induced by SDS. Consistent with the experimental results, the network

pharmacological analysis also indicate that PNE may play an anti-IBD role through MAPK-JNK pathway. Taken together, the above results suggest that JNK signaling pathway may be a potential target for PNE treatment of IBD.

Network pharmacology can be used to understand the complexities between biological systems, drugs, and diseases from a network perspective [72, 73]. Moreover, we can identify drug targets in a high-throughput manner through network pharmacology. Widely targeted metabolomics was utilized to analyze the chemical compositions and medicinal properties of PNE, leading to the identification of 476 ingredients categorized into 11 types. Subsequently, network pharmacology was applied to investigate the anti-IBD effects of PNE and elucidate the associated molecular mechanisms in potential signaling pathways. The findings from network pharmacology and molecular docking indicated that PNE4, PNE5, and PNE9 serve as the primary active constituents in PNE responsible for its anti-IBD activity. It was revealed that PNE potentially exerts its anti-IBD effects by targeting SRC and MAPK1 to modulate the MAPK-JNK signaling pathway (Figure 3C). These results were consistent with the GO enrichment and KEGG analyses which demonstrated the involvement of the MAPK signaling pathway in the therapeutic effects of PNE on IBD (Figure 3A, 3B). The MAPK signaling pathway, comprising crucial components such as ERK, p38, and MAPK-JNK, plays a pivotal role in various cellular processes including proliferation, differentiation, and development [74]. Particularly, the JNK pathway has been found to regulate cell growth and proliferation, with significant implications in the development of diverse organisms, including *Drosophila* [41]. In addition, the molecular docking results showed that the PNE9-JAK1 was the most stable binding complex, suggesting that PNE may also be involved in the activation of JAK-STAT pathway. The JAK-STAT pathway is also known to be involved in the maintenance of intestinal homeostasis induced by toxic compounds [75].

The results combined in silico and in vivo experimental provided an insight into the use of PNE. In summary, our findings suggest that PNE, as a potential new therapeutic agent for IBD, can maintain intestinal homeostasis by regulating JNK signaling pathways and improve the survival rate of IBD *Drosophila* (Figure 10). In addition, future studies in rodent models will help us further understand the mechanism of PNE in the treatment of IBD.

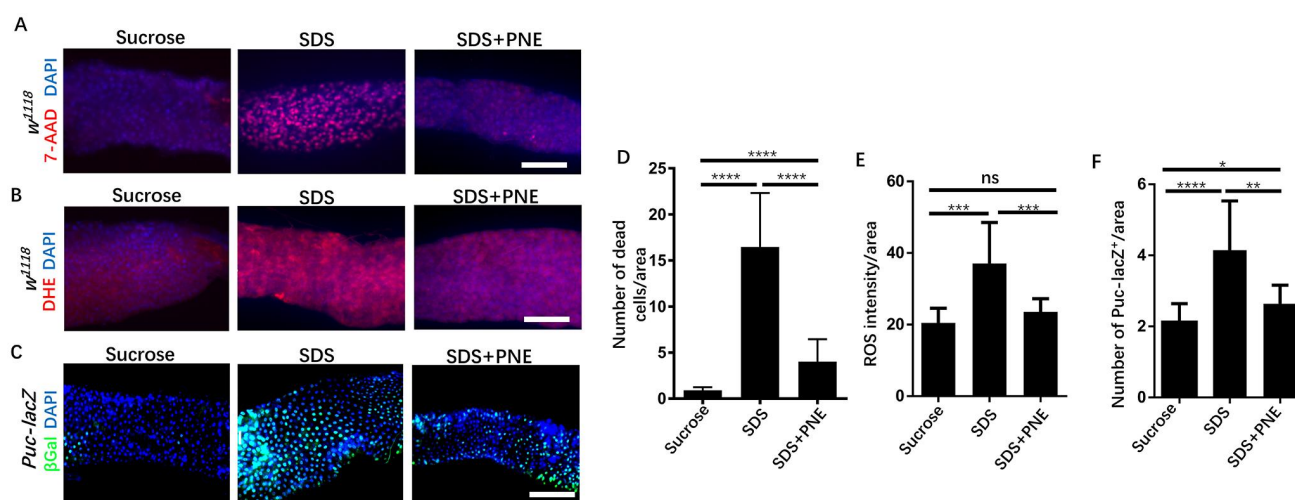


Figure 9 PNE relieves SDS-induced intestine damage by preventing oxidative stress-associated JNK signaling. (A, D) The number of dead cells in adult females' midguts of *w¹¹¹⁸* was detected by using 7-AAD (red). The dead cells were significantly increased following SDS treatment, 10% PNE supplementation significantly rescued the dead cells; the number of dead cells was quantified in (D). (B) DHE was used to monitor the ROS level (red). ROS levels were significantly increased in the midgut after SDS treatment. 10% PNE supplementation restored the elevated ROS levels induced by SDS. (C) Immunofluorescence images of the midguts of *Puc-lacZ* (green), which is a JNK pathway reporter. After treatment with SDS, the *Puc-lacZ* levels were remarkably increased. 10% PNE significantly reduced the expression of *Puc-lacZ* in the SDS-treated midguts. (E) The ROS intensity per unit area in the midgut in (B). (F) Quantification of the number of *Puc-lacZ⁺* cells in the midgut in (C). Three independent experiments were performed ($n = 30$). $P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. Scale bars represent 100 μ m. ns, no significance. PNE, *Panax notoginseng*; SDS, sodium dodecyl sulfate; DAPI, 4',6-diamidino-2-phenylindole; DHE, dihydroethidium; 7-AAD, 7-aminoactinomycin.

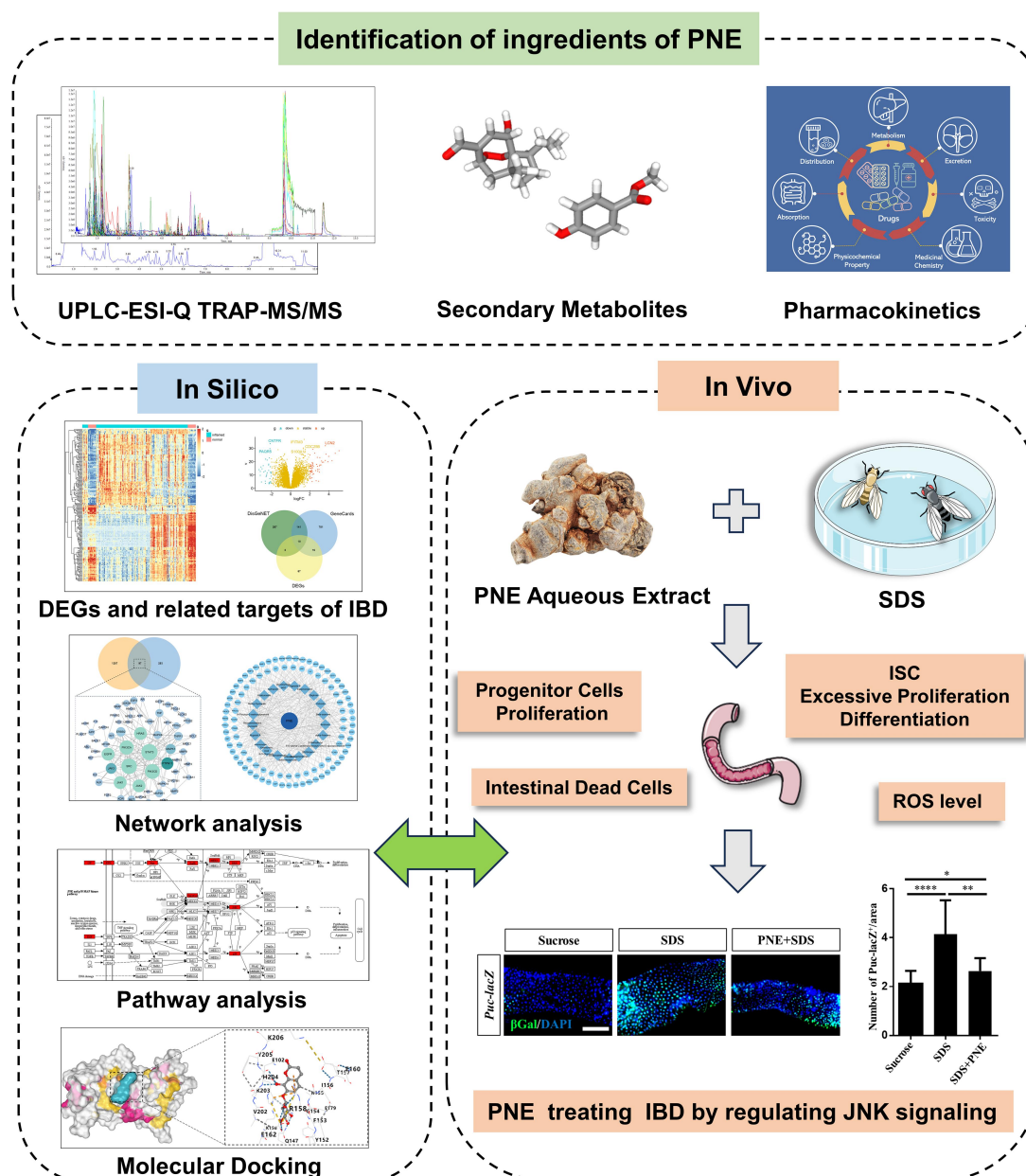


Figure 10 Graphic abstract of PNE in treatment of IBD. Chemical characterization, bioinformatics analysis and experimental with drosophila IBD model. PNE, *Panax notoginseng*; SDS, sodium dodecyl sulfate; JNK, jun-Nterminal kinase; IBD, inflammatory bowel disease; DEGs, differentially expressed genes; ISC, intestinal stem cell; ROS, reactive oxygen species.

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