Psychopharmacology
https://doi.org/10.1007/s00213-025-06796-w

ORIGINAL INVESTIGATION

Senegenin ameliorates diabetic encephalopathy via promoting mitophagy and repressing NLRP3 inflammasome activation

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Received: 27 October 2024 / Accepted: 18 April 2025

Abstract

Rationale Diabetic encephalopathy (DE) remains a severe complication of diabetes in central nervous system with limited effective therapy.

Objectives This study investigated the beneficial effect of senegenin on DE and its possible mechanisms.

Methods Type 2 diabetes mellitus mouse model and high-glucose (HG)-stimulated PC-12 cells were used as the in vivo and in vitro DE models. Learning and memory ability was evaluated by MWM test. Pathological changes in the brain tissues were determined by HE staining. Cell viability was detected by CCK-8. Mitochondrial membrane potential was measured by JC-1 probe. Target protein levels were assessed by Western blotting. Nucleotide-binding domain-like receptor protein 3 (NLRP3) expression was observed by immunofluorescent staining.

Results Cognitive impairment and obvious pathological changes were found in DE mice, which were effectively attenuated by senegenin treatment. In addition, senegenin induced mitophagy and maintained homeostasis of mitochondrial dynamics to relieve mitochondrial dysfunction. Moreover, NLRP3 inflammation activation induced by DE was inhibited by senegenin. Finally, inhibition of mitophagy counteracted senegenin-mediated inactivation of NLRP3 inflammation and neuroprotection.

Conclusions Senegenin relieved diabetic encephalopathy via inducing mitophagy to inactivate NLRP3 inflammasome. Senegenin might be an effective therapy for treating DE.

Keywords Diabetic encephalopathy · Senegenin · NLRP3 inflammation · Mitophagy · Mitochondrial dysfunction

Introduction

Diabetic encephalopathy (DE) is a chronic cognitive impairment and accompanying moto dysfunction caused by diabetes mellitus (Belenichev et al. 2023). DE patients are more likely to suffer both dementia and postural perturbations (Oh et al. 2021; Strand et al. 2024). So far, DE has been considered as a frequent central nervous system complication of diabetes mellitus. However, there is currently no effective treatments for DE because of its complicated pathological mechanisms (Watroba et al. 2024). Therefore, it is crucial to clarify the pathogenesis and identify effective therapies for DE.

Senegenin is one of bioactive constituents extracted from Polygala tenuifolia Willd root (Chen et al. 2022). Senegenin has been demonstrated to exert multiple pharmacological effects, such as suppressing inflammation (Fan et al. 2017), anti-depressive disorder (Li et al. 2017), anti-acute lung injury (Fu et al. 2016), and so on. Notably, senegenin can pass through the blood–brain barrier attributed to

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Published online: 26 April 2025

its lipophilicity and small molecular weight, which could exhibit marked improvement in cognitive function (Cai et al. 2013; Chen et al. 2010). However, the role of senegenin in the treatment of DE remains unknown.

Mitophagy is a specific type of autophagy that is responsible for the maintenance of mitochondrial quantity control (Chen et al. 2016). Mitophagy disruption has been shown to participate in various diabetic complications. For example, mitophagy was restrained in diabetic neuropathy, and activation of PINK1-Parkin-mediated mitophagy could effectively relieve diabetic neuropathy in rats (Yang et al. 2024). A previous study documented that mitochondrial dynamics disorder was involved in the pathogenesis of cognitive impairment in DE (Han et al. 2024). In this respect, mitophagy induction might represent an effective intervention for DE.

Nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome is composed of NLRP3, ASC and caspase- 1 (Choe and Kim 2017). NLRP3 inflammasome activation takes part in a variety of disorders, including DE (Hu et al. 2020). Studies have suggested that inactivation of NLRP3 inflammasome conferred protection against DE (Zhai et al. 2018; Zhang et al. 2023). Senegenin has been reported to possess anti-depressant effect in mice through inhibiting NLRP3 inflammasome activation (Li et al. 2017). Besides, senegenin attenuated dopaminergic neuron inflammation by restraining NLRP3 inflammasome (Fan et al. 2017). However, whether senegenin alleviated DE through inhibiting NLRP3 inflammasome has not been explored.

This study aimed to investigate whether senegenin can relieve DE via regulation of mitophagy and NLRP3 inflammasome in the in vivo and in vitro models of DE. Our findings provide evidence that senegenin may serve as a promising therapy for treating DE.

Materials and methods

Animal model

Six- to eight-week-old male C57BL/6 mice (weighing 18–22 g) were purchased from HFK Bioscience Co. Ltd (Beijing, China). After adjustable feeding for 7 days, the mice were randomly divided into 4 groups (n = 6 per group): control, DE model, DE model + senegenin low concentration (L), DE model + senegenin high concentration (H). To establish DE model, the mice were fed with a high fat diet (Research Diets, USA) for 12 weeks, followed by intraperitoneal injection with streptozocin (STZ, 40 mg/kg, Solarbio, Beijing, China) for 5 consecutive days. The control mice were fed with normal chow diet and injected with citric acid buffer. The mice with blood glucose level > 11.1 mmol/L were considered as diabetes. The mice in senegenin L and senegenin H groups were received intragastrical administration with 2 mg/kg and 4 mg/kg senegenin once a day for 12 weeks (Cai et al. 2013), respectively. After drug treatment, all mice were subjected to euthanasia via cervical dislocation, and brain tissues were collected. All experimental procedures were approved by the Ethics Committee of Shenyang Medical College.

Morris water maze (MWM)

The spatial learning and memory ability of mice was evaluated by MWM test before euthanasia as previously described (Qi et al. 2024). The MWM assay was conducted in a round water pool containing four quadrants and a hidden platform. For training, the mice swam in the pool to find the platform. If the mice could not find the platform within 60 s, they were guided to the platform. The trials were carried out four times daily over 5 days. Then, the platform was placed in a new position, and the paths and the time spending to find the platform were recorded. Moreover, the paths and the number of crossing the platform within 1 min were detected.

Hematoxylin and eosin (HE) staining

The brain tissues were fixed in 4% paraformaldehyde, embedded in paraffin and sliced to 4-μm sections. Then, the sections were subjected to HE staining to determine the morphological changes using the HE Stain Kit (Solarbio), following the protocol. The stained sections were examined under a light microscope (Olympus, Tokyo, Japan).

Immunofluorescent staining

The expression of NLRP3 in the brain sections was assessed by immunofluorescent staining. Repair antigen was performed on the brain sections using the boiling sodium citrate buffer. The sections were blocked with 1% bovine serum albumin (BSA) for 1 h, and then probed with primary antibody against NLRP3 (A12694, 1:50, Abclonal, Wuhan, China) at 4 °C overnight. Thereafter, the sections were incubated with the Cy3-conjugated Goat anti-Rabbit IgG (AS007, 1:100, ABclonal) for 1 h. After incubation with the DAPI agent (Solarbio), the sections were photographed under a fluorescence microscope (Olympus).

Cell culture and treatment

Rat adrenal pheochromocytoma PC- 12 cells were purchased from American Type Culture Collection (USA) were cultured in RPMI- 1640 Medium (Gibco, USA) containing 10% fetal bovine serum (Gibco) at 37 °C with 5% CO_2. To simulate diabetes in vitro, PC- 12 cells were stimulated with high glucose (HG, 100 mM) for 48 h. The control cells were maintained with normal glucose (NG, 5 mM). For senegenin

treatment, PC- 12 cells were treated with 30 μM or 60 μM senegenin and HG stimulation simultaneously (Ren et al. 2022; Tian et al. 2022).

Cell transfection

Short hairpin RNA (shRNA) targeting PINK1 (sh-PINK1) and negative control shRNA (sh-NC) were purchased from GeneChem (Shanghai, China). Senegenin-treated PC- 12 cells were transfected with sh-PINK1 or sh-NC using Lipofectamine 2000 (Thermo Fisher).

Western blotting

Protein samples were isolated from brain tissues and PC-12 cells using the cell lysis buffer (CST, USA) added with PMSF (Beyotime). The BCA Protein Assay Kit (Solarbio) was adopted to determine protein concentration. Equal amount of protein extracts was separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis, and then then blotted onto the polyvinylidene fluoride membranes. After blocking with 5% nonfat milk, the membranes were incubated with primary antibodies against OPA1 (#80,471, 1:1000, CST), MFN1 (A9880, 1:500, ABclonal), MFN2 (A19678, 1:500, ABclonal), p-DRP1 (Ser616, #63,940, 1:500, CST), FIS1 (#32525S, 1:1000, CST), PINK1 (A7131, 1:1000, ABclonal), Parkin (#32,833, 1:1000, CST), LC3 (A19665, 1:500, ABclonal), NLRP3 (A12694, 1:500, ABclonal), ASC (A1170, 1:500, ABclonal), cleaved Caspase-1 (PA5 - 119,528, 1:500, Thermo Fisher), IL-1β (ab283818, 1:1000, Abcam, UK), IL- 18 (A23076, 1:2000, ABclonal), and GAPDH (#2118, 1:1000, CST) at 4 °C overnight. Subsequently, the membranes were probed with the secondary antibodies (AS014, 1:2000, ABclonal) for 1 h. Protein bands were detected using the SuperSignal West Pico PLUS (Thermo Fisher).

Cell-counting-kit 8 (CCK- 8)

Cell viability was measured using the CCK- 8 assay kit (Beyotime, Haimen, China). PC12 cells were seeded into 96-well plates (3000 cells per well). After various treatments, CCK- 8 reagent (10 μL) was added into each well, followed by incubation for 2 h. The result was detected on a microplate reader (Thermo Fisher, USA) at the absorbance of 450 nm.

Mitochondrial membrane potential evaluation

Mitochondrial membrane potential was evaluated using the mitochondrial membrane potential assay kit with JC- 1 (Beyotime). PC12 cells were planted into 6-well plates (3×10^5 cells per well). Subsequently, the cells with various treatments were incubated with JC- 1 solution for 20 min at 37 °C. After washing with culture medium for 3 times, the cells were analyzed by flow cytometry (BD Biosciences, USA).

Screening of targets for senegenin against DE

The canonical SMILE of senegenin was acquired from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The canonical SMILE of senegenin was submitted into Swiss Target Prediction database (http://www.swisstargetprediction.ch/), Super-PRED database (https://prediction.charite.de/) and Pharmmapper database (https://prediction.charite.de/) to predict potential targets of senegenin. The DE-related genes were obtained from the Genecard database (https:/www.genecards.org/), OMIM database (https:/www.genecards.org/) and PharmGKB database (https://www.pharmgkb.org/).

Network construction

The protein–protein interaction (PPI) network of the intersection targets was generated using the STRING database (https://cn.string-db.org/). PPI networks were visualized and analyzed using the Cytoscape (version 3.8.2) software.

Gene ontology (GO) and Kyoto encyclopaedia of genes and genomes (KEGG)

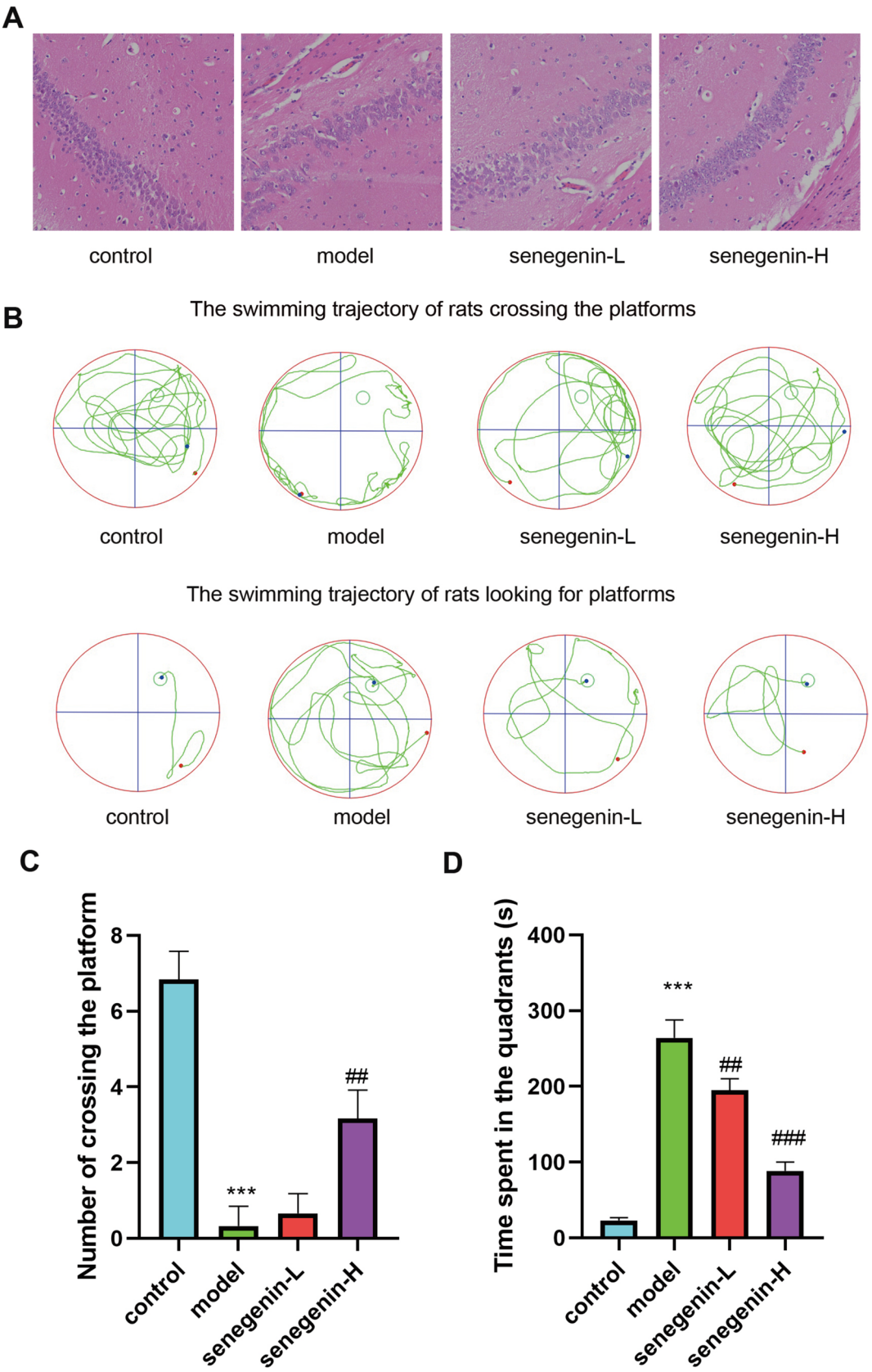
To analyze the biological significance of the targets, enrichment analysis of GO and KEGG was performed using the DAVID database (https://david.ncifcrf.gov/). The p-values (≤ 0.05) were ranked from the smallest to the largest.

Molecular docking

To evaluate the potential binding modes of senegenin with target proteins, AutoDock software was adopted to conduct molecular docking analysis. The protein data bank (PDB) file was downloaded from the PDB database (https://www.rcsb.org/), and the 3D structure of senegenin was acquired from the PubChem database. The docking results were visualized using PyMol 4.6.0.

Statistical analysis

Data are presented as mean ± standard deviation (SD). GraphPad Prism 6.0 was adopted for statistical analysis using Student's t test for two group or One-Way ANOVA for multiple group comparison. A p-value less than 0.05 was considered as statistically significant.


A
control
model
senegenin-L
senegenin-H
B
The swimming trajectory of rats crossing the platforms
control
model
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senegenin-H
The swimming trajectory of rats looking for platforms
control
model
senegenin-L
senegenin-H
C
Number of crossing the platform
0
2
4
6
8
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##
control
model
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Time spent in the quadrants (s)
0
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senegenin-H

◂**Fig. 1** Senegenin treatment protected against cognitive impairment of DE mice. **A** The pathological alterations in the hippocampal tissues were observed by HE staining. **B** Swimming trajectories of the MWM test. **C** The number of crossing the platform. **D** Time spent for searching the platform. *** $p < 0.001$, compared with the control group. ## $p < 0.01$, ### $p < 0.001$, compared with the model group

Results

Senegenin treatment alleviated cognitive impairment of DE mice

First, we evaluated the role of senegenin in the pathological alterations in the brains of DE mice. HE staining indicated that the neuronal cells were damaged, arranged irregularly, and pyknotic in the hippocampal tissues of DE model group, whereas senegenin-H treatment obviously attenuated these pathological changes (Fig. 1A). Furthermore, learning and memory functions of mice were determined by the MWM assay. As shown in Fig. 1B-D, DE mice spent more time to find the hidden platform, while had a smaller number of crossing the platform, which could be partly reversed by administration with senegenin. These results demonstrated that senegenin effectively improved the cognitive function of DE mice.

Senegenin induced mitophagy to maintain homeostasis of mitochondrial dynamics in DE mice

Mitochondria have been recognized as the main place for glucose metabolism and energy production, and synaptic mitochondria dysfunction was reported to drive DE progression (Han et al. 2024). As mitochondria dynamics is responsible for the maintenance of mitochondrial function, we further investigated the effect of senegenin on the fusion and fission of mitochondria. The results showed that the protein levels of fusion-associated proteins OPA1, MFN1, and MFN2 were down-regulated, while fission-associated proteins p-DRP1 and FIS1 were up-regulated in the hippocampal tissues of DE mice. However, senegenin treatment partially counteracted the above changes (Fig. 2A-F). In addition, DE led to reduction in the expression of mitophagy-related proteins PINK1 and Parkin, as well as LC3II/I ratio in the hippocampal tissues, which was strikingly enhanced by senegenin intervention (Fig. 2G-J). Taken together, senegenin could improve DE-induced dysfunction of mitochondrial dynamics and damaged mitophagy.

Senegenin inactivated NLRP3 inflammasome in DE mice

NLRP3 inflammasome overactivation has been documented to participate in the pathogenesis of DE (Zhang et al. 2023). We found that the positive staining of NLRP3 in the hippocampal tissues of DE mice was evidently enhanced, whereas senegenin treatment could significantly inhibit NLRP3 expression (Fig. 3A). Besides, the protein levels of NLRP3, ASC, cleaved caspase- 1, IL-1β, and IL- 18 were raised in DE group, which were partly abolished in senegenin treatment groups (Fig. 3B-G). The above observations suggested that DE-induced NLRP3 inflammasome activation was weakened by senegenin.

Senegenin relieved mitochondrial dysfunctionviainducing mitophagy in HG-stimulated PC- 12 cells

We further established an in vitro model of DE by stimulation with HG in PC- 12 cells. As presented in Fig. 4A, HG-induced decrease in cell viability was restored by senegenin intervention. Additionally, the decreased mitochondrial membrane potential after HG stimulation was remarkably enhanced in senegenin-treated PC- 12 cells (Fig. 4B). Furthermore, HG-mediated down-regulation of PINK1, Parkin, and LC3II/I ratio in PC- 12 cells was strikingly recovered after senegenin treatment (Fig. 4C-F). Collectively, senegenin protected against HG-triggered mitochondrial dysfunction via inducing mitophagy in PC- 12 cells in vitro.

Senegenin triggered mitophagy to restrain NLRP3 inflammasome activation of HG-challenged PC- 12 cells

Previous study has revealed that enhancing mitophagy could inactivate NLRP3 inflammasome (Lei et al. 2024). Thus, we further explored whether senegenin-mediated mitophagy could suppress NLRP3 inflammasome activation in PC- 12 cells upon HG exposure. As assessed by Western blotting, senegenin treatment effectively reversed the enhanced protein levels of NLRP3, ASC, cleaved caspase- 1, IL-1β, and IL- 18 in HG-stimulated PC- 12 cells (Fig. 5A-F). Next, sh-NC or sh-PINK1 was transfected into senegenin-treated PC- 12 cells in the presence of HG. CCK- 8 data indicated that the elevation of cell viability mediated by senegenin was abrogated by PINK1 knockdown (Fig. 5G). Besides, senegenin-induced down-regulation of NLRP3, ASC, cleaved caspase- 1, IL-1β, and IL- 18 in HG-stimulated PC- 12 cells was counteracted after sh-PINK1 transfection (Fig. 5H-M). Therefore, senegenin inactivated NLRP3 inflammasome via mitophagy induction, thereby conferring protection against DE.

Network pharmacology analysis of senegenin against DE

Swiss Target Prediction, Super-PRED, and Pharmmapper databases identified 100 potential targets for senegenin,

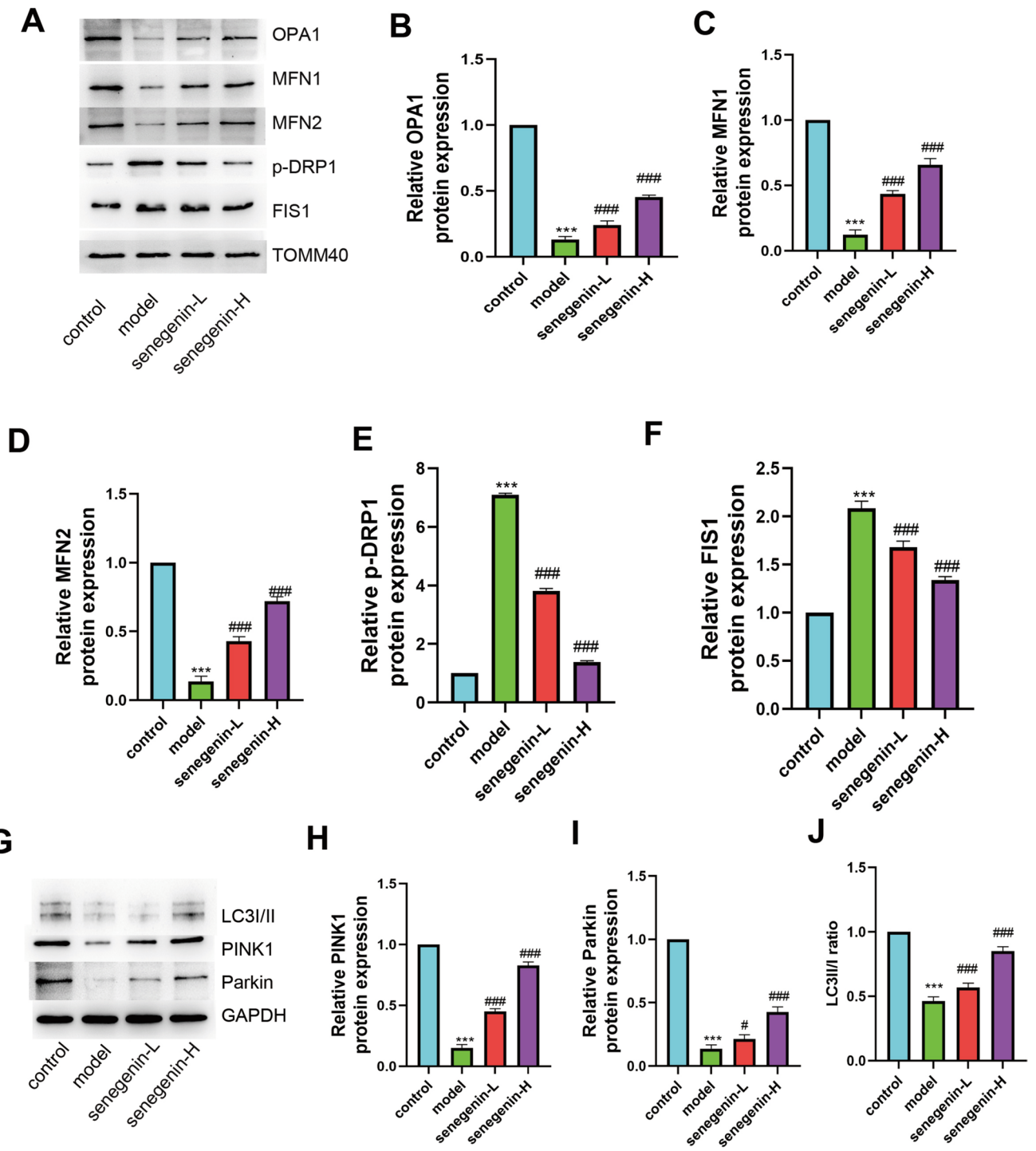

Fig. 2 Senegenin contributed to mitophagy and homeostasis of mitochondrial dynamics in DE mice. **A** Western blotting analysis of OPA1, MFN1, MFN2, p-DRP1, and FIS1 protein abundance in the hippocampal tissues of mice. **B-F** Quantification of the protein bands. **G** The protein levels of PINK1, Parkin, and LC3I/II were assessed by Western blotting. **H-J** Quantification of the protein bands. *** $p < 0.001$, compared with the control group. # $p < 0.05$, ### $p < 0.001$, compared with the model group

and 6779 DE-related targets were obtained from Genecard, OMIM, and PharmGKB databases. The PPI network of senegenin targets was illustrated in Fig. 6A. Furthermore, the core targets of senegenin were screened out via degree values, betweenness centrality (BC), and closeness centrality (CC) higher than the average (Fig. 6B). The top- 14 targets of senegenin are ESR1, CASP3, PTGS2, EGFR, CREBBP, APP, PARP1, RELA, CDK2, CYP1 A1, CYP19 A1,

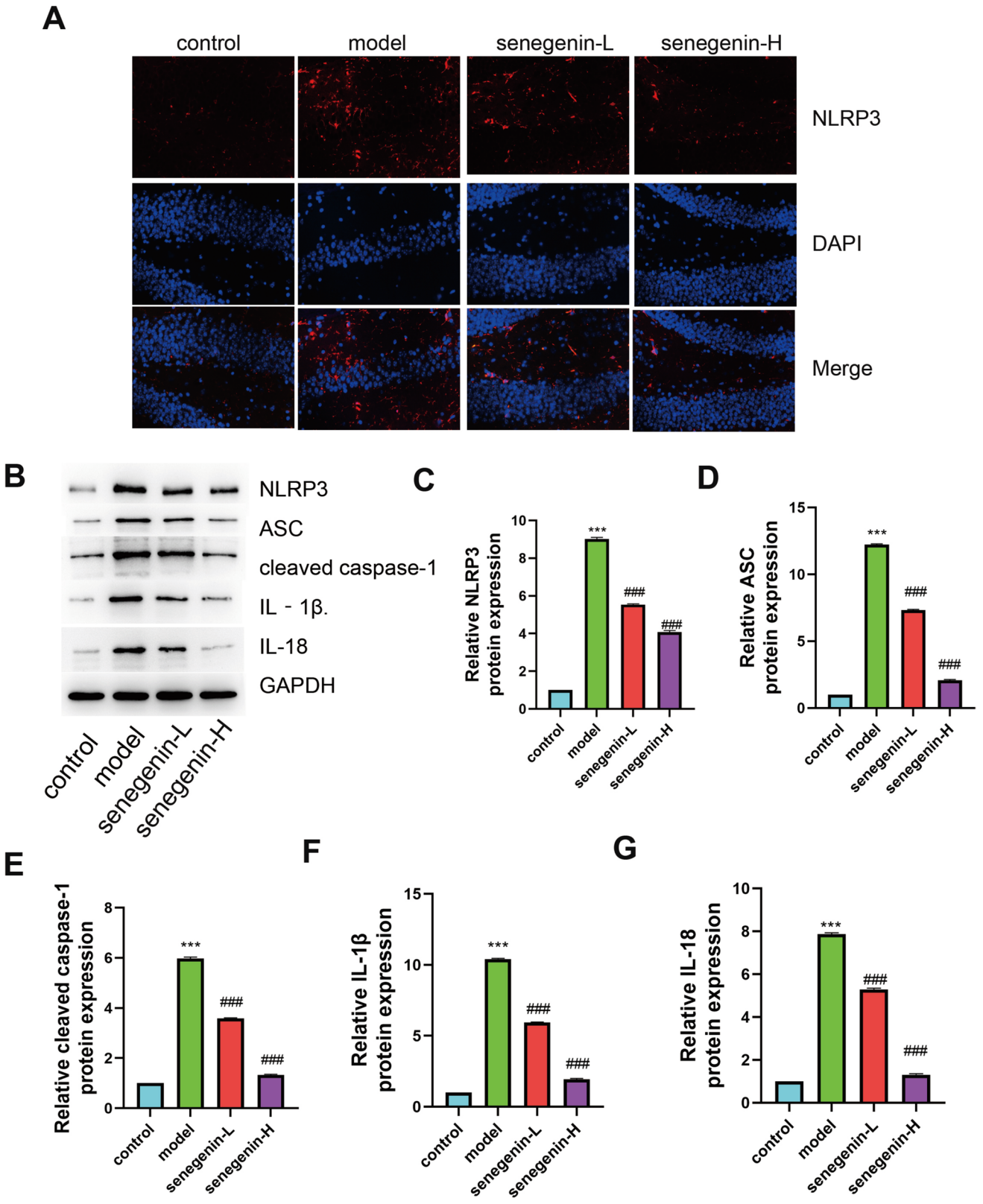

Fig. 3 Senegenin resulted in inactivation of NLRP3 inflammasome in DE mice. **A** NLRP3 expression in the hippocampal tissues was evaluated by immunofluorescent staining. **B** The protein levels of NLRP3, ASC, Cleaved Caspase-1, IL-1β, and IL- 18 were determined by Western blotting. **C-G** Quantification of the protein bands. *** $p < 0.001$, compared with the control group. ### $p < 0.001$, compared with the model group

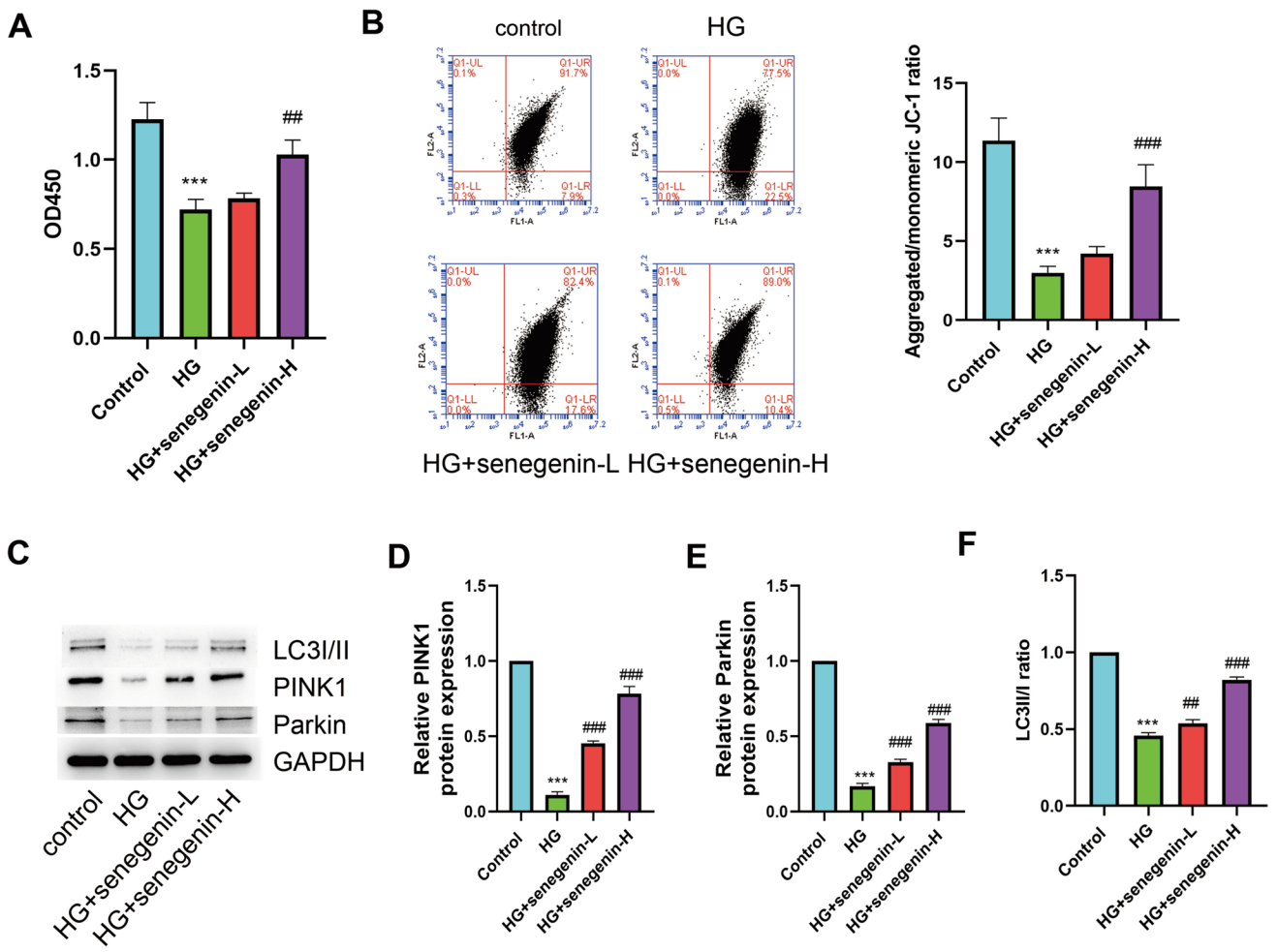

Fig. 4 Senegenin triggered mitophagy to attenuate HG-induced mitochondrial dysfunction of PC- 12 cells. PC- 12 cells were treated with 30 μM or 60 μM senegenin together with stimulation with 100 mM HG for 48 h. **A** Cell viability of PC- 12 cells was measured by CCK-8. **B** Mitochondrial membrane potential of PC- 12 cells was detected by JC- 1 staining. **C** Western blotting analysis of PINK1, Parkin, and LC3I/II protein levels in PC- 12 cells. **D-F** Quantification of the protein bands. *** $p < 0.001$, compared with the control group. ## $p < 0.01$, ### $p < 0.001$, compared with the HG group

MAPK14, CTSB, and CYP1 A2. By overlapping the results via Venn diagram, there were 82 intersection targets between senegenin and DE (Fig. 6C). The intersection target network diagram of senegenin and DE was constructed by Cytoscape software (Fig. 6D). Subsequently, the 82 intersection targets were uploaded to the STRING website to generate a PPI network diagram (Fig. 6E). According to cytoHubba analysis, 10 key targets of senegenin for treating DE are CASP3, MCL1, SRC, MAPK14, KDR, MMP2, SERPINE1, EP300, GSK3B, and PDGFRB (Fig. 6F).

GO and KEGG enrichment analysis

To evaluate the functional association of senegenin-targeted genes in DE, the GO enrichment analysis was performed. The results showed that the biological processes, including mitochondria, phosphorylation, angiogenesis, negative regulation of the apoptosis process are included (Fig. 7A). In addition, the KEGG analysis identified 10 enriched pathways, including diabetic cardiomyopathy, AGE-RAGE signaling Pathway in diabetic complications, and proteoglycan in cancer, and so on (Fig. 7B). These data uncovered the molecular mechanisms underlying the beneficial action of senegenin on DE.

Molecular docking analysis

To validate the interaction between senegenin and key targets, molecular docking analysis was carried out. The binding energy with less than − 5.0 kcal/mol indicates a favorable binding affinity. The binding energies of senegenin to key targets are as follow: MCL1 (− 7.3 kcal/mol), CASP3(− 9.5 kcal/mol), SRC (− 6.1 kcal/mol), PDGFRB (− 6.9 kcal/mol), MAPK14 (− 8.0 kcal/mol), KDR

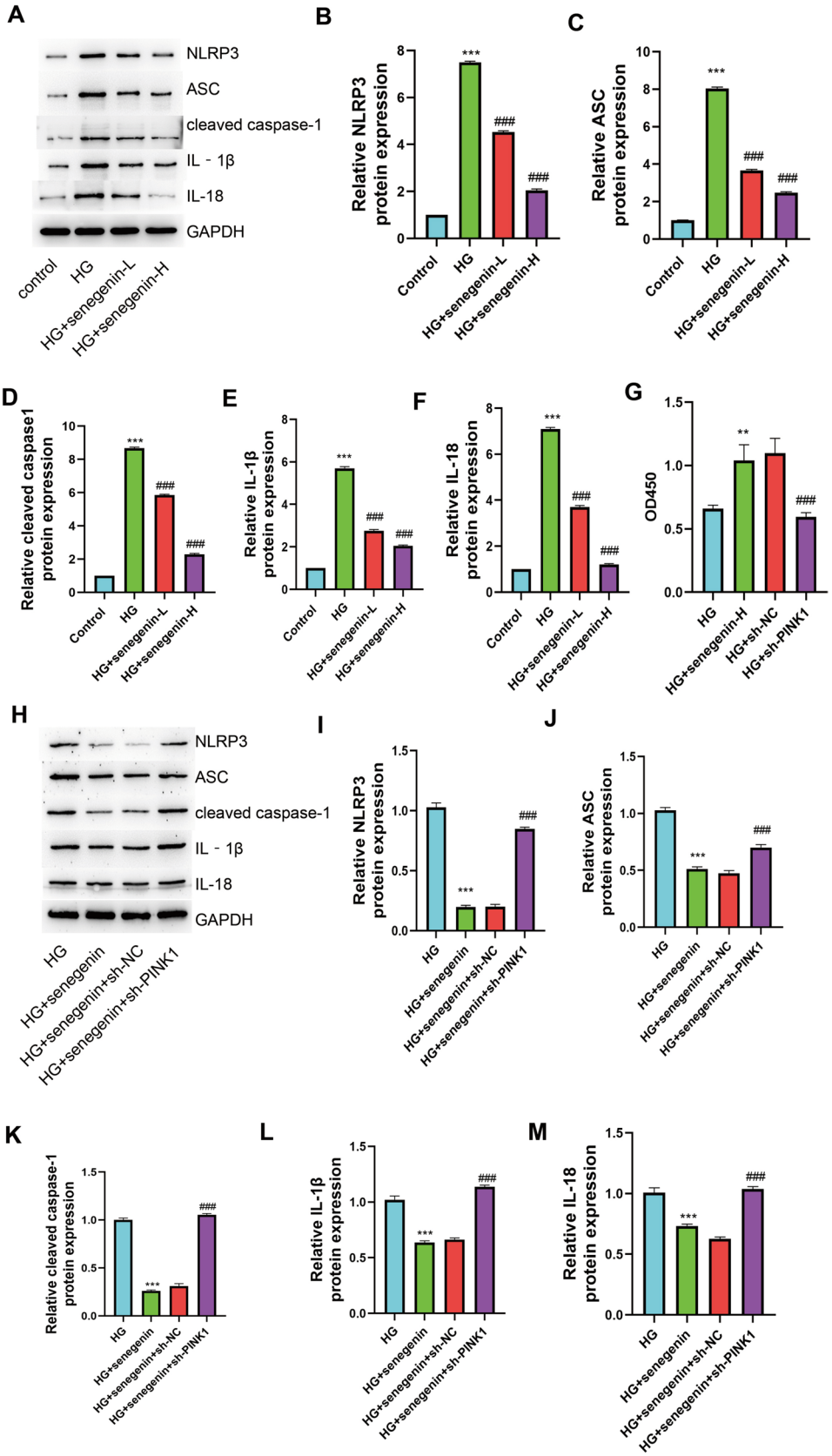

Fig. 5 Senegenin promoted mitophagy to repress NLRP3 inflammasome overactivation of HG-exposed PC- 12 cells. PC- 12 cells were treated with 30 μM or 60 μM senegenin together with stimulation with 100 mM HG for 48 h. **A** The protein levels of NLRP3, ASC, Cleaved Caspase-1, IL-1β, and IL- 18 in PC- 12 cells were measured by Western blotting. **B-F** Quantification of the protein bands. PC- 12 cells treated with 60 μM senegenin were transfected with sh-NC or sh-PINK1 in the presence of HG exposure. **G** PC- 12 cell viability was assessed by CCK- 8. **H** Western blotting analysis of NLRP3, ASC, Cleaved Caspase-1, IL-1β, and IL- 18 protein abundance. **I-M** Quantification of the protein bands. *** $p < 0.001$, compared with the control group. ### $p < 0.001$, compared with the HG group

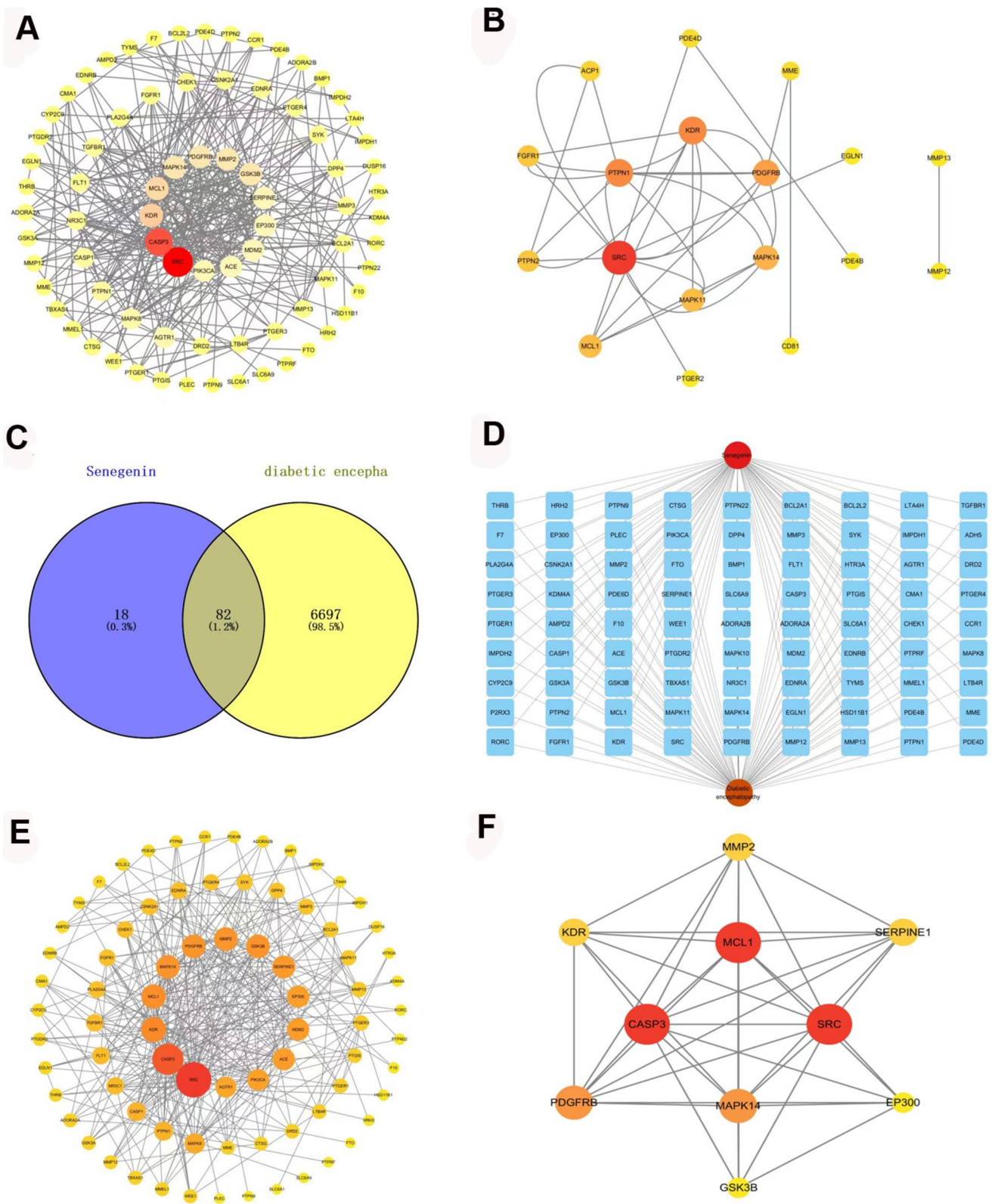

Fig. 6 Network pharmacology analysis. **A** A PPI network of targets regulated by senegenin. **B** The core targets of senegenin with average BC, CC and degree values higher than the average. **C-D** 82 intersection targets of DE and senegenin. **E** The PPI network of DE and senegenin intersection targets. **F** Key targets of senegenin for treating DE based on cytoHubba analysis

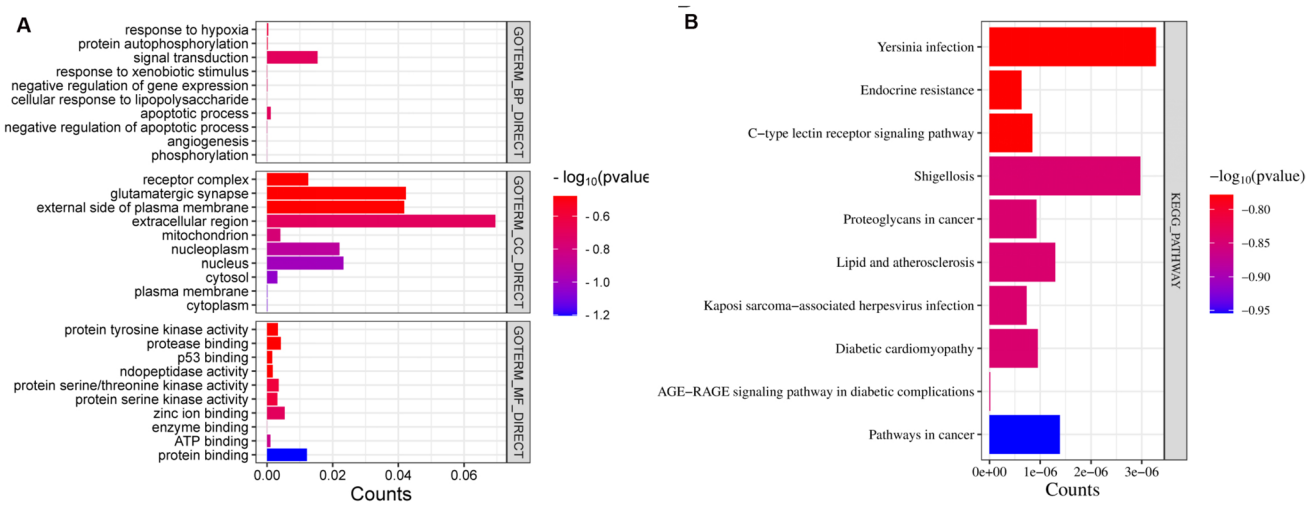

Fig. 7 GO and KEGG pathway enrichment analysis. **A** GO enrichment analysis. **B** KEGG pathway analysis of core target genes

(− 8.5 kcal/mol), MMP2(− 7.5 kcal/mol), SERPINE1 (− 7.6 kcal/mol), EP300 (− 7.3 kcal/mol), and GSK3B (− 6.7 kcal/mol) (Fig. 8). To sum up, senegenin possessed a favorable binding affinity with these key targets during the therapeutic action on DE.

Discussion

DE, characterized by cognitive impairment, has been recognized as a main contributor to death of diabetic patients (Li et al. 2023). Currently, there is limited effective drug for DE due to its unclear pathogenesis. Senegenin has been reported to relieve cognitive impairment caused by hepatic ischemia–reperfusion injury (Xie et al. 2012) and ovariectomy (Cai et al. 2013). However, the biological function and related mechanism of senegenin on DE remain uncovered. In our study, in vivo and in vitro explorations were conducted to evaluate the specific mechanisms of senegenin on attenuating the cognitive dysfunction in DE. We found that senegenin ameliorated cognitive impairment via activating mitophagy and maintaining homeostasis of mitochondrial dynamics. Moreover, NLRP3 inflammasome was inactivated by senegenin treatment. Our study provided first evidence that senegenin might serve as a potential drug for alleviating DE.

Homeostasis of mitochondrial dynamics depends on the balance of fusion and fission, which exerts a key role in the maintenance of mitochondrial function (Vasileiou et al. 2019). The fusion and fission processes can be modulated by a series proteins, including OPA1, MFN1/2 for fusion, and p-DRP1 and FIS1 for fission (Mishra and Chan 2014). Previous studies have reported the changes in mitochondrial dynamics during DE. For instance, DRP1 expression was documented to be highly expressed in the hippocampus of DE rats (Zhou et al. 2018). Another study found that mitochondria were fragmented in the hippocampal tissues of DE rats as confirmed by decreasing OPA1 and MFN1/2 expression, and increasing p-DRP1 and FIS1 expression (Han et al. 2024). In this study, we demonstrated that senegenin treatment effectively reversed down-regulation of OPA1 and MFN1/2, and up-regulation of p-DRP1 and FIS1, indicating the maintenance of mitochondrial dynamics homeostasis. As a specific type of autophagy, mitophagy is a crucial biological process for mitochondrial quality control (Li et al. 2024). Mitophagy is responsible for removal of damaged mitochondria and maintenance of homeostasis of mitochondrial dynamics (Xu et al. 2020). The PINK1/Parkin pathway is a classical regulatory mechanism of mitophagy, which plays a critical role in removing dysfunctional mitochondria after injury (Ajoolabady et al. 2022). Declined membrane potential inhibits the entrance of PINK1 into inner membrane, resulting in accumulation of PINK1 in the outer membrane. Subsequently, PINK1 can be activated via autophosphorylation, which consequently recruits Parkin to induce mitophagy (Okatsu et al. 2013). In this work, mitophagy was repressed in DE model as evidenced by decreasing PINK1 and Parkin expression; however, senegenin intervention remarkably activated mitophagy. Thus, our findings indicated that senegenin improved DE-induced cognitive dysfunction via inducing mitophagy to maintain mitochondrial dynamics homeostasis.

NLRP3 inflammasome overactivation has been demonstrated to participate in the pathogenesis of DE (Zhang et al. 2023). NLRP3, ASC and caspase- 1 are the major components of NLRP3 inflammasome. NLRP3 can interact with ASC to catalyze the transition of inactive procaspase- 1 to active caspase- 1, which further facilitates

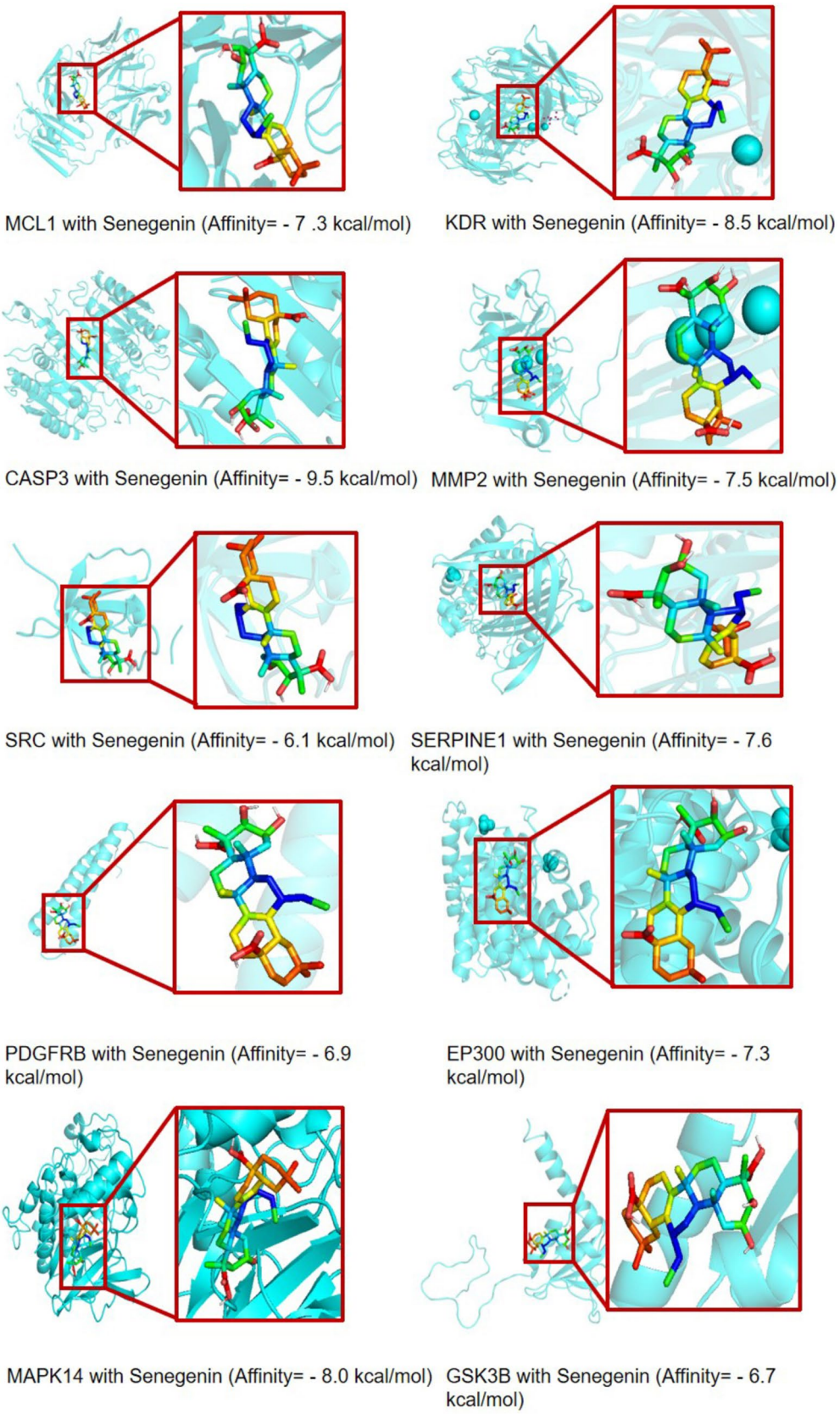

Fig. 8 Molecular docking of senegenin complexed with CASP3, MCL1, SRC, MAPK14, KDR, MMP2, SERPINE1, EP300, GSK3B, and PDGFRB

the maturation and secretion of IL- 1β and IL- 18 (Lu et al. 2024). As reported, HG led to activation of NLRP3 inflammasome in the hippocampal cells (Ward and Ergul 2016). Recent evidence proved that inhibition of NLRP3 inflammasome activation exhibited the protection against cognitive dysfunction in DE mice (Zhang et al. 2023). Li et al. documented that senegenin could mitigate depression in mice through inactivation of NLRP3 inflammasome (Li et al. 2017). In this study, elevated expression of NLRP3, ASC, active caspase- 1, IL- 1β and IL- 18 were found in DE models, while senegenin treatment obviously reversed the above changes, indicating that NLRP3 inflammasome inactivation took part in the beneficial effect of senegenin against DE. The correlation between the inactivation of NLRP3 inflammasome and mitophagy has been identified. For example, Ginkgolide B could relieve neuropathic pain in rats via repressing NLRP3 inflammasome activation by mitophagy induction (Liang et al. 2024). Consistently, our results indicated that inhibiting mitophagy by PINK1 knockdown counteracted senegenin-mediated NLRP3 inflammasome inactivation and neuroprotection. Therefore, senegenin relieved DE via triggering mitophagy to restrain NLRP3 inflammasome overactivation.

To further explore the detailed mechanism underlying the protective effect of senegenin on DE, network pharmacology identified several key targets of senegenin, including CASP3, MCL1, SRC, MAPK14, KDR, MMP2, SERPINE1, EP300, GSK3B, and PDGFRB. The binding affinity of senegenin with these key targets was further validated by molecular docking analysis. Among these key targets, MCL1, SRC, and MAPK14 have been identified as modulators of mitophagy (Gui et al. 2024; Hirota et al. 2015; Zheng et al. 2023). However, whether senegenin can induce mitophagy through modulation of MCL1, SRC, and MAPK14 in DE needs to be elucidated in the future.

In conclusion, our findings demonstrated that senegenin effectively ameliorated cognitive impairment in DE. Senegenin facilitated mitophagy by the activation of PINK1/Parkin pathway, which consequently suppressed NLRP3 inflammasome. This study suggests senegenin as a potential neuroprotective drug for attenuating DE in clinical practice.

Authors contributions **Xiao-dan** Yan: Data curation, Investigation, Methodology, Writing – original draft. Yu Yang: Formal analysis, Data curation, Writing – review & editing. Wan-ting Zhang and Qing-quan Kong: Visualization, Investigation. Xi-tong Zheng: Methodology, Investigation, Software, Validation. Qing Yu and Lin-sen Li: Conceptualization, Supervision, Validation, Writing – review & editing.

Funding This work was supported by basic scientific research project of Liaoning Provincial Department of Education (Grant number: LJKMZ20221777) and science and technology joint plan of Liaoning Province (Grant number: 2024-MSLH- 450).

Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethical approval This research received approval from the Ethics Committee of Shenyang Medical College.

Competing interests The authors declare no conflict of interests.

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