doi: 10.4149/gpb_2025032

Saponin of *Rhizoma Polygonati* inhibits neuronal apoptosis and ameliorates cognitive deficits in Alzheimer's disease mice by modulating c-Abl/MST1 pathway

Changying Zhang[1,*], Xudong Tang[2,*] and Shixing Wu[1]

[1] *Department of Neurology, The Second People's Hospital of Hunan Province (Brain Hospital of Hunan Province), Changsha, Hunan Province, China*
[2] *Department of clinical Laboratory, The Second People's Hospital of Hunan Province (Brain Hospital of Hunan Province), Changsha, Hunan Province, China*

Abstract. Saponin of *Rhizoma Polygonati* (SRP) is a key bioactive component obtained from *Rhizoma Polygonati*, has neuroprotection, antioxidant and anticancer effects. This research aimed to explore whether SRP improves cognitive deficits in Alzheimer's disease (AD) mice. Behavioral testing was performed by the Morris water maze test. Histopathological changes and neuronal apoptosis in mouse hippocampus were observed by Hematoxylin-eosin, Nissl staining, and TUNEL staining. The impact of SRP on amyloid β-protein (Aβ) and neurofibrillary tangles (NFTs) production was determined by immunofluorescence and Glycine silver staining. Western blot detected c-Abl/Mammalian sterile 20 (STE20)-like kinase 1 (MST1) pathway, apoptosis-related protein, and Tau phosphorylation level. AD mice exhibit cognitive deficits compared to normal mice, and SRP ameliorates cognitive deficits. The hippocampal tissues of AD mice showed neuronal damage and apoptosis, with Aβ deposition, loss of Nissl bodies and formation of NFTs. SRP attenuated neuronal damage in AD mice, inhibited Aβ deposition and NFTs formation. Additionally, SRP blocked the c-Abl/MST1 pathway in AD mice. c-Abl inhibitor enhanced the neuroprotective effect of SRP in AD mice, while c-Abl agonist weakened this effect. SRP inhibits hippocampal histopathological damage and enhances cognitive ability in AD mice through blocking the c-Abl/MST1 pathway.

Key words: Saponin of *Rhizoma Polygonati* — Alzheimer's disease — Cognitive defect — Neuron damage — c-Abl/MST1 pathway

Highlights:

- Saponin of *Rhizoma Polygonati Rhizoma Polygonati* (SRP) attenuates cognitive deficits and reduces neurofibrillary tangle formation in brain tissue in AD mice.
- SRP inhibits Tau protein hyperphosphorylation and suppresses amyloid β-protein deposition in hippocampal tissue of AD mice.
- SRP ameliorates hippocampal histopathological damage and inhibits hippocampal neuronal apoptosis in AD mice.
- SRP inhibits the c-Abl/MST1 pathway, thereby attenuating cognitive deficits and ameliorating hippocampal histopathological damage and neuronal apoptosis in AD mice.

* These authors contributed equally to this work.
Correspondence to: Shixing Wu, Department of Neurology, The Second People's Hospital of Hunan Province (Brain Hospital of Hunan Province), Changsha, Hunan Province, China
E-mail: ZCY851675tzd@hotmail.com

Introduction

As the population continues to age, the morbidity and mortality rates of age-related incapacitating diseases have risen significantly (Dominguez et al. 2021; Gonzales et al. 2022). Alzheimer's disease (AD) stands as the most prevalent type of dementia in old age, according to statistics, there are approximately 50 million people with AD globally in 2018, and it is expected that this number could rise to 150 million in 2050 (Scheltens et al. 2021; Rostagno 2022). As a common clinical neurodegenerative disease, the primary symptoms of AD include progressive loss of cognitive function and behavioral abnormalities (Knopman et al. 2021; Monteiro et al. 2023). In recent years, scientists have conducted numerous studies and found that the typical pathological changes in AD are hippocampal atrophy, amyloid β-protein (Aβ) deposition, Tau protein hyperphosphorylation, and neurofibrillary tangles (NFTs) formation (Huang et al. 2024; Zheng and Wang 2025). Despite intensive research by scientists, AD remains an incurable disease in clinical practice, posing significant challenges to patients and their families, as well as to society as a whole (Breijyeh and Karaman 2020; Passeri et al. 2022). As a result, it is crucial to create medications that are both safe and effective for AD treatment.

In recent years, natural plants have received a surge of interest from researchers because of their high safety profile and rich bioactivity with multiple targets of action (Varshney and Siddique 2021; Wang et al. 2022; Shen et al. 2024; Liu et al. 2025; Sharma et al. 2025). Plant macromolecules such as flavonoids, saponin, polysaccharide, and phenols have been reported to have therapeutic potential for AD, and screening for effective drugs against AD from such natural plant extracts may be a promising new option (Chen et al. 2022; Varshney and Siddique 2024). *Rhizoma Polygonati* is a traditional Chinese medicinal herb used in anti-aging, immunomodulation, neuroprotection and anti-cancer applications (Nurtay et al. 2021; Wang et al. 2023). Saponin of *Rhizoma Polygonati* (SRP), as the main active ingredient of Rhizoma Polygonati, also has a wide range of pharmacological effects (Huang et al. 2024; Wu et al. 2024). SRP has been reported to regulate the intestinal flora of type 2 diabetic mice and improve glucose tolerance, activate glucose transporter protein 4, and effectively reduce blood glucose (Chen et al. 2022). Zhao et al. (2024) found that SRP improved immune function, declined inflammatory factors levels, and improved the intestinal mucosal barrier function in immunosuppressed mice. Additionally, SRP down-regulated the expression of miR-142-3p, which could protect bone marrow hematopoietic stem cells and thus improve hematopoiesis in myelosuppressed mice (Gu et al. 2021). Notably, research indicates that polysaccharides from *Rhizoma Polygonati* could attenuate cognitive deficits in AD model mice by remodeling the gut microbiota (Luo et al. 2022). However, at present, the influence of SRP on cognitive impairments in AD mice and the underlying mechanisms remain unclear.

c-Abl is part of the Abl family of non-receptor tyrosine kinases in mammals and is extensively present in cells of various human tissues (Motaln and Rogelj 2023). c-Abl is able to interact with other proteins through its own tyrosine kinase structural domain to mediate cell proliferation, cell cycle, skeleton remodeling, inflammatory response, apoptosis, cell signaling, tumorigenesis, and posttranscriptional processing (Bohio et al. 2020; González-Martín et al. 2021). It has been shown that c-Abl is important in neurodegenerative diseases, and down-regulation of c-Abl improves memory capacity, inhibits astrocyte activation, and attenuates neuronal damage in AD mice (Giesert 2021; Feng et al. 2022; Leon et al. 2023). Mammalian sterile 20 (STE20)-like kinase 1 (MST1), a member of the STE20-like kinase subfamily, is ubiquitously present in various mammalian tissues (Li et al. 2023; Shao et al. 2023). MST1 is a core component of the Hippo pathway, which is crucial for tissue regeneration and tumorigenesis in the liver, intestine, and heart, and is critical for regulating apoptosis, cell proliferation, and differentiation (He et al. 2024; Zhou et al. 2024). It has been demonstrated that MST1 overexpression induces an AD phenotype in mice, leading to cognitive deficits and neuronal apoptosis (Wang et al. 2022). Importantly, c-Abl induces phosphorylation of MST1 (Xiao et al. 2011), so we hypothesized that inhibition of the c-Abl/MST1 pathway might be an important strategy to improve AD. However, whether SRP can inhibit AD progression by inhibiting the c-Abl/MST1 pathway has not yet been explored. Therefore, this research investigated the impacts of SRP on cognitive deficits and neuronal damage in AD mice and whether it acts by inhibiting the c-Abl/MST1 pathway. This research aims to lay a theoretical basis for the clinical use of SRP and AD drug development.

Materials and Methods

Laboratory animals and handling

Amyloid precursor protein (APP)/presenilin 1 (PS1) double transgenic AD mice (ten months old) and wild-type (WT) C57BL/6J mice (control) were acquired from Cyagen Biotechnology (Suzhou, Jiangsu, China). The experimental mice were kept in a standard indoor clean environment with the ambient temperature regulated at 20 ± 2°C, relative humidity levels maintained between 50% and 60%, and a light/dark cycle of 12 h. During the rearing period, mice can freely ingest feed and drinking water. The rearing cages used in the experiments need to be regularly replaced with mats and disinfected to ensure a clean and safe rearing environment.

Following a week of adaptation to their surroundings, APP/PS1 double transgenic AD mice were split into AD group, AD+SRP-L group, AD+SRP-M group, AD+SRP-H group, AD+SRP+DPH group, and AD+SRP+IkT group in a random manner (n = 6). Mice in the AD+SRP-L group received daily intraperitoneal injections of SRP (20 mg/kg, PHL89810, Sigma-Aldrich, St. Louis, MO, USA), mice in the AD+SRP-M group were administered SRP (40 mg/kg), and mice in the AD+SRP-H group were administered SRP (80 mg/kg) for five weeks (Gu et al. 2021). Mice in the AD+SRP+DPH group received daily intraperitoneal injections of SRP (40 mg/kg), along with the c-Abl kinase agonist 30 mg/kg DPH (HY-12070, MedChemExpress, Monmouth Junction, NJ, USA) every 2 days for 5 consecutive weeks (Shapiro et al. 2017). Mice in the AD+SRP+IkT group were *intraperitoneally* injected with SRP (40 mg/kg) daily, along with the c-Abl kinase inhibitor IkT-148009 (50 mg/kg, MedChemExpress) daily for 5 weeks (Karuppagounder et al. 2023). Mice in the AD group and control mice were injected *intraperitoneally* with an equal dose of saline daily for 5 weeks. At the conclusion of the drug treatment, behavioral tests were conducted to assess the cognitive function of the mice. Mice were executed through sodium pentobarbital injection at the end of the behavioral test, and intact brain tissues were isolated by tissue shears for subsequent experiments.

Behavioral tests

The Morris water maze system for mice consists of a cylindrical black pool (diameter of 100 cm) and a circular platform with a diameter of 10 cm that can be moved in position and adjusted in height. A computerized tracking system (Noldus, EthoVision XT 19) was used to automatically record the swimming time and path of the mice."

The pool is split into four equal parts, 1, 2, 3, and 4 with the circular hidden platform located in the middle of part 1. Fresh water was poured into the pool until it was 2 cm above the platform, and water temperature was controlled between 20 and 25°C. The experimental environment was quiet and soundproof, and the light was stabilized. The test was carried out for six consecutive days, with the first 5 days being the training period, and each mouse was subjected to 4 rounds of experiments each day. Each mouse was carefully dropped into the water from four different locations to train its spatial memory ability, and each round of the experiment lasted 90 s. The experiment was stopped if the mouse found the platform and stayed on the platform for more than 3 s within the specified time, and recorded the duration it took to find the platform (escape latency). If the mouse failed to locate the platform in 90 s, it was assisted in finding the platform and remained there for 15 s, with the escape latency noted as 90 s. The swimming distance of the mouse was recorded. The interval between two experiments was not less than 30 min, and each time the mice were removed from the water, and dried. On day 6, the platform was taken away, and the duration the mice spent in the part 1 within 90 s was recorded, as well as the crossovers numbers (She et al. 2024; Zeng et al. 2024).

Hematoxylin-eosin (HE) staining

Intact mouse brain tissues were removed on ice with tissue shears, made into coronal sections and transferred to 4% paraformaldehyde (P885233, Macklin, Shanghai, China) for overnight immersion. The brain tissue was dehydrated using gradient ethanol (100%, 95%, 75%, and 50%), and the dehydrated brain tissue was embedded in paraffin and prepared as 4 μm sections. The samples were placed in a 75°C oven for 30 min, deparaffinized by submersion in xylene (247642, Sigma-Aldrich) for 20 min, hydrated with gradient ethanol, and then washed using distilled water. Referring to the study of Zhai et al. (2023), the histopathological changes in the CA1 area of the brain hippocampus were assessed through HE staining Kit (C0105S, Beyotime, Shanghai, China). The liquid on the surface of the sections was aspirated, hematoxylin stain was added dropwise, and rinsed with tap water after 8 min, then placed in differentiation solution (C0161s, Beyotime) for 30 s to differentiate the nuclei color to blue. Staining was performed by adding appropriate drops of eosin to the sections (about 1 min), and the staining was stopped by washing with water to color the cytoplasm. The samples were rinsed in anhydrous ethanol for 4 min, followed by a 5-min immersion in xylene to enhance transparency, then sealed with neutral balsam (C0173, Beyotime), and finally viewed through an inverted microscope (DM IL LED, Leica, Heidelberg, Germany).

Nissl staining

Paraffin sections of mouse brain tissue were spread flat in warm water to fully expand, and then fished out with slides after the tissue was stretched. The samples were set inside a drying oven and baked at 65°C for 1.5 h. Xylene deparaffinization was performed for 20 min, and rehydration was performed in gradient alcohol for 5 min each time. The liquid on top of the sections was aspirated, after which Nissl staining solution (C0117, Beyotime) was gently dripped onto the surface of the sections, followed by 10-min incubation. After sufficiently rinsed with tap water, it was immersed in 95% ethanol and dehydrated for 4 min, and the dehydration was completed by transparency in xylene, and a neutral balsam was added to seal the film. Finally, the samples were observed by inverted microscope and the area of damaged neurons was assessed with ImageJ software (v 1.46, Wavne Resband, National Institute of Mental Health, USA).

Immunofluorescence

Paraffin sections of mouse brain tissue were placed in PBS (phosphate-buffered saline) and rinsed three times, and treated with 0.3% Triton X-100 (X100, Sigma-Aldrich) for 10 min. After shaking off the solution, 5% bovine serum protein (BSA, A801320, Macklin) was added dropwise on the surface of sections and closed for 1 h. The blocking solution was shaken off and treated with Aβ primary antibody (36-6900, 1:50, Invitrogen, Carlsbad, CA, USA) overnight at 4°C. After that, the sections were rinsed with PBS and exposed to FITC-labeled goat anti-rabbit secondary antibody (F-2765, 1:500, Invitrogen) for 1 h. Sections were sealed with AntiFade Mounting Medium (HY-K1047, MedChemExpress) containing DAPI, avoiding air bubbles during the sealing process, then observed under a microscope.

Glycine silver staining

Referring to Xiao et al. (2022), NFTs were assessed by a glycine silver staining kit (R23708, Saintbio, Shanghai, China). Paraffin sections of mouse brain tissue were deparaffinized, hydrated, and washed 3 times with distilled water. It was put into Glycine Silver Stain Solution C and treated for 5 min, then washed with distilled water for 3 times. Then put it into the preheated silver glycine staining solution B at 37°C for 3 min. The sections were removed, the residual staining solution on the surface was shaken off, and put into the preheated silver glycine stain A at 45°C to observe the reduction effect, and after a few seconds, the sections were removed and the staining solution was quickly shaken off and the sections were rinsed with distilled water. The samples underwent dehydration using anhydrous ethanol, followed by a 5-min treatment with xylene for transparency, and were then sealed with neutral balsam prior to microscopic examination.

TdT-mediated dUTP Nick-End Labeling (TUNEL) staining

Paraffin sections of mouse brain tissue were deparaffinized, and subsequently dehydrated with gradient ethanol. Sections were exposed to 0.1% Triton X-100 for 2 min at room temperature to increase permeability. Added dropwisly DNase-free proteinase K (20 μg/ml, HY-108717A, MedChemExpress), and left to incubate for half an hour at room temperature. After that, the sections were incubated with TUNEL assay solution (HY-K1078, MedChemExpress) for 1.5 h in a light-protected environment."

The slices were sealed with AntiFade Mounting Medium containing DAPI, observed and photographed using fluorescence microscopy.

Western blot

RIPA lysis buffer (P0013B, Beyotime) was mixed well with the mouse brain tissue samples and the tissues were lysed sufficiently to extract proteins. After lysis was completed, the protein levels of the obtained samples were assessed through the BCA Protein Assay Kit (B917925, Macklin). Next, the extracted protein supernatant was mixed with SDS-PAGE protein upsampling buffer, separated by SDS-PAGE electrophoresis, then transferred to polyvinylidene difluoride membranes (88518, Invitrogen) and closed with 5% BSA for 2 h. Subsequently, the membranes were exposed to primary antibodies Tau (PA5-27287, 1:3000, Invitrogen), pTau (S396) (44-752G, 1:1000, Invitrogen), pTau (S404) (44-758G, 1:1000, Invitrogen), p-Tau (T181) (701530, 1:500, Invitrogen), p-Tau (T205) (44-738G, 1:1000, Invitrogen), Cytochrome C (Cyto C) (710627, 1:2500, Invitrogen), Bax (MA5-35342, 1:2000, Invitrogen), Bcl2 (PA5-27094, 1:500, Invitrogen), caspase3 (700182, 1:1000, Invitrogen), Cleaved-caspase3 (ab32042, 1:500, Abcam, Cambridge, MA, USA), c-Abl (PA5-86077, 1:500, Invitrogen), p-c-Abl (44-250, 1:1000, Invitrogen), p-Y433-MST1 (PA5-104616, 1:2000, Invitrogen) or MST1 (PA5-119762, 1:1000, Invitrogen) at 4°C overnight. The following day, after rinsing 3 times, the membrane were exposed to goat anti-rabbit antibody (65-6120, 1:2000, Invitrogen) for 2 h. Developing solution (HY-K2005, MedChemExpress) was prepared and dropped evenly on the membrane, which was scanned with a gel imaging system (iBright CL1500, Invitrogen). GAPDH (MA5-35235, 1:50,000, Invitrogen) was used as an internal reference. The relative level was calculated by obtaining the gray value of each protein band after processing the image with ImageJ software.

Statistical analysis

Every experiment was conducted a minimum of 3 times, and experimental data were presented as mean ± standard deviation. The statistical analysis of all data was utilized using SPSS version 26.0 (IBM SPSS Statistics 26), while Graphpad Prism 9 software was utilized to drawn the statistical graphs. The Shapiro-Wilk test was utilized to evaluate the normal distribution of the data. When the data followed a normal distribution, an independent samples *t*-test was employed to assess the statistical variations between the two groups. $p < 0.05$ was considered statistically significant.

Results

SRP treatment improves cognitive deficits in AD mice

Cognitive deficits and memory loss are typical characteristics of AD. Therefore, we conducted the Morris water maze

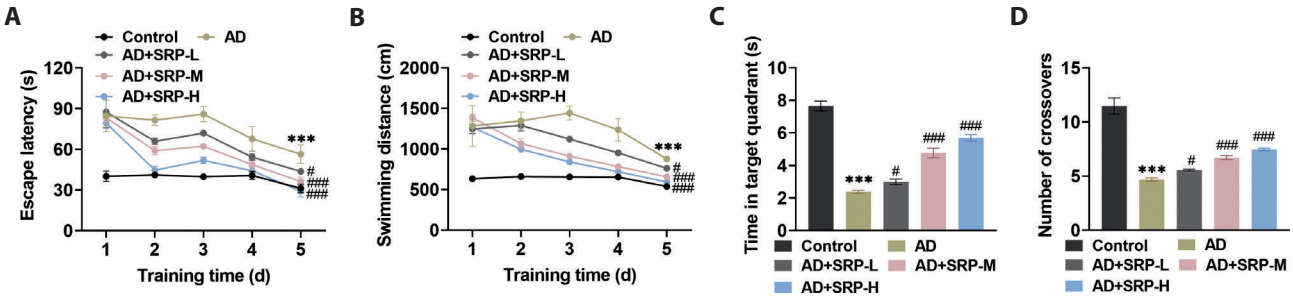


Figure 1. SRP attenuates cognitive deficits in AD mice. Morris water maze experiment was conducted to assess cognitive ability of mice, in which the escape latency (**A**), swimming speed (**B**), the duration the mice remained in the target quadrant (**C**), and the crossovers numbers the mice traversed the hidden platform in 90 s (**D**) were recorded ($n = 6$). *** $p < 0.001$ *vs.* Control; # $p < 0,05$, ## $p < 0,01$, ### $p < 0,001$ *vs.* AD

experiment to evaluate how SRP influences cognitive impairments in mice with AD. AD mice found platforms with significantly longer escape latencies and longer swimming distances than other mice. SRP treatment dose-dependently significantly shortened the escape latency and swimming distance in AD mice (Fig. 1A,B). Meanwhile, AD mice showed a marked decrease in the duration spent in the target quadrant and number of passes through the platform's original location within 90 s, whereas SRP treatment increased the residence time and number of passes, suggesting significant recovery of their cognitive deficits and memory capacity (Fig. 1C,D). These results suggested that AD mice showed significant cognitive deficits, and that SRP attenuated cognitive deficits in AD mice.

SRP ameliorates pathological damage to neurons in AD mice

The hippocampus plays an essential role in memory and learning, prompting us to explore how SRP treatment impacts the pathological damage observed in the hippocampus of mice with AD. HE staining revealed that the hippocampal neurons of control mice were tightly arranged, with clear boundaries between nucleus and cytoplasm and obvious nucleoli. In contrast, hippocampal neuronal cells of AD mice showed deepened staining, neurons showed crumpled state, and nuclei and cytoplasm were blurred, whereas SRP treatment caused a marked rise in the number and density of neurons, and the morphology of neuronal cells tended to be normalized (Fig. 2A). Nissl staining labels neurons with a basic dye for Nissl bodies, a characteristic neuronal structure that reflects cellular protein synthesis; normal Nissl bodies are abundant and clearly stained (Piavchenko et al. 2022). Nissl staining showed that hippocampal neurons in AD mice were loose and disorganized, with severe loss of Nissl bodies and severe neuronal vacuolization. The morphology and arrangement of hippocampal neurons in mice were improved after administration of SRP, suggesting that SRP is beneficial in increasing neuronal survival and reducing neuronal damage (Fig. 2B,C). The accumulation of Aβ plaques and the excessive phosphorylation of Tau protein are the most typical pathological features of AD (Scheltens et al. 2021). Immunofluorescence findings indicated that Aβ fluorescence intensity in the hippocampal tissue of AD mice was markedly increased, whereas SRP treatment notably reduced Aβ level (Fig. 2D,E). The excessive phosphorylation of Tau proteins can also lead to NFTs formation, and Glycine silver staining is a common way to detect the formation of NFTs (Xiao et al. 2022). NFTs were notably increased in the hippocampal tissue of AD mice, but SRP treatment inhibited the formation of NFTs (Fig. 2F). In addition, Western blot results indicated a notable rise in Tau phosphorylation levels in AD mice, and SRP treatment effectively ameliorated the aberrant phosphorylation of Tau (Fig. 2G–K). These results confirmed that SRP was effective in improving the pathological manifestations of hippocampal tissue in AD mice and attenuating Aβ plaque deposition and Tau hyperphosphorylation.

SRP inhibits hippocampal neuronal apoptosis in AD mice

It has been shown that the degree of neuronal apoptosis is directly related to the level of cognitive impairment and is one of the diagnostic criteria for AD (Zang et al. 2018; Guo et al. 2023). As a result, we explored the impacts of SRP on neuronal apoptosis in AD mice. TUNEL staining specifically labels cells undergoing apoptosis by detecting breaks in nuclear DNA during apoptosis (Giridharan et al. 2020). We found that a large quantity of apoptotic neurons appeared in AD mice, and SRP caused a decline in TUNEL-positive cells, suggesting that SRP ameliorated neuronal apoptosis in AD mice (Fig. 3A,B). Additionally, the apoptotic proteins Cyto C, Bax and Cleaved-caspase3/caspase3 levels were elevated, and the anti-apoptotic protein Bcl-2 level was decreased in brain tissues of AD mice, while SRP treatment ameliorated the abnormal expression of these proteins (Fig. 3C–G). This further confirmed that SRP attenuates neuronal apoptosis in AD mice.

SRP modulates the c-Abl/MST1 pathway and inhibits hippocampal neuronal apoptosis in AD mice

c-Abl is crucial in the process of neuronal death, and it facilitates the phosphorylation of MST1, blocking the c-Abl/MST1 pathway attenuates neuronal cell death (Xiao et al. 2011). We therefore evaluated the impact of SRP on the c-Abl/MST1 pathway. In the brain tissues of AD mice, the phosphorylation levels for both c-Abl and MST1 were notably elevated, and SRP treatment dose-dependently

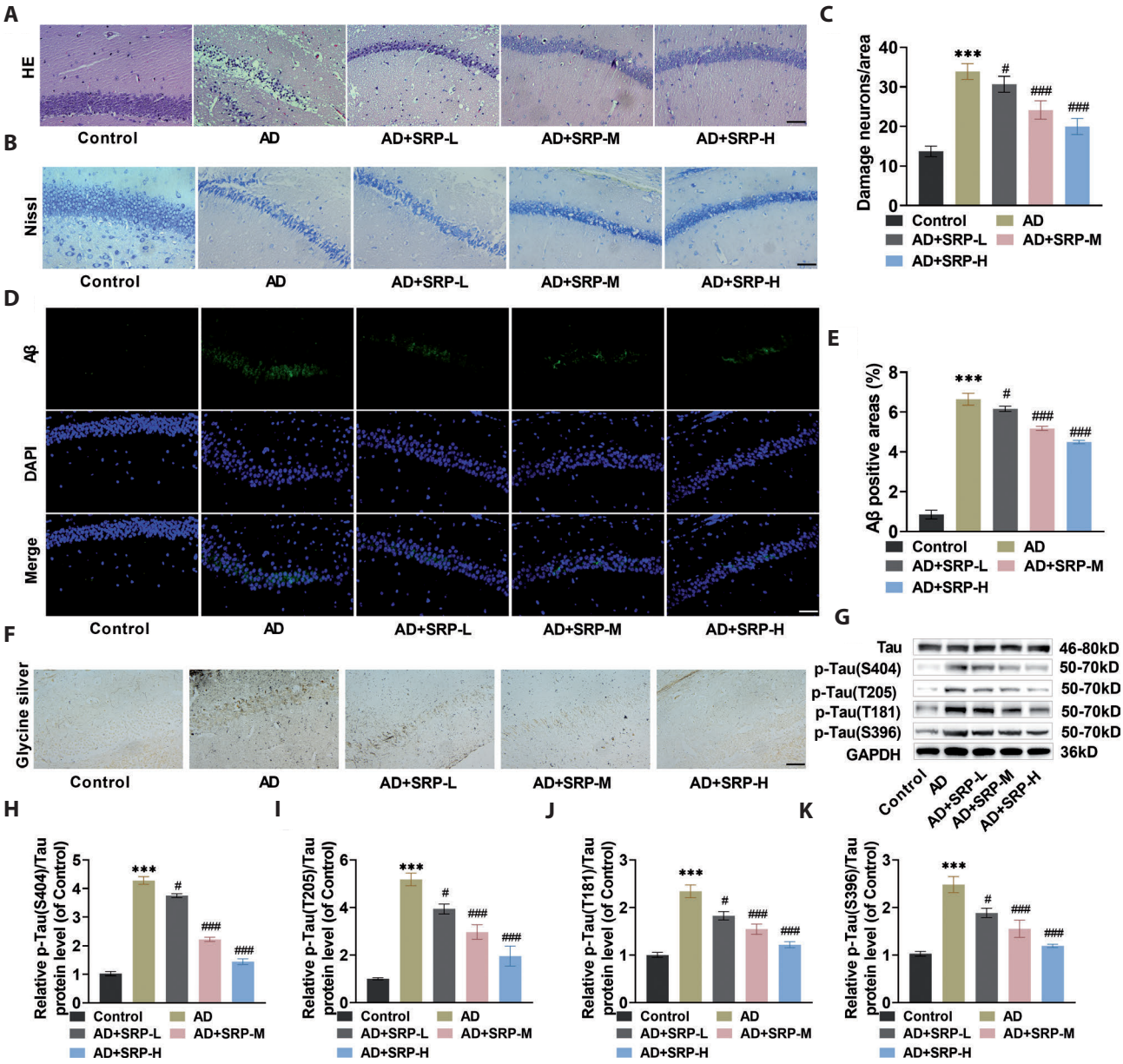


Figure 2. SRP ameliorates pathological damage to neurons in AD mice. **A.** HE staining showed that hippocampal neurons in AD mice were wrinkled and the nuclei and cytoplasm were blurred, and SRP treatment led to normalization of neuronal morphology (40×, 50 μm). **B,C.** Nissl staining showed loose and disorganized hippocampal neurons with severe loss of Nissl bodies in AD mice, and the morphology and arrangement of neurons improved after SRP treatment (40×, 50 μm). **D,E.** The fluorescence intensity of Aβ in the hippocampal tissue of AD mice was elevated as measured through immunofluorescence, and SRP treatment decreased the fluorescence intensity of Aβ (40×, 50 μm). **F.** Glycine silver staining results showed an increase in NFTs in AD mice, and SRP treatment inhibited the formation of NFTs (40×, 50 μm). **G–K.** Tau phosphorylation level in AD mice was elevated as measured by Western blot, and SRP treatment declined Tau phosphorylation level ($n = 6$). *** $p < 0.001$ *vs.* Control; # $p < 0.05$, ### $p < 0.001$ *vs.* AD.

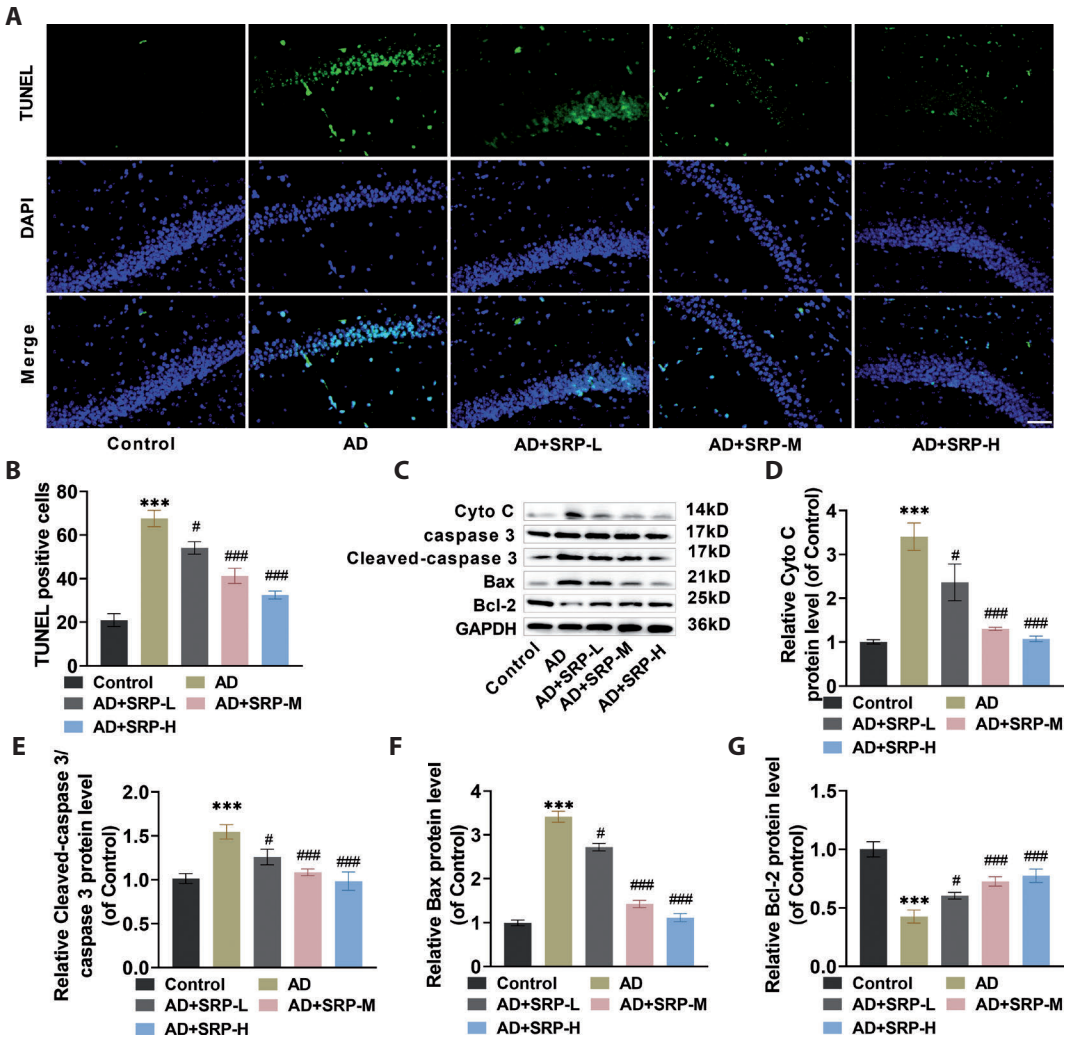
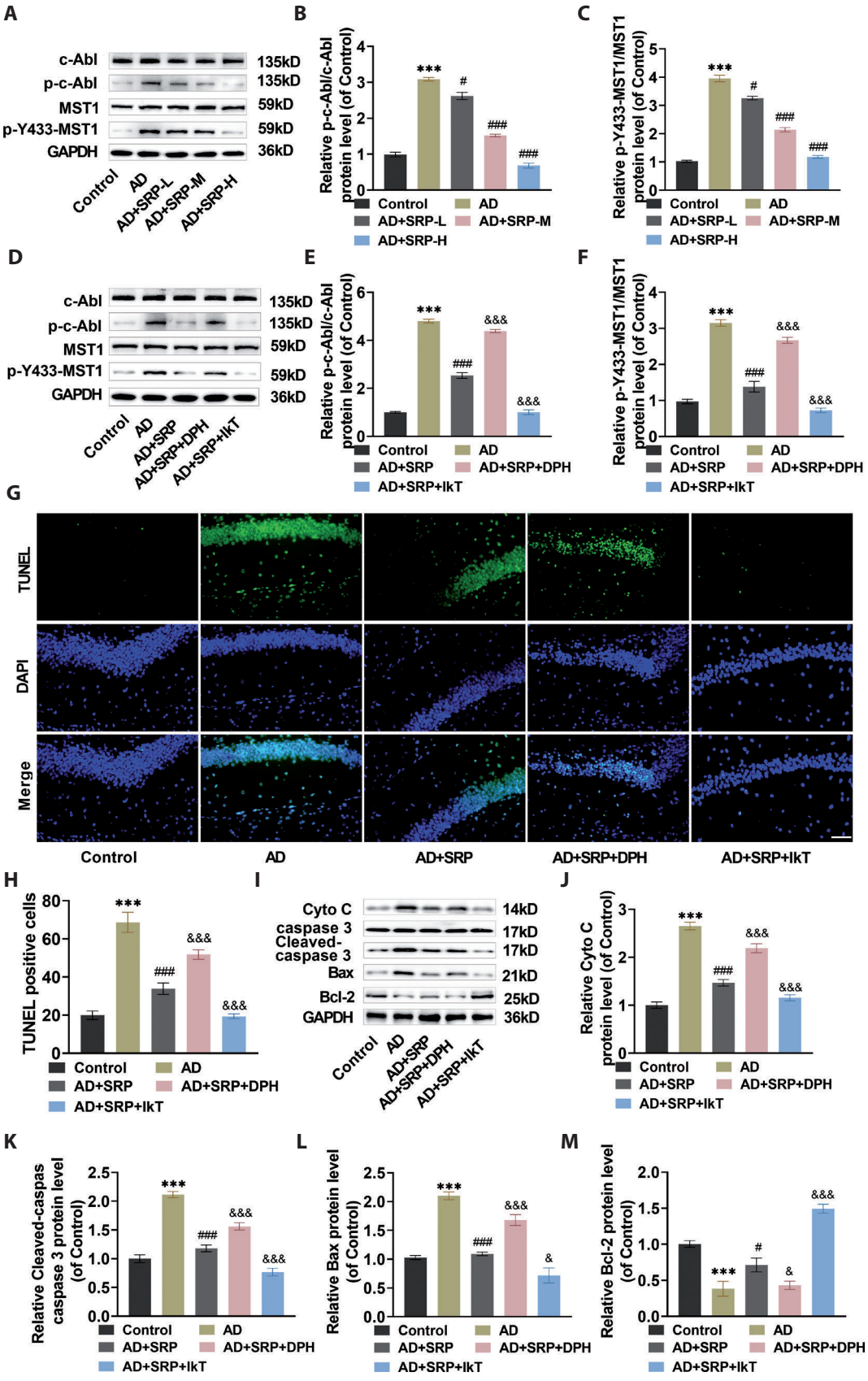


Figure 3. SRP inhibits hippocampal neuronal apoptosis in AD mice. **A,B.** TUNEL staining revealed elevated TUNEL-positive cells in the brain tissue of AD mice, and SRP caused a decrease in TUNEL-positive cells (40×, 50 μm). **C–G.** Western blot measured elevated Cyto C, Bax and Cleaved-caspase3/caspase3 levels, and decreased Bcl-2 protein levels in brain tissue of AD mice, which was reversed by SRP treatment ($n = 6$). *** $p < 0.001$ *vs.* Control; # $p < 0.05$, ### $p < 0.001$ *vs.* AD.

ameliorated the aberrant expression of these proteins (Fig. 4A–C). SRP at 40 mg/kg already had a significant inhibitory impact on the c-Abl/MST1 pathway, so we chose this dose for further experiments. Next, we explored the impacts of the c-Abl kinase agonist DPH and the inhibitor IkT on the effects of SRP action. We found that intraperitoneal injection of DPH weakened the inhibitory effect of SRP on the c-Abl/MST1 pathway, causing a notable rise in c-Abl and MST1 phosphorylation levels in mouse brain tissues, but intraperitoneal injection of IkT increased the inhibitory effect of SRP on the c-Abl/MST1 pathway (Fig. 4D–F). In addition, DPH attenuated the suppressive impact of SRP on neuronal apoptosis, resulting in a notably higher number of TUNEL-positive cells, whereas IkT markedly declined TUNEL-positive cells in AD mice (Fig. 4G,H). Not only that, DPH also increased apoptotic proteins Cyto C, Bax, and Cleaved-caspase3/caspase3 levels and declined Bcl-2 level in the AD+SRP group, and IkT had the opposite effect (Fig. 4I–M). The above results confirmed that SRP treatment suppressed the c-Abl/MST1 pathway activation in AD mice, which in turn inhibited hippocampal neuronal apoptosis.

SRP ameliorates pathological neuronal damage in hippocampus of AD mice through c-Abl/MST1 signaling pathway

Next, we explored whether SRP ameliorates pathological damage in hippocampal tissue of AD mice by modulating


A
c-Abl
p-c-Abl
MST1
p-Y433-MST1
GAPDH
135kD
135kD
59kD
59kD
36kD
Control
AD
AD+SRP-L
AD+SRP-M
AD+SRP-H
B
Relative p-c-Abl/c-Abl protein level (of Control)
C
Relative p-Y433-MST1/MST1 protein level (of Control)
D
AD+SRP
AD+SRP+DPH
AD+SRP+IkT
E
F
G
TUNEL
DAPI
Merge
H
TUNEL positive cells
I
Cyto C
caspase 3
Cleaved-caspase 3
Bax
Bcl-2
14kD
17kD
17kD
21kD
25kD
J
Relative Cyto C protein level (of Control)
K
Relative Cleaved-caspas caspase 3 protein level (of Control)
L
Relative Bax protein level (of Control)
M
Relative Bcl-2 protein level (of Control)

the c-Abl/MST1 pathway. The c-Abl kinase agonist DPH exacerbated the pathological damage to hippocampal tissues with severe loss of Nissl bodies in the AD+SRP group of mice, whereas the c-Abl kinase inhibitor IkT increased the protective effect of SRP on hippocampal tissues, resulting in a normalization of neuronal morphology and a significant reduction in the area of neuronal damage (Fig. 5A–C). SRP treatment significantly reduced the fluorescence intensity of Aβ in the hippocampal tissues of AD mice and inhibited the formation of NFTs, whereas DPH intervention attenuated the effect of SRP, resulting in a notable rise in Aβ level and leading to a marked increase in NFTs, and IkT further enhanced the effect of SRP (Fig. 5D–F). In addition, SRP significantly reduced the aberrant phosphorylation of Tau in hippocampal tissues of AD mice, whereas DPH intervention attenuated the effect of SRP, resulting in a marked elevation in Tau phosphorylation, and IkT ameliorated the aberrant phosphorylation of Tau (Fig. 5G–K). This confirmed that SRP ameliorated pathological damage in hippocampal tissue of AD mice by inhibiting c-Abl/MST1 pathway.

SRP ameliorates cognitive deficits in AD mice through c-Abl/MST1 signaling pathway

Finally, we explored whether SRP mitigates cognitive deficits in AD mice through regulating the c-Abl/MST1 pathway. SRP treatment significantly shortened the escape latency and swimming distance in AD mice, but DPH reduced the effects of SRP and prolonged the escape latency and swimming distance, whereas the effects of IkT were opposite to those of DPH (Fig. 6A,B). Meanwhile, SRP treatment increased the residence time in the target quadrant and the number of crossings by the AD mice in the quadrant where the platform was originally located within 90 s. However, DPH reduced the effect of SRP, whereas IkT treatment further increased the residence time and the crossovers number (Fig. 6C,D). The above results indicated that the c-Abl kinase agonist DPH impaired the ameliorative impact of SRP on cognitive deficits in AD mice, whereas the c-Abl kinase inhibitor IkT did the opposite, suggesting that SRP ameliorates cognitive deficits in AD mice through inhibiting the c-Abl/MST1 pathway.

Discussion

AD is one of the global public health priorities for prevention and control. The number of existing dementia in China is 13,140,400 accounting for about 25.5% of the world. Age-standardized prevalence rate of 788.3 *per* 100,000 and age-standardized case-fatality rate of 23.3 *per* 100,000, which is slightly elevated compared to the global level (Ren et al. 2022). The pathogenesis of AD have not yet been fully clarified, and many factors may be involved, including but not limited to genetic factors, neurotransmitter imbalance, traumatic brain injury, and immune factors, etc. There is no specific drug treatment, and the clinical practice is mostly based on symptomatic treatment and improvement of symptoms, making the development of new treatment strategies especially crucial (Breijyeh and Karaman 2020; Passeri et al. 2022). APP/PS1 double transgenic mice are mice overexpressing APP and PS1 with typical AD symptoms, including neurological impairments and learning memory deficits, and are commonly used as AD models (Park et al. 2021; Cheng et al. 2024). In the present study, APP/PS1 double transgenic mice showed significant cognitive deficits with pathological damage and neuronal apoptosis in the hippocampal tissue, which is consistent with AD symptoms and allows for subsequent experiments. In recent years, natural herbs have become a research hotspot due to their unique biological activities. *Rhizoma Polygonati*, a traditional Chinese herb, is also gaining attention for its possible benefits in enhancing cognitive impairment. It has been shown that polysaccharides extracted from *Rhizoma Polygonati* can protect the integrity of the intestinal barrier, effectively alleviate neurological disorders caused by intestinal inflammation and improve cognitive impairment (Jiang et al. 2024). Existing studies have provided reference evidence for the safety of SRP: treatment with 40 μM SRP for 48 h shows no adverse effects on the viability of bone marrow hematopoietic stem cells, after daily intraperitoneal injection of SRP (40 mg/kg) for two consecutive weeks, the thymus index of mice increases and their hematopoietic function improves, at this dose, SRP exhibits therapeutic potential for cyclophosphamide-induced myelosuppression, and no adverse reactions in mice during SRP injection were reported in that study (Gu et al. 2021). Based on the above

◀ **Figure 4.** SRP modulates the c-Abl/MST1 pathway and inhibits hippocampal neuronal apoptosis in AD mice. **A–C.** Western blot measured elevated phosphorylation levels of c-Abl and MST1 in AD mice, while SRP treatment decreased the phosphorylation levels of c-Abl and MST1. **D–F.** Western blot measured that the c-Abl agonist DPH attenuated the effect of SRP, while the c-Abl inhibitor IkT increased the impact of SRP. **G,H.** TUNEL staining showed that SRP caused a decline in TUNEL-positive cells in AD mice, whereas DPH attenuated the effect of SRP, and IkT further decreased TUNEL-positive cells (40×, 50 μm). **I–M**. Western blot measured that SRP decreased Cyto C, Bax and Cleaved-caspase3/caspase3 levels, and raised Bcl-2 levels in AD mice, which was reversed by DPH treatment, and IkT increased the effect of SRP ($n = 6$). *** $p < 0.001$ *vs.* Control; $^{\#}$ $p < 0.05$, $^{\#\#\#}$ $p < 0.001$ *vs.* AD; $^{\&}$ $p < 0.05$, $^{\&\&\&}$ $p < 0.001$ *vs.* AD+SRP.

research, in this experiment, we referred to a similar dose range, and found that SRP was effective in ameliorating cognitive deficits and alleviating hippocampal histopathological damage, as well as neuronal apoptosis in AD mice, which confirms that SRP could serve as a promising therapeutic option for AD.

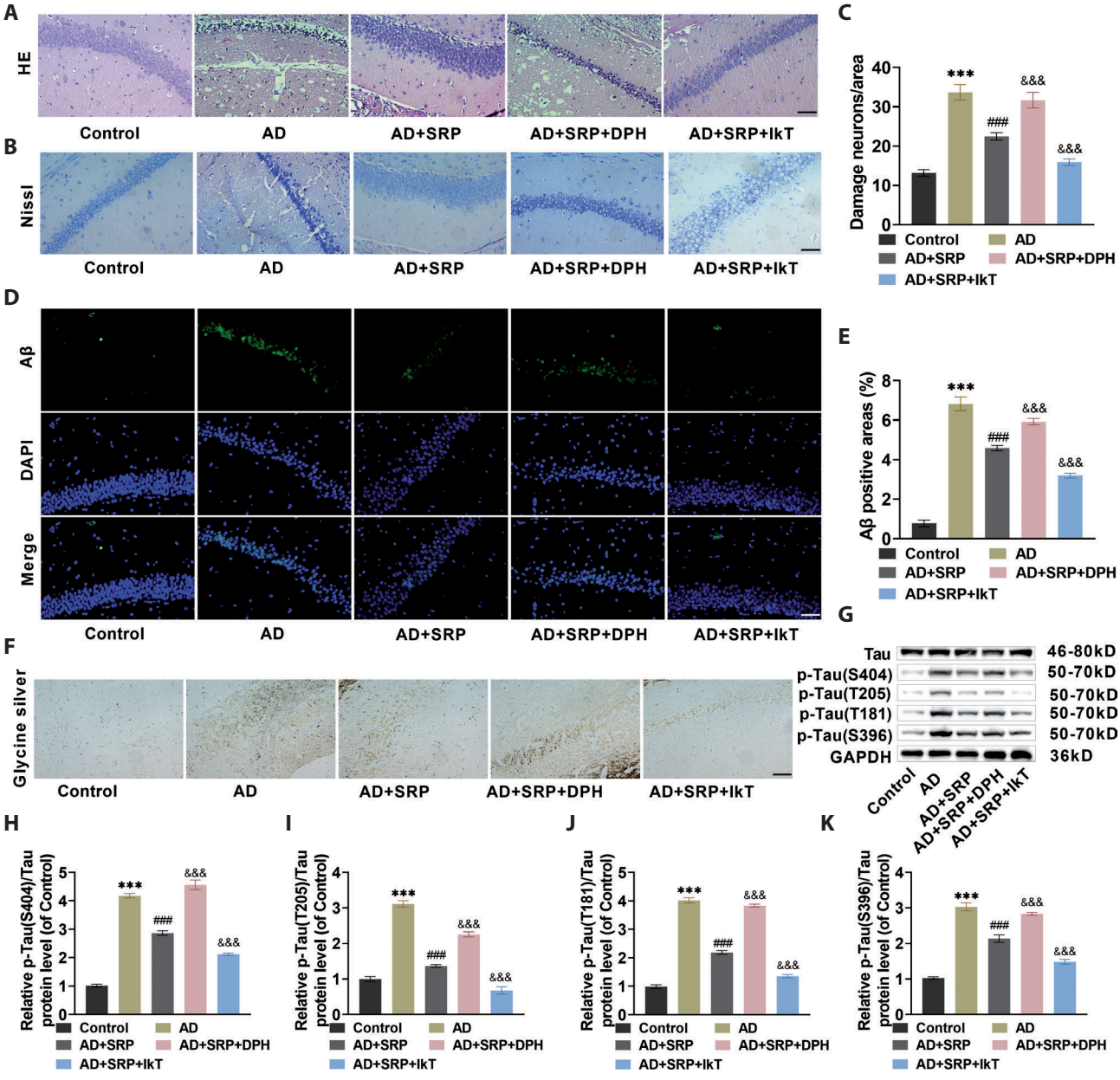


Figure 5. SRP ameliorates pathological neuronal damage in hippocampus of AD mice through c-Abl/MST1 signaling pathway. **A.** HE staining showed that SRP treatment attenuated neuronal damage in the hippocampal tissue of AD mice, whereas DPH attenuated the effect of SRP, and IkT increased the effect of SRP and further reduced neuronal damage (40×, 50 μm). **B,C.** Nissl staining showed that SRP treatment improved hippocampal neuron morphology and arrangement in AD mice, whereas DPH attenuated the effect of SRP and IkT had the opposite effect of DPH (40×, 50 μm). **D,E.** Immunofluorescence measured that SRP treatment decreased the fluorescence intensity of Aβ in hippocampal tissues of AD mice, DPH caused a rise in Aβ fluorescence intensity, and IkT decreased Aβ fluorescence intensity (40×, 50 μm). **F.** Glycine silver staining revealed that SRP inhibited the formation of NFTs in the hippocampal tissues of AD mice, DPH intervention caused a rise in the number of NFTs, and IkT further inhibited the formation of NFTs (40×, 50 μm). **G–K.** Western blot measured that SRP treatment decreased Tau phosphorylation level in AD mice, while DPH intervention elevated Tau phosphorylation level, and the effect of IkT was opposite to that of DPH ($n = 6$). *** $p < 0.001$ *vs.* Control; ### $p < 0.001$ *vs.* AD; &&& $p < 0.001$ *vs.* AD+SRP.

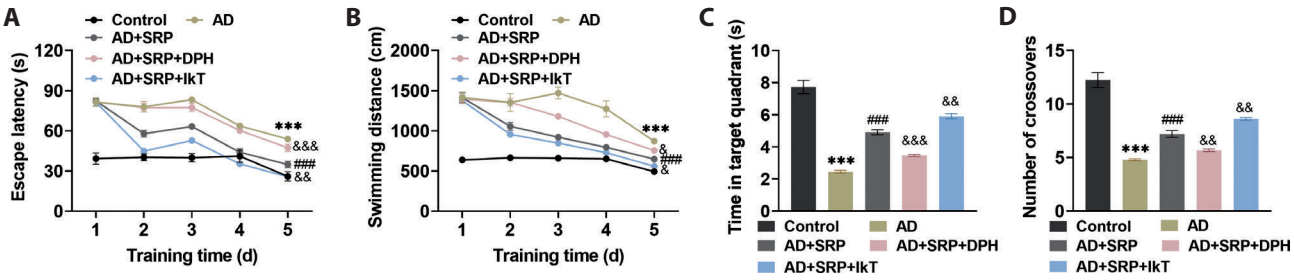


Figure 6. SRP ameliorates cognitive deficits in AD mice through c-Abl/MST1 pathway. The cognitive abilities of mice in different groups were tested by the Morris water maze experiment, in which the escape latency (**A**), swimming speed (**B**), the duration the mice remained in the target quadrant (**C**), and the crossovers numbers the mice traversed the hidden platform within 90 s (**D**) were recorded ($n = 6$). *** $p < 0.001$ *vs.* Control; $^{###}$ $p < 0.001$ *vs.* AD; $^{\&}$ $p < 0.05$, $^{\&\&}$ $p < 0.01$, $^{\&\&\&}$ $p < 0.001$ *vs.* AD+SRP.

The amyloid hypothesis suggests that APP can produce multiple biologically active fragments via amyloidogenic and non-amyloidogenic pathways (Orobets and Karamyshev 2023). In the amyloidogenic process, APP undergoes sequential cleavage by β-secretase and γ-secretase, ultimately generating Aβ, which can aggregate to form oligomers, protofibrils, and plaques after its release, interfering with signaling between neurons, which in turn triggers neuroinflammation, ultimately leading to neuronal death (Cho et al. 2022). Increased Tau kinase activity leads to aberrant phosphorylation of Tau, which in turn impacts the transport of APP to the cell membrane; therefore, aberrantly phosphorylated Tau is also considered a pathological feature of AD (Congdon et al. 2023). Not only that, excessive accumulation of phosphorylated Tau induces synaptic damage, subsequently causing neuronal dysfunction and the NFTs formation (Drummond et al. 2020). Xu et al. (2023) reported that Aβ plaque deposition was present in AD model mice and that Tau showed aberrant phosphorylation at several sites (T181, S396, T205, and S404). In the present study, Aβ fluorescence intensity and phosphorylation levels of Tau were elevated in the hippocampal tissues of AD mice, and a large number of NFTs were formed. SRP treatment significantly reduced these indicators, improved Aβ plaque deposition and Tau hyperphosphorylation, and inhibited NFTs formation. This result confirmed that SRP can effectively inhibit the pathological manifestations of AD and provides an important scientific basis for creating new therapeutic approaches for AD.

The core pathological process of AD is closely related to neuronal apoptosis, which is not only a key mechanism for neuronal loss and synaptic function impairment in AD brain, but also a central hub connecting pathological events such as Aβ deposition, Tau protein hyperphosphorylation, and neuroinflammation (Kumari et al. 2023). Cyto C is a carrier for transferring electrons in the respiratory chain of mitochondria, which is vital in apoptosis, and the release of Cyto C from the mitochondria to the cytoplasmic cells is an essential step in initiating apoptosis (Zhou et al. 2024). The caspases are the major family of proteases that perform apoptosis in mammalian cells, and at least 15 family members have been identified. Normally exists as an inactive zymogen in living cells, which is activated upon apoptotic stimulation by protein hydrolysis of its Aspartate residue sites, of which caspase-3 is the most important (Eskandari

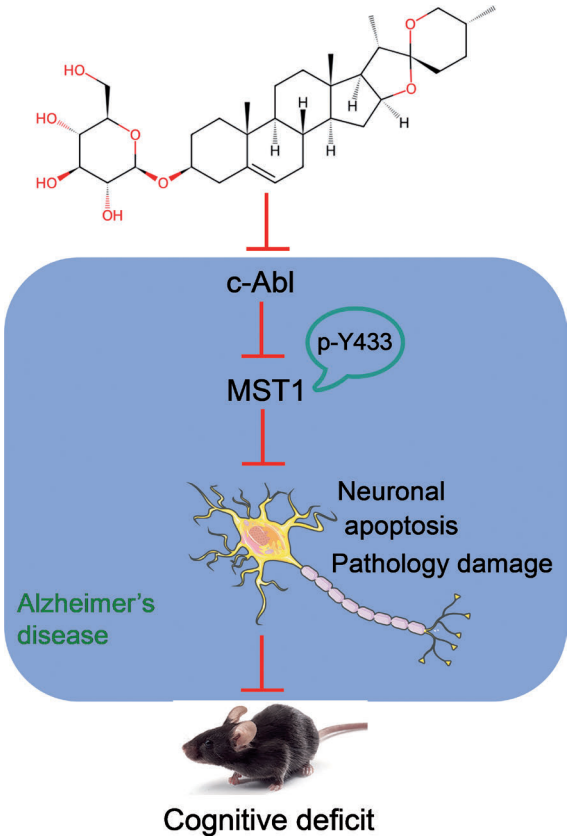


Figure 7. Mechanism diagram. SRP hinders c-Abl/MST1 pathway in the brain tissue of AD mice, thereby inhibiting hippocampal histopathological damage and neuronal apoptosis, and improving cognitive deficits.

and Eaves 2022). Aggregated Aβ activates caspase-3, disrupts mitochondrial membrane potential and releases Cyto C, which is neurotoxic to brain cells in the hippocampus, promotes neuroinflammation, and ultimately leads to apoptosis of neuronal cells (Mangalmurti and Lukens 2022; Rajesh and Kanneganti 2022). Our analysis revealed a higher count of TUNEL-positive cells, elevated expression of the apoptotic proteins Cyto C, Bax, and Cleaved-caspase3/caspase3, and declined Bcl-2 level in AD mice, confirming apoptosis of neurons in the brain tissues of AD mice. In contrast, SRP reversed the aberrant levels of these proteins and decreased TUNEL-positive cells numbers, suggesting that SRP can effectively inhibit neuronal apoptosis.

To further elucidate how SRP functions to alleviate AD progression, we evaluated the relevant signaling pathways. Research suggests that c-Abl has a crucial function in various neurodegenerative diseases, like AD and Parkinson's disease (Feng et al. 2022; Gutiérrez et al. 2023). Notably, downregulation of c-Abl effectively inhibited AD progression and improved memory deficits and spatial learning in AD mice (Zhong et al. 2024). MST1 is also crucial in the advancement of AD, and inhibition of MST1 rescued astrocyte senescence in the brain tissue of AD mice and improved cognitive function (Xu et al. 2021). c-Abl mediates the phosphorylation of MST1, and inhibition of the c-Abl/MST1 pathway attenuates neuronal cell death caused by oxidative stress (Xiao et al. 2011). These studies reveal the important role of the c-Abl/MST1 pathway in the physiopathology process of AD. In the present study, c-Abl and MST1 phosphorylation levels of were increased in the brain tissues of AD mice. SRP treatment decreased the phosphorylation levels of c-Abl and MST1, suggesting that SRP inhibits the c-Abl/MST1 pathway activation. Importantly, treatment with the c-Abl agonist DPH attenuated the ameliorative impacts of SRP on neuronal apoptosis and cognitive deficits in AD mice and elevated Aβ and p-Tau levels. The c-Abl inhibitor IkT, on the other hand, improved the therapeutic efficacy of SRP, and these results confirm that SRP may ameliorate cognitive deficits and neuronal apoptosis in AD mice by blocking the c-Abl/MST1 pathway, thereby inhibiting AD progression.

In summary, SRP can ameliorate cognitive deficits, alleviate neuronal pathological damage, and inhibit neuronal apoptosis in AD mice through modulating the c-Abl/MST1 signaling pathway (Fig. 7). This research elucidates the potential mechanism of action of SRP in inhibiting AD progression, expands the pharmacological role of SRP, and provides a novel approach for AD clinical treatment. Nonetheless, this investigation has its limitations; the current study is based only on the APP/PS1 mouse model, and the difference in efficacy of SRP has not yet been validated in models with multiple genetic backgrounds or different pathological stages. Subsequent studies could further validate the effect of SRP in different AD models. Additionally, we did not find any significant adverse reactions in mice during the experimental period. In the future, we will supplement the systematic toxicological evaluation of SRP to more comprehensively verify its safety and clinical translational potential.

Conflicts of interest. The authors affirm that they do not have any financial conflicts of interest.

Funding. Scientific research project of the Health Commission of Hunan Province (No. 202203073473, No. 20200919).

Author contribution. ChZ: Conducted and designed the research, carried out experiments, and analyzed findings. Edited and refined the manuscript with a focus on critical intellectual contributions. XT: Participated in collecting, assessing, and interpreting the data; made significant contributions to data interpretation and manuscript preparation. SW: Provided substantial intellectual input during the drafting and revision of the manuscript. The final version of the manuscript has been reviewed and approved by all authors.

References

Bohio AA, Wang R, Zeng X, Ba X (2020): cAblmediated tyrosine phosphorylation of DNA damage response proteins and implications in important cellular functions (Review). Mol Med Rep. **22,** 612-619 https://doi.org/10.3892/mmr.2020.11156

Breijyeh Z, Karaman R (2020): Comprehensive review on Alzheimer's disease: Causes and treatment. Molecules **25,** 5789 https://doi.org/10.3390/molecules25245789

Chen YY, Liu QP, An P, Jia M, Luan X, Tang JY, Zhang H (2022): Ginsenoside Rd: A promising natural neuroprotective agent. Phytomedicine **95,** 153883 https://doi.org/10.1016/j.phymed.2021.153883

Chen Z, Luo J, Jia M, Chai Y, Bao Y (2022): Polygonatum sibiricum saponin exerts beneficial hypoglycemic effects in type 2 diabetes mice by improving hepatic insulin resistance and glycogen synthesis-related proteins. Nutrients **14,** 5222 https://doi.org/10.3390/nu14245222

Cheng M, Yuan C, Ju Y, Liu Y, Shi B, Yang Y, Jin S, He X, Zhang L, Min D (2024): Quercetin attenuates oxidative stress and apoptosis in brain tissue of APP/PS1 double transgenic AD mice by regulating Keap1/Nrf2/HO-1 pathway to improve cognitive impairment. Behav. Neurol. **2024,** 5698119 https://doi.org/10.1155/2024/5698119

Cho Y, Bae HG, Okun E, Arumugam TV, Jo DG (2022): Physiology and pharmacology of amyloid precursor protein. Pharmacol. Ther. **235,** 108122 https://doi.org/10.1016/j.pharmthera.2022.108122

Congdon EE, Ji C, Tetlow AM, Jiang Y, Sigurdsson EM (2023): Tau-targeting therapies for Alzheimer disease: current status and future directions. Nat. Rev. Neurol. **19,** 715-736 https://doi.org/10.1038/s41582-023-00883-2

Dominguez LJ, Veronese N, Vernuccio L, Catanese G, Inzerillo F, Salemi G, Barbagallo M (2021): Nutrition, physical activity, and

other lifestyle factors in the prevention of cognitive decline and dementia. Nutrients **13,** 4080
https://doi.org/10.3390/nu13114080

Drummond E, Pires G, MacMurray C, Askenazi M, Nayak S, Bourdon M, Safar J, Ueberheide B, Wisniewski T (2020): Phosphorylated tau interactome in the human Alzheimer's disease brain. Brain **143,** 2803-2817
https://doi.org/10.1093/brain/awaa223

Eskandari E, Eaves CJ (2022): Paradoxical roles of caspase-3 in regulating cell survival, proliferation, and tumorigenesis. J. Cell Biol. **221,** e202201159
https://doi.org/10.1083/jcb.202201159

Feng L, Fu S, Yao Y, Li Y, Xu L, Zhao Y, Luo L (2022): Roles for c-Abl in postoperative neurodegeneration. Int. J. Med. Sci. **19,** 1753-1761
https://doi.org/10.7150/ijms.73740

Giesert F (2021): c-Abl phosphorylation primes PARIS for neurodegeneration. Brain **144,** 3555-3557
https://doi.org/10.1093/brain/awab412

Giridharan VV, Karupppagounder V, Arumugam S, Nakamura Y, Guha A, Barichello T, Quevedo J, Watanabe K, Konishi T, Thandavarayan RA (2020): **3,**4-dihydroxybenzalacetone (DBL) prevents aging-induced myocardial changes in senescence-accelerated mouse-prone 8 (SAMP8) mice. Cells **9,** 597
https://doi.org/10.3390/cells9030597

Gonzales MM, Garbarino VR, Pollet E, Palavicini JP, Kellogg DL, Jr., Kraig E, Orr ME (2022): Biological aging processes underlying cognitive decline and neurodegenerative disease. J. Clin. Invest. **132,** e158453
https://doi.org/10.1172/JCI158453

González-Martín A, Moyano T, Gutiérrez DA, Carvajal FJ, Cerpa W, Hanley JG, Gutiérrez RA, Álvarez A R (2021): c-Abl regulates a synaptic plasticity-related transcriptional program involved in memory and learning. Prog. Neurobiol. **205,** 102122
https://doi.org/10.1016/j.pneurobio.2021.102122

Gu X, Zhu LY, Xu ZY, Shen KP (2021): Astragaloside IV and sof Rhizoma Polygonati cure cyclophosphamide-induced myelosuppression in lung adenocarcinoma via down-regulating miR-142-3p. Front. Oncol. **11,** 630921
https://doi.org/10.3389/fonc.2021.630921

Guo K, Huang W, Chen K, Huang P, Peng W, Shi R, He T, Zhang M, Wang H, Hu J, et al. (2023): Fibroblast growth factor 10 ameliorates neurodegeneration in mouse and cellular models of Alzheimer's disease via reducing tau hyperphosphorylation and neuronal apoptosis. Aging Cell **22,** e13937
https://doi.org/10.1111/acel.13937

Gutiérrez DA, Chandía-Cristi A, Yáñez MJ, Zanlungo S, Álvarez AR (2023): c-Abl kinase at the crossroads of healthy synaptic remodeling and synaptic dysfunction in neurodegenerative diseases. Neural. Regen. Res. **18,** 237-243
https://doi.org/10.4103/1673-5374.346540

He X, Huang S, Yu C, Chen Y, Zhu H, Li J, Chen S (2024): Mst1/Hippo signaling pathway drives isoproterenol-induced inflammatory heart remodeling. Int. J. Med. Sci. **21,** 1718-1729
https://doi.org/10.7150/ijms.95850

Huang R, He X, Wang X, Li X, Liu Y, Tan P (2024): The analysis of raw and processed Polygonatum kingianum saponins and stimulatory mechanism in Caenorhabditis elegans. Fitoterapia **179,** 106242
https://doi.org/10.1016/j.fitote.2024.106242

Huang YY, Gan YH, Yang L, Cheng W, Yu JT (2024): Depression in Alzheimer's disease: Epidemiology, mechanisms, and treatment. Biol. Psychiatry **95,** 992-1005
https://doi.org/10.1016/j.biopsych.2023.10.008

Jiang Y, Zeng X, Dai H, Luo S, Zhang X (2024): Polygonatum sibiricum polysaccharide regulation of gut microbiota: A viable approach to alleviate cognitive impairment. Int. J. Biol. Macromol. **277,** 134494
https://doi.org/10.1016/j.ijbiomac.2024.134494

Karuppagounder SS, Wang H, Kelly T, Rush R, Nguyen R, Bisen S, Yamashita Y, Sloan N, Dang B, Sigmon A, et al. (2023): The c-Abl inhibitor IkT-148009 suppresses neurodegeneration in mouse models of heritable and sporadic Parkinson's disease. Sci. Transl. Med. **15,** eabp9352
https://doi.org/10.1126/scitranslmed.abp9352

Knopman DS, Amieva H, Petersen RC, Chételat G, Holtzman DM, Hyman BT, Nixon RA, Jones DT (2021): Alzheimer disease. Nat. Rev. Dis. Primers **7,** 33
https://doi.org/10.1038/s41572-021-00269-y

Kumari S, Dhapola R, Reddy DH (2023): Apoptosis in Alzheimer's disease: insight into the signaling pathways and therapeutic avenues. Apoptosis **28,** 943-957
https://doi.org/10.1007/s10495-023-01848-y

León R, Gutiérrez DA, Pinto C, Morales C, de la Fuente C, Riquelme C, Cortés B I, González-Martin A, Chamorro D, Espinosa N, et al. (2023): c-Abl tyrosine kinase down-regulation as target for memory improvement in Alzheimer's disease. Front. Aging Neurosci. **15,** 1180987
https://doi.org/10.3389/fnagi.2023.1180987

Li T, Wen Y, Lu Q, Hua S, Hou Y, Du X, Zheng Y, Sun S (2023): MST1/2 in inflammation and immunity. Cell Adh. Migr. **17,** 1-15
https://doi.org/10.1080/19336918.2023.2276616

Liu J, Zing Hern T, Khe Jia S, Yang S, Lu A JH (2025): Disrupting cancer metabolism: Molecular docking insights into andrographolides as a competitive inhibitors of selected lipogenic enzymes (Fatty Acid Synthase, ATP Citrate Lyase, and Acetyl-CoA Carboxylase). JCBT **2,** 55-64
https://doi.org/10.62382/jcbt.v2i2.62

Luo S, Zhang X, Huang S, Feng X, Zhang X, Xiang D (2022): A monomeric polysaccharide from Polygonatum sibiricum improves cognitive functions in a model of Alzheimer's disease by reshaping the gut microbiota. Int. J. Biol. Macromol. **213,** 404-415
https://doi.org/10.1016/j.ijbiomac.2022.05.185

Mangalmurti A, Lukens J R (2022): How neurons die in Alzheimer's disease: Implications for neuroinflammation. Curr. Opin. Neurobiol. **75,** 102575
https://doi.org/10.1016/j.conb.2022.102575

Monteiro A R, Barbosa D J, Remião F, Silva R (2023): Alzheimer's disease: Insights and new prospects in disease pathophysiology, biomarkers and disease-modifying drugs. Biochem. Pharmacol. **211,** 115522
https://doi.org/10.1016/j.bcp.2023.115522

Motaln H, Rogelj B (2023): The role of c-Abl tyrosine kinase in brain and its pathologies. Cells **12,** 2041

https://doi.org/10.3390/cells12162041
Nurtay L, Sun Q, Mu C, Cao Z, Wang Q, Liang Z, Ma C, Li X, Amin A, Xie Y (2021): Rhizoma Polygonati from Mount Tai: nutritional value and usefulness as a traditional Chinese medicine, source of herbzyme, and potential remediating agent for COVID-19 and chronic and hidden hunger. Acupunct. Herb. Med. **1,** 31-38
https://doi.org/10.1097/HM9.0000000000000008
Orobets KS, Karamyshev A L (2023): Amyloid precursor protein and alzheimer's disease. Int. J. Mol. Sci. **24,** 14794
https://doi.org/10.3390/ijms241914794
Park MW, Cha HW, Kim J, Kim JH, Yang H, Yoon S, Boonpraman N, Yi SS, Yoo I D, Moon JS (2021): NOX4 promotes ferroptosis of astrocytes by oxidative stress-induced lipid peroxidation via the impairment of mitochondrial metabolism in Alzheimer's diseases. Redox Biol. **41,** 101947
https://doi.org/10.1016/j.redox.2021.101947
Passeri E, Elkhoury K, Morsink M, Broersen K, Linder M, Tamayol A, Malaplate C, Yen F T, Arab-Tehrany E (2022): Alzheimer's disease: Treatment Strategies and their limitations. Int. J. Mol. Sci. **23,** 13954
https://doi.org/10.3390/ijms232213954
Piavchenko G, Soldatov V, Venediktov A, Kartashkina N, Novikova N, Gorbunova M, Boronikhina T, Yatskovskiy A, Meglinski I, Kuznetsov S (2022): A combined use of silver pretreatment and impregnation with consequent Nissl staining for cortex and striatum architectonics study. Front. Neuroanat. **16,** 940993
https://doi.org/10.3389/fnana.2022.940993
Rajesh Y, Kanneganti T D (2022): Innate immune cell death in neuroinflammation and Alzheimer's disease. Cells **11,** 1885
https://doi.org/10.3390/cells11121885
Ren R, Qi J, Lin S, Liu X, Yin P, Wang Z, Tang R, Wang J, Huang Q, Li J, et al. (2022): The China Alzheimer report 2022. Gen. Psychiatr. **35,** e100751
https://doi.org/10.1136/gpsych-2022-100751
Rostagno A A (2022): Pathogenesis of Alzheimer's disease. Int. J. Mol. Sci. **24,** 104
https://doi.org/10.3390/ijms24010107
Scheltens P, De Strooper B, Kivipelto M, Holstege H, Chételat G, Teunissen C E, Cummings J, van der Flier W M (2021): Alzheimer's disease. Lancet **397,** 1577-1590
https://doi.org/10.1016/S0140-6736(20)32205-4
Shao Y, Wang Y, Sun L, Zhou S, Xu J, Xing D (2023): MST1: A future novel target for cardiac diseases. Int. J. Biol. Macromol. **239,** 124296
https://doi.org/10.1016/j.ijbiomac.2023.124296
Shapiro LP, Omar M H, Koleske AJ, Gourley S L (2017): Corticosteroid-induced dendrite loss and behavioral deficiencies can be blocked by activation of Abl2/Arg kinase. Mol. Cell Neurosci. **85,** 226-234
https://doi.org/10.1016/j.mcn.2017.10.007
Sharma KK, Gupta S, Bisen PS (2025): Enhancing gastrointestinal (GI) cancer therapies with Ganoderma Lucidum: A review of mechanisms and efficacy. Journal of Cancer Biomoleculars and Therapeutics **2,** 15-44
https://doi.org/10.62382/jcbt.v2i1.28
She L, Sun J, Xiong L, Li A, Li L, Wu H, Ren J, Wang W, Liang G, Zhao X (2024): Ginsenoside RK1 improves cognitive impairments and pathological changes in Alzheimer's disease via stimulation of the AMPK/Nrf2 signaling pathway. Phytomedicine **122,** 155168
https://doi.org/10.1016/j.phymed.2023.155168
Shen Y, Liu F, Zhang M (2024): Therapeutic potential of plant-derived natural compounds in Alzheimer's disease: Targeting microglia-mediated neuroinflammation. Biomed. Pharmacother. **178,** 117235
https://doi.org/10.1016/j.biopha.2024.117235
Varshney H, Siddique YH (2021): Role of natural plant products against Alzheimer's disease. CNS Neurol Disord Drug Targets **20,** 904-941
https://doi.org/10.2174/1871527320666210420135437
Varshney H, Siddique YH (2024): Effect of natural plant products on Alzheimer's disease. CNS Neurol. Disord. Drug Targets **23,** 246-261
https://doi.org/10.2174/1871527322666230228102223
Wang H, Shang Y, Wang E, Xu X, Zhang Q, Qian C, Yang Z, Wu S, Zhang T (2022): MST1 mediates neuronal loss and cognitive deficits: A novel therapeutic target for Alzheimer's disease. Prog. Neurobiol. **214,** 102280
https://doi.org/10.1016/j.pneurobio.2022.102280
Wang S, He F, Wu H, Xiang F, Zheng H, Wu W, Li S (2023): Health-promoting activities and associated mechanisms of Polygonati Rhizoma polysaccharides. Molecules **28,** 1350
https://doi.org/10.3390/molecules28031350
Wang Y, Wang K, Yan J, Zhou Q, Wang X (2022): Recent progress in research on mechanisms of action of natural products against Alzheimer's disease: Dietary plant polyphenols. Int. J. Mol. Sci. **23,** 13886
https://doi.org/10.3390/ijms232213886
Wu Y, Zheng H, Zheng T, Jiang J, Xu Y, Jia F, He K, Yang Y (2024): Quantitative changes and transformation mechanisms of saponin components in chinese herbal medicines during storage and processing: A review. Molecules **29,** 4486
https://doi.org/10.3390/molecules29184486
Xiao L, Chen D, Hu P, Wu J, Liu W, Zhao Y, Cao M, Fang Y, Bi W, Zheng Z, et al. (2011): The c-Abl-MST1 signaling pathway mediates oxidative stress-induced neuronal cell death. J. Neurosci. **31,** 9611-9619
https://doi.org/10.1523/JNEUROSCI.0035-11.2011
Xiao M, Xiang W, Chen Y, Peng N, Du X, Lu S, Zuo Y, Li B, Hu Y, Li X (2022): DHA Ameliorates Cognitive ability, reduces amyloid deposition, and nerve fiber production in Alzheimer's disease. Front. Nutr. **9,** 852433
https://doi.org/10.3389/fnut.2022.852433
Xiao M, Yao C, Liu F, Xiang W, Zuo Y, Feng K, Lu S, Xiang L, Li M, Li X, et al. (2022): Sialic acid ameliorates cognitive deficits by reducing amyloid deposition, nerve fiber production, and neuronal apoptosis in a mice model of Alzheimer's disease. NeuroSci. **3,** 28-40
https://doi.org/10.3390/neurosci3010002
Xu QQ, Su ZR, Yang W, Zhong M, Xian YF, Lin ZX (2023): Patchouli alcohol attenuates the cognitive deficits in a transgenic mouse model of Alzheimer's disease via modulating neuropathology and gut microbiota through suppressing C/EBPβ/AEP pathway. J. Neuroinflammation **20,** 19
https://doi.org/10.1186/s12974-023-02704-1

Xu X, Shen X, Wang J, Feng W, Wang M, Miao X, Wu Q, Wu L, Wang X, Ma Y, et al. (2021): YAP prevents premature senescence of astrocytes and cognitive decline of Alzheimer's disease through regulating CDK6 signaling. Aging Cell **20,** e13465 https://doi.org/10.1111/acel.13465

Zang G, Fang L, Chen L, Wang C (2018): Ameliorative effect of nicergoline on cognitive function through the PI3K/AKT signaling pathway in mouse models of Alzheimer's disease. Mol. Med. Rep. **17,** 7293-7300 https://doi.org/10.3892/mmr.2018.8786

Zeng Y, Xiong L, Tang H, Chen L, Yu Q, Li L, Chen F, Li L, Zheng Y, Sun J, et al. (2024): Norboldine improves cognitive impairment and pathological features in Alzheimer's disease by activating AMPK/GSK3β/Nrf2 signaling pathway. J. Ethnopharmacol. **333,** 118498 https://doi.org/10.1016/j.jep.2024.118498

Zhai L, Pei H, Shen H, Yang Y, Han C, Guan Q (2023): Paeoniflorin suppresses neuronal ferroptosis to improve the cognitive behaviors in Alzheimer's disease mice. Phytother. Res. **37,** 4791-4800 https://doi.org/10.1002/ptr.7946

Zhao D, Liu H, Yan C, Teng Y, Zou Y, Ren X, Xia X (2024): Polygonatum sibiricum saponin prevents immune dysfunction and strengthens intestinal mucosal barrier function in cyclophosphamide-induced immunosuppressed BALB/c mice. Foods **13,** 934 https://doi.org/10.3390/foods13060934

Zheng Q, Wang X (2025): Alzheimer's disease: insights into pathology, molecular mechanisms, and therapy. Protein Cell **16,** 83-120 https://doi.org/10.1093/procel/pwae026

Zhong M, Xu QQ, Hu Z, Yang W, Lin ZX, Xian YF (2024): Tianma-Gouteng pair ameliorates the cognitive deficits on two transgenic mouse models of Alzheimer's disease. J. Ethnopharmacol. **328,** 118113 https://doi.org/10.1016/j.jep.2024.118113

Zhou J, Li L, Wu B, Feng Z, Lu Y, Wang Z (2024): MST1/2: Important regulators of Hippo pathway in immune system associated diseases. Cancer Lett. **587,** 216736 https://doi.org/10.1016/j.canlet.2024.216736

Zhou Z, Arroum T, Luo X, Kang R, Lee Y J, Tang D, Hüttemann M, Song X (2024): Diverse functions of cytochrome c in cell death and disease. Cell Death Differ. **31,** 387-404 https://doi.org/10.1038/s41418-024-01284-8

Received: May 16, 2025
Final version accepted: July 18, 2025