

Lycopene Treatment Ameliorates Amyloid Plaque Deposition and Memory Impairment in the APP/PS1 Mice

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Abstract

Alzheimer's disease (AD) is a neurodegenerative condition associated with oxidative stress and neuroinflammation. Lycopene has previously been shown to ameliorate neuroinflammation and exert protection against oxidative damage in neuroblastoma cells. The role of this compound in reversing cognitive dysfunction in AD has yet to be determined. The present study investigates the role of lycopene in AD with an *in vitro* A β 1-42-induced cell cytotoxicity model as well as the *in vivo* APP/PS1 mouse model. The activation of Nrf2 signal pathway was assessed using western blot and RT-PCR. MDA, 8-OHdG, ROS, SOD, GHS and GSSG measurements were carried out using the specialized assay kits. The Morris water maze was used to examine qualitative assessment of memory and spatial learning. Immunofluorescence was used to visualize astrocytes and microglia activation as well as brain β -amyloid (A β) deposition. The NeuN positive cells were detected by immunofluorescence and western blot. Levels of cerebral cytokines were quantified using RT-PCR. Lycopene ameliorates oxidative damage in the A β 1-42-triggered cell cytotoxicity model via Nrf2-ARE signal pathway activation, which is regulated by AKT-GSK3 β pathway. In addition, lycopene improves the cognitive impairment and reduces the A β deposition. Mechanistically, lycopene attenuates neuron loss, decreases chronic inflammation and activates cerebral Nrf2-ARE signaling pathway in APP/PS1 mice. The results suggest that lycopene alleviates oxidative stress via AKT- Nrf2-ARE pathway. And early administration of lycopene improves cognitive deficits by reducing A β deposition, neuronal loss and decreasing the degree of chronic inflammation.

Introduction

Alzheimer's disease (AD) is one of the most common dementia [1,2]. The pathogenesis of AD is recognized by amyloid- β (A β) deposition, abnormal Tau phosphorylation, brain atrophy and decreased in neurons and synapses along with neuroinflammation^[3]. It is reported that oxidative stress induced by A β affects cellular homeostasis, causing neurotoxicity and neuronal death which in turn promotes the formation and deposition of A β . This vicious cycle forms the basis for the development of AD [4]. Neuroinflammation is also a key component in AD pathogenesis [5] which causes nerve cell damage and increases neuronal degeneration in brain

Previous studies have demonstrated lycopene, a fat-soluble carotenoid found in tomatoes and other red vegetables and fruits, exerts a plethora of anti-inflammatory and anti-oxidative benefits [6,7]. For example, several experimental models have demonstrated the anti-oxidative effects of lycopene in both the peripheral and central nervous systems [8-10]. Our previous studies proved that lycopene shielded SH-SY5Y cells against oxidative toxicity through decreased endoplasmic reticulum stress^[11]. The anti-neuroinflammatory effect of lycopene has been demonstrated in primary cultured rodent microglia [12,13]. Furthermore, studies show that lycopene ameliorates AD-associated neuropathological changes in cultured rat cortical neurons [14], and decreases the behavioral deficit in Parkinson's disease mice and colchicine-induced memory impairment in rats [15,16]. These findings indicate that lycopene exerts

multiple effects and may be a potential therapy for neurodegenerative diseases. However, whether lycopene affects cognitive deficit, neuroinflammation and neuronal death in the APP/PS1 mice remains unknown.

Here, we report that lycopene decreases loss of hippocampal neurons and neuroinflammation and reverses cognitive deficit in the APP/PS1 mice, which could be a potential therapeutic target for AD treatment.

Material And Methods

1.1 Cell Culture and treatment

The Conservation Genetics of the Chinese Academy of Sciences Kunming Cell Bank provided SH-SY5Y cells which were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with high glucose, 1% penicillin/streptomycin solution and 10% fetal bovine serum (FBS) (all products from ThermoFisher Scientific, OR, USA) at 37 °C under 5% CO₂. β-Amyloid (Aβ) [1-42] (Human) (Invitrogen Scientific, OR, USA) was first dissolved in ddH₂O at 6mg/mL and diluted to 1mg/mL with PBS prior to undergoing a 6 hours incubation period at 37 °C. 0.025% butylated hydroxytoluene enhanced tetrahydrofuran (Sigma-Aldrich, MO, USA) was used to dilute lycopene (Sigma-Aldrich, MO, USA) to form a 10 mM stock solution. PBS was used for further dilution to improve solution stability and uptake^[17]. SH-SY5Y cells were subjected to a 2 h-treatment period in the absence or presence of lycopene (5 μM) before being further incubated for 24 h with Aβ1-42 (20 μM). Cellular products were then harvested for further investigation.

1.2 Animals and treatment

Four-month old wild-type mice along with age-matched transgenic APP/PS1 (APP^{swe}PSEN1^{dE9}) mice from the same litter were procured from Nanjing Biomedical Research Institute of Nanjing University (Nanjing, China). All mice were allowed water and food *ad libitum* and reared in standard laboratory conditions for one month before further treatment. Animal experimental protocols were created in compliance to the regulations of the Institutional Animal Care and Use Committee of Zhongshan School of Medicine, Sun Yat-sen University and were approved by the relevant ethics committee.

After a month of environmental adaptation, APP/PS1 mice or age-matched littermates were fed 2 mg/kg lycopene (Sigma, MO, USA) diluted in corn oil or pure corn oil once a day for 12 weeks via gavage. 0.025% butylated hydroxytoluene enhanced tetrahydrofuran (Sigma, MO, USA) was used to dilute lycopene in order to produce a 10 mM stock solution before the addition into corn oil. The control group received only THF via the same method.

1.3 Morris Water Maze

A computerized tracking system of the Morris water maze (MT-200, Chengdu, China) was performed 12 weeks later to determine mice memory and spatial learning abilities. A tank of 50-cm-height and 120-cm-diameter containing opacified water at a temperature of 24 ± 1 °C was used. A 10-mm platform located 1 cm underwater was centered in the tank target quadrant after its division into four equal quadrants. Mice was first allowed to explore the tank four times a day for 120 s per trial over six consecutive days in the task acquisition phase. The escape latency was documented during the initial part of the test. Mice which failed to find the platform in 120 s were manually placed on it and kept in place for 20 s. The probe trial took place 7 d post-platform removal. The mice were allowed to swim for 120 s. Time spent in the target quadrant and number of times crossing the original platform area were recorded.

1.4 Tissue preparation

All mice were sacrificed immediately after the behavioural test. 50mls of cold normal saline was transfused intracardially and mice brains were harvested. All right and left cerebrums were divided. 4% paraformaldehyde was used to fix one hemisphere for histological analysis. Liquid nitrogen was used to freeze the isolated cortex and hippocampus from the other hemisphere. Samples were preserved at -80°C for further experiments.

1.5 Immunofluorescence

Paraformaldehyde-treated hemisphere was immersed in phosphate buffer saline (PBS) containing 30% sucrose for 2 days before a freezing microtome (Leica SM2000R) was used to produce 40 µm sections. Sliced sections were rinsed thrice with PBS before being immersed for 30 mins in 1% bovine serum albumin (BSA) at 37°C. Primary antibodies were then exposed overnight to the sections at 4°C. Immunofluorescent secondary antibodies was then added the next morning after rinsing the samples thrice with PBS for 1h at 37°C. Cell nuclei were counterstained for 30s with Hoechst solution. The primary antibodies incorporated comprised of mouse anti- A β (1:1000, A5213, Sigma-Aldrich), rabbit anti-Iba-1 (1:500, 019-19741, Wako Chemical), rabbit anti-GFAP (1:100, G9269, Sigma-Aldrich), mouse anti-NeuN (1:1000, MAB377, Chemicon International). The fluorescent secondary antibodies included: Alexa Fluor 488 goat anti-mouse (1:200, SA00006-1, Proteintech), Alexa Fluor 594 goat anti-rabbit (1:200, SA00006-4, Proteintech).

Microscopic analyses of A β /Iba-1, A β /GFAP, NeuN signal were imaged using a Zeiss LSM 800 confocal laser scanning microscope. Microscopic analyses of A β deposition were imaged using a Zeiss Axio Scan Z1 automatic digital slide scanning system. The fluorescence of each special signal was captured using the same parameter. The percentages of specially stained areas were determined by imaging three coronal sections spaced 200µm apart through the regions of interest (hippocampus or cerebral cortex) per mouse. Images were converted to digital quantification using the Image J software.

1.6 Western blot

Western blot was used to assess protein levels. SDS-PAGE gels were used to separate protein samples prior to immunoblotting onto polyvinylidene difluoride (PVDF) membranes. This was followed by endogenous reaction blocking for an hour at room temperature using bovine serum albumin (BSA) or 5% non-fat dry milk in TBST. Primary antibodies were then added onto the membranes before they were left to incubate overnight. The next morning, species specific HRP-conjugated secondary antibody was exposed at room temperature to the samples for an hour. A chemiluminescence substrate was used to develop samples. The corresponding bands were visualized with GE AI600 Imaging System (GE, USA). The primary antibodies used included Keap1(1:1000, ab66620, Abcam), Nrf2(1:1000, 14596S, Cell Signaling Technology), Gclc(1:1000, ab207777, Abcam), Gclm(1:1000, ab124827, Abcam), GSK-3 β (1:1000, 12456, Cell Signaling Technology), p-GSK-3 β (Ser9)(1:000, 5558, Cell Signaling Technology), AKT(1:1000, 9272, Cell Signaling Technology), p-AKT(Ser473)(1:1000, 4058, Cell Signaling Technology).

1.7 Quantitative real-time PCR

qRT-PCR was used to assess relative gene expressions. The HiPure Total RNA Mini Kit (Magen, Guangzhou, China) allowed for total RNA extraction, while the PrimeScriptTMRT Master Mix (Takara, Shiga, Japan) was used to cDNA production. Quantitative real-time PCR was then performed with the TB GreenTM Premix Ex TaqTM (Takara, Shiga, Japan) in compliance to manufacturer protocols. The CFX96 Real-Time PCR Detection System (BIO-RAD, USA) with SYBR Green[®] Master (Takara, Shiga, Japan) was used to carry out all reactions. The $\Delta\Delta C_q$ method was used to assess all data collected using the Bio-Rad CFX manager 3.1 software. The chosen internal control was GAPDH.

1.8 MDA assay

The malondialdehyde (MDA) Assay Kit (Beyotime, China) was used for this experiment. Cells were harvested after treatment as described previously. The lysates were sonicated on ice and centrifuged for 10 min at 1600 rpm at 4°C. The Bicinchoninic Acid Protein Assay (BCA) kit (Beyotime, China) was used to quantify concentration of the supernatants. The MDA content in the supernatants was measured using a spectrophotometer at 532 nm (Tecan, Switzerland) prior to calculation of relative protein levels.

1.9 8-OHdG assay

An enzyme-linked immunosorbent assay kit (Cloud-Clone, USA) was used to determine 8-hydroxydeoxyguanosine (8-OHdG) levels. Cells were placed onto 6-well plates and treated as described previously. The supernatants were extracted from the differentially treated cells and were assayed following the supplier's instructions. Supernatant 8-OHdG content was measured spectrophotometrically at 450 nm.

1.10 ROS assay

Reactive oxygen species (ROS) was analyzed using the ROS Assay Kit (Beyotime, China). Briefly, cells were planted onto 96-well plates and preincubated with lycopene at concentration of 5 μ M for 2 h, followed by a 24 h exposure to A β 1-42. The cells were then rinsed with PBS before and incubation with

ROS substrate solution for 30min at 37 °C under 5% CO₂ protecting from light. The absorbance was read at 490nm using a multifunctional microplate reader (SpectraMax M5, USA).

1.11 SOD assay

SOD concentration was analyzed with the help of a SOD Assay Kit (Beyotime, China). Cells were harvested after treatment as described previously. The lysates were sonicated on ice and centrifuged at 1600 rpm for 10 min at 4°C. The harvested supernatant was subjected to analysis using the BCA kit (Beyotime, China). The SOD concentration in the supernatants was measured spectrophotometrically at 450 nm (Tecan, Switzerland) and analyzed in relation to the protein levels.

1.12 GSH and GSSH assay

Glutathione (GSH) and GSSH were analyzed using the GSH and GSSH Assay Kit (Beyotime, China). In short, 6-well plates were used to seed cells which were pretreated as described previously. After incubation, cells were collected and GSH and GSSH were detected follow the instruction of manufacturer. A microplate reader was used to assess absorbance at 412 nm (Tecan, Switzerland).

1.13 Statistical analysis

The SPSS 20.0 software (IBM, NY, USA) was used to carry out all data analysis. The two-way repeated-measures ANOVA, followed by LSD post hoc comparisons, allowed for the assessment of escape latency data. Comparisons between two or more groups were carried out using the one-way ANOVA test followed by LSD post hoc comparison. Mean±SEM were used to express data. Statistical significance was achieved when $P \leq 0.05$.

Results

2.1 Lycopene attenuates A β 1-42-induced oxidative damage in the SH-SY5Y cells.

8-OHdG, MDA and ROS were suppressed in the lycopene-treated group in contrast to the lycopene-naïve group (Fig. 1A-C). Conversely, SOD activity was heightened in the cells treated with lycopene compared to the control group (Fig. 1D). Moreover, the content of GSH levels were markedly raised in cells treated with lycopene in contrast to the control group, while the converse was true with regards to GSSG (Fig. 1E, F).

2.2 Lycopene activated Nrf2 through AKT-GSK-3 β pathway in cytotoxicity cell model induced by A β 1-42.

There was no marked difference in Keap1 protein expressions between lycopene-treated and untreated cells. There was a raised ratio of p-GSK-3 β / GSK-3 β in the lycopene-treated group compared to cells which were not treated with lycopene (Fig. 2A, B). Nrf2 protein expression as well as levels of p-AKT/AKT and p-GSK-3 β /GSK-3 β in the lycopene-treated group are augmented in cells treated with lycopene compared to those which were not. Interestingly, administration with LY294002 (an AKT-specific inhibitor) significantly reversed these effects (Fig. 2C, D).

2.3 Lycopene ameliorated the spatial memory deficits in the APP/PS1 mice.

There was a marked reduction in escape latency in the abilities of lycopene-treated mice to find the hidden platform at day 2 and day 6 during the 6-days acquisition phase, in contrast to APP/PS1 mice exposed only to the vehicle (Fig. 3A). Lycopene-treated mice were noted to perform better in comparison to vehicle-treated APP/PS1 mice in the probe trial phase as evidenced by the increased times that mice crossed the area that the platform was formerly placed (Fig. 3B, C). The average time duration of times that the mice spent in all other quadrants were also notably reduced in APP/PS1 mice treated with lycopene in contrast to those treated with the vehicle alone (Fig. 3D). Moreover, there was no significant differences between body weight, food and water intake among the three groups (data not shown).

2.4 Lycopene reduced A β deposition in the APP/PS1 mice.

The percentage area and A β plaques density in cortical and hippocampal cerebral regions of APP/PS1 mice was assessed to dissect the impact of lycopene on A β plaque deposition. Immunofluorescence was used to visualize cerebral A β deposition (Fig. 4A). Lycopene markedly reduced the cortical percentage area of A β plaque, with no statistically significant hippocampal reduction, in APP/PS1 mice exposed to lycopene compared to those which were not (Fig. 4B). In addition, the A β plaque density in both the hippocampus and cortex were markedly reduced with lycopene treatment (Fig. 4C).

2.5 Lycopene attenuated neuroinflammation in the APP/PS1 Mice.

We found increased accumulation of reactive astrocytes and microglia around the A β plaques in the cortex of APP/PS1 mice, compared to the lycopene-treated APP/PS1 mice (Fig. 5A-D). However, there is no differences for the activity of microglia and astrocytes between the APP/PS1 control mice and lycopene-treated APP/PS1 mice in the hippocampus (data not showed). In addition, we investigated gene expression of pro-inflammatory and anti-inflammatory mediators in the cortex and hippocampus. The data showed that the level of IL-4 was significantly increased while the level of TNF- α was remarkably decreased both in hippocampus and cortex in lycopene-treated APP/PS1 mice, compared with those in APP/PS1 mice (Fig. 6).

2.6 Lycopene attenuated neuronal cell loss in the APP/PS1 mice.

The immunofluorescence and immunohistofluorescence results showed that the NeuN positive cells as well as levels of the NeuN protein in the hippocampus of lycopene-treated mice was much higher in comparison to APP/PS1 mice (Fig. 7A-D).

2.7 Lycopene activated Nrf2 signaling pathway in both APP/PS1 mice and a cytotoxicity cell model induced by A β 1–42.

Both protein and mRNA expressions of Gclm and Gclc were significantly upregulated in lycopene-exposed APP/PS1 mice in contrast to the vehicle-treated APP/PS1 mice. Nrf2 mRNA levels were upregulated in lycopene-exposed APP/PS1 mice in contrast to APP/PS1 mice, despite no statistical significance

observed between the two groups with regards to Nrf2 protein levels (Fig. 8A-C). An A β 1-42-induced cytotoxic cell model was established using SH-SY5Y cells in attempt to mimic the intracerebral environment of AD. This model was then used to assess the protein and mRNA expressions of Nrf2-ARE pathway related genes. Protein expressions of Nrf2, Gclm and Gclc were markedly upregulated in the lycopene-exposed group in contrast to the lycopene-naïve group. The mRNA expressions of Gclm and Gclc were also upregulated in the lycopene-treated group in contrast to the lycopene-naïve group, while the mRNA expression of Nrf2 demonstrated no statistical differences between the two groups (Fig. 8D-F).

Discussion

Here, we demonstrate that lycopene improves the cognitive function of APP/PS1 mice. Lycopene significantly ameliorates A β deposition and attenuates activation of microglia and astrocytes, inhibits neuroinflammation and reduces neuronal loss in the hippocampus.

Lycopene have been shown to possess a myriad of therapeutic effects and neuroprotection on AD [18–22]. For example, cognitive deficit in mice treated with high-fructose and high-fat diets has been attenuated with lycopene treatment, a phenomenon that was attributed to reduced inflammation and reversal of lipid metabolism dysfunction and insulin resistance [23]. Interestingly, lycopene suppresses choroid plexus A β induced inflammation in the early AD phases in rats, as evidenced by reductions of TNF- α , IL-1 β and IL-6 β [24]. In addition, lycopene can also inhibit neuronal apoptosis and restore mitochondrial function [25, 26]. In the LPS-induced mouse model, lycopene has neuroprotective effects such as attenuating amyloidosis and amyloid deposition [27].

Chronic oxidative stress causes neuroinflammation and cognitive dysfunction in neurodegenerative diseases [28]. The Nrf2-ARE pathway is a well-known mediator of oxidative stress [29]. Gclc and Gclm, two subunits composing of glutamate cysteine ligase (GCL), are target genes of Nrf2 [30]. In our previous study, we demonstrated that lycopene alleviated oxidative stress through Nrf2 pathway in an M146L AD cell model that was double transfected with human APP and presenlin-1 genes [31]. To further understand the underlying mechanisms of lycopene in AD, we set up an *in vitro* cell model of AD. A β 1–42 served as a trigger of oxidative stress in this model. We found that lycopene restored oxidative balance by activating AKT-GSK3 β pathway and reducing Nrf2 degradation. A previous *in vitro* study using vascular endothelial cells demonstrated that lycopene activated the AKT pathway by promoting the phosphorylation of AKT and exhibited a cardiovascular protection effect [32]. On top of that, lycopene has been found to activate the AKT pathway directly in an oxidative neurotoxicity model triggered by tert-butyl hydroperoxide [33].

We hypothesized that Nrf2 pathway may also be involved in lycopene-mediated cognitive improvements seen in the APP/PS1 mice. These findings allude to the strong ability of lycopene in activating the Nrf2-ARE pathway as evidenced by raised hippocampal Nrf2, Gclc and Gclm levels in the APP/PS1 mice in contrast to lycopene-naïve APP/PS1 mice. Similarly in a mouse model of bilateral common carotid artery occlusion, lycopene was also found to impart neuroprotective effects through stimulation of the Nrf2/HO-

1 signaling pathway^[34]. In addition, lycopene has been reported to ameliorated neuroinflammation as well as cognitive impairment in the fructose-drinking insulin resistant rats^[35].

Glial cell activation is well established in AD and other neurodegenerative diseases. Microglia and astrocytes are activated by A β fibrils and are involved in the activation of inflammatory cascades via secretion of proinflammatory cytokines. Ultimately, these processes lead to chronic neuroinflammation^[36]. In addition, accumulated cytokines have been implicated in the insufficient degradation and clearance of A β by microglia and astrocytes^[37]. Glial cell activation might therefore occur before A β deposition in the AD^[38]. Therefore, early lycopene administration in the APP/PS1 mice may serve to halt disease progression. Lycopene might alter glial cell phenotypes, phagocytosis and cytokines secretion in the early stage of APP/PS1 mice. Furthermore, lycopene exhibits some interesting biological activities, such as inhibition of microglia and astrocyte and suppression of neuroinflammation, events which are strongly linked to cognitive improvement and reduction of A β deposition.

In summary, the present study demonstrates that lycopene improves spatial memory and reduces amyloid deposition and neuronal loss. This effect likely stems from the inhibition of glial activation and suppression of neuroinflammation, both of which are mediated by oxidative stress and activation of the AKT- Nrf2-ARE signaling. Our study strongly supports the neuroprotective effects of lycopene on AD.

Abbreviations

AD: Alzheimer's disease; A β : β -amyloid; DME: AKT: Protein kinase B; Dulbecco' modified eagle medium; DMSO: Dimethyl sufoxide; FBS: Fetal bovine serum; GCLC: Glutamate cysteine ligase catalytic subunit; GCLM: Glutamate cysteine ligase modifier subunit; GSH: Glutathione; GSK-3 β : Glycogen synthase kinase 3 beta; HO-1: Heme oxygenase-1; IL-1 β : Interleukin-1 beta; MDA: Malonadialdehyde; Nrf2: Nuclear factor (erythroid-derived 2)-like 2; PBS: Phosphate buffer saline; PI3K: Phosphatidyl inositol 3-kinase; PVDF: Polyvinylidene difluoride; ROS: Reactive oxygen species; SDS: Sodium dodecyl sulfate ; SOD: Superoxide dismutase; TNF- α : Tumor necrosis factor alpha; 8-OHdG: 8-hydroxy-2'-deoxyguanosine.

Declarations

Ethics approval and consent to participate:

This study was approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University.

Consent for publication:

Not applicable

Availability of data and material:

All data generated or analysed during this study are included in this published article.

Competing interests:

The authors declare no competing interest.

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Author Contributions:

Conceptualization, Shanshan Ou, Kaihua Guo and JieXu; Data curation, Shanshan Ou and Yinchao Fang; Formal analysis, Yinchao Fang; Funding acquisition, Kaihua Guo and Jie Xu; Investigation, Shanshan Ou; Methodology, Shanshan Ou and Yinchao Fang; Resources, Shanshan Ou and Yinchao Fang; Supervision, Kaihua Guo and Jie Xu; Validation, Tong Wu; Writing – original draft, Shanshan Ou and Yinchao; Writing – review & editing, Kaihua Guo.

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Figures

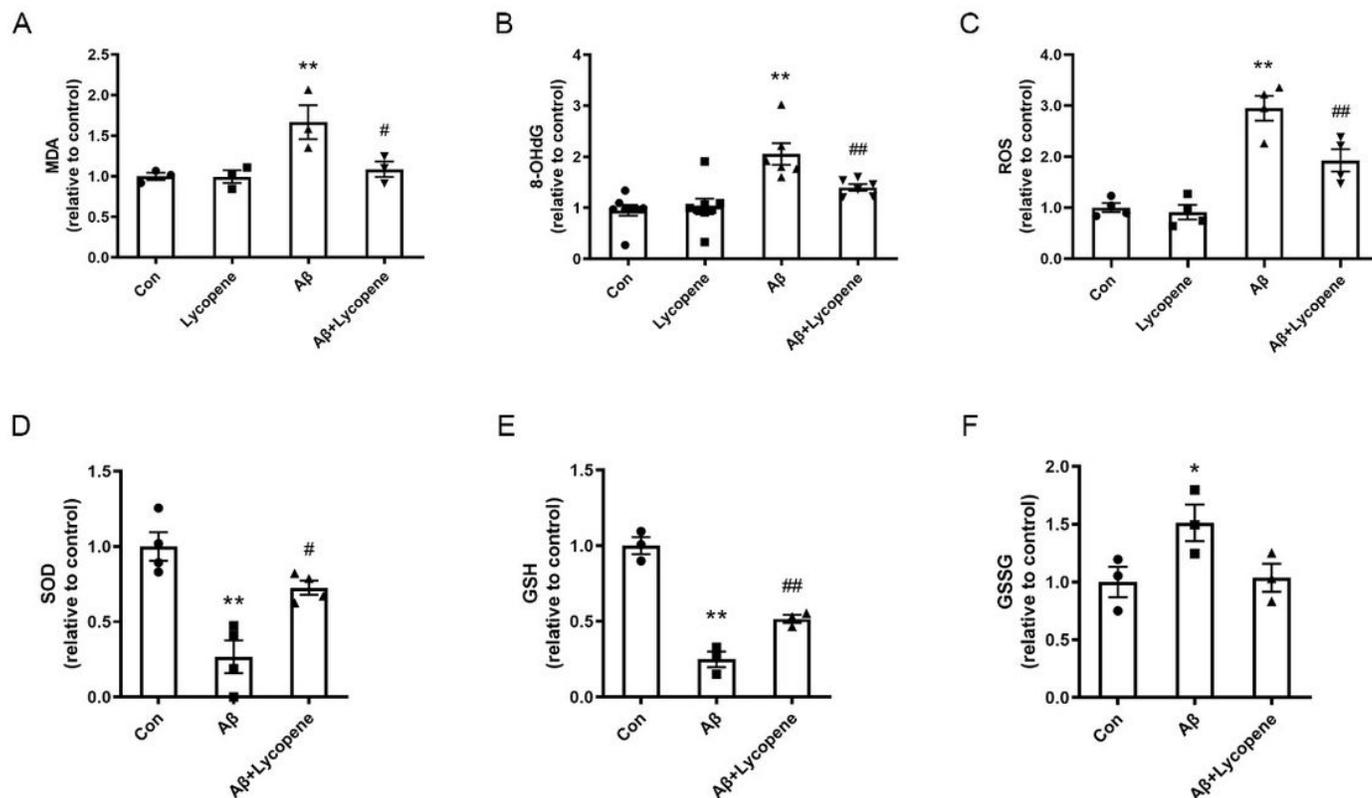


Figure 1

Lycopene up-regulated antioxidants and rescued the disruption of oxidative damage induced by Aβ 1-42 in the SH-SY5Y cells. (A-C) Lycopene treatment reduced MDA and 8-OHdG concentration and the level of ROS. (D-F) Lycopene treatment up-regulated the SOD activity and GSH concentration, but had not effects on GSSG concentration. The results are all presented as the Mean± SEM, *P<0.05, ** P<0.01, vs. control group; ##P<0.01, #P<0.05, vs. Aβ group.

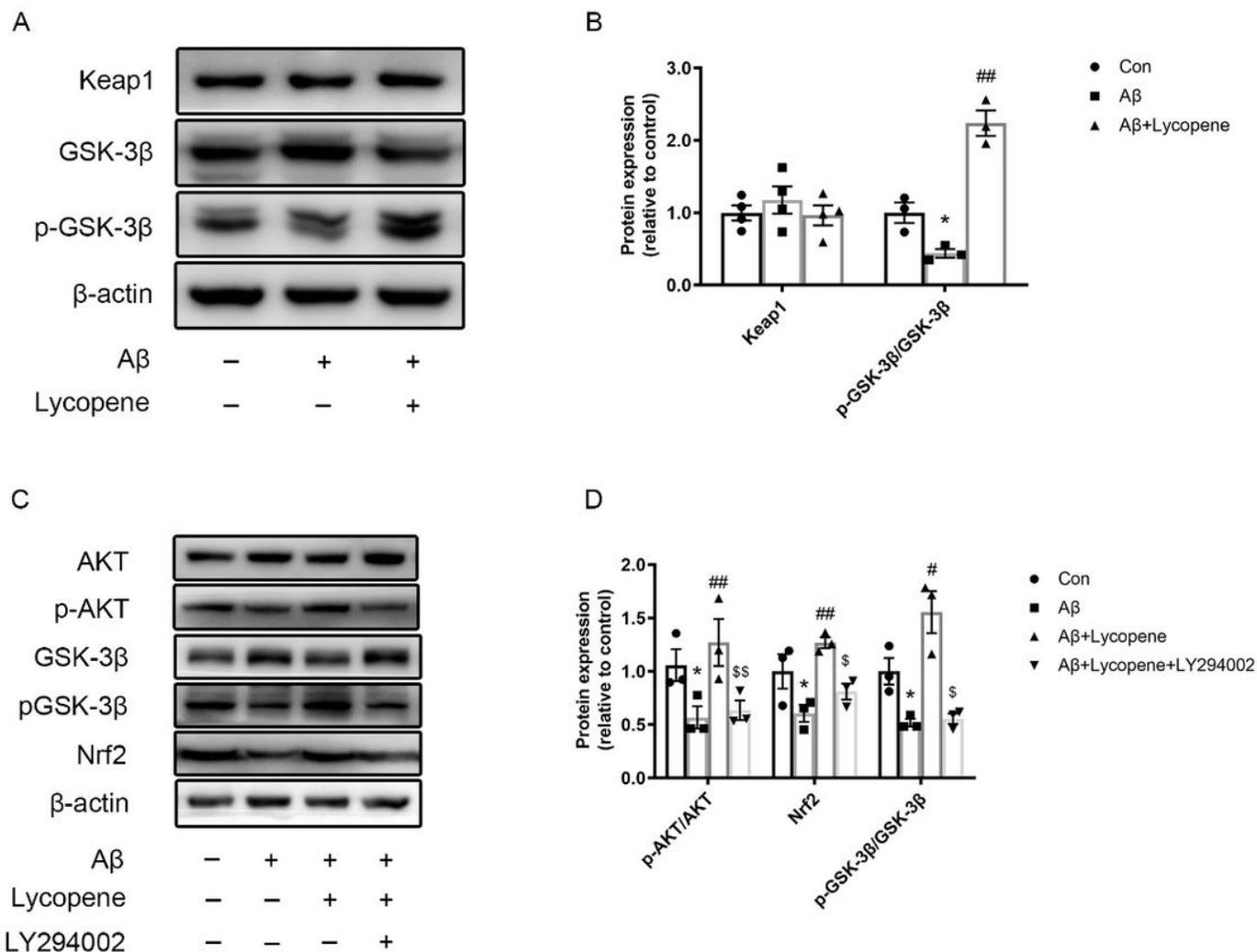


Figure 2

Lycopene activated Nrf2 through AKT-GSK-3β pathway in the SH-SY5Y cells incubating with Aβ1-42. (A) Protein expressions of Keap1, GSK-3β and p-GSK-3β in SH-SY5Y cell were determined by Western blot; (B) β-actin was used to normalize densitometric analysis. (C) Protein expressions of AKT, p-AKT, GSK-3β, p-GSK-3β and Nrf2 were determined by Western blot; (D) Densitometric analysis was normalized to β-actin. *P<0.05, **P<0.01, vs. control group; #P<0.05, ##P<0.01, vs. Aβ group. *P<0.05, vs. CON group; #P<0.05, ##P<0.01, vs. Aβ group; \$P<0.05, \$\$P<0.01, vs. Aβ+lycopene group.

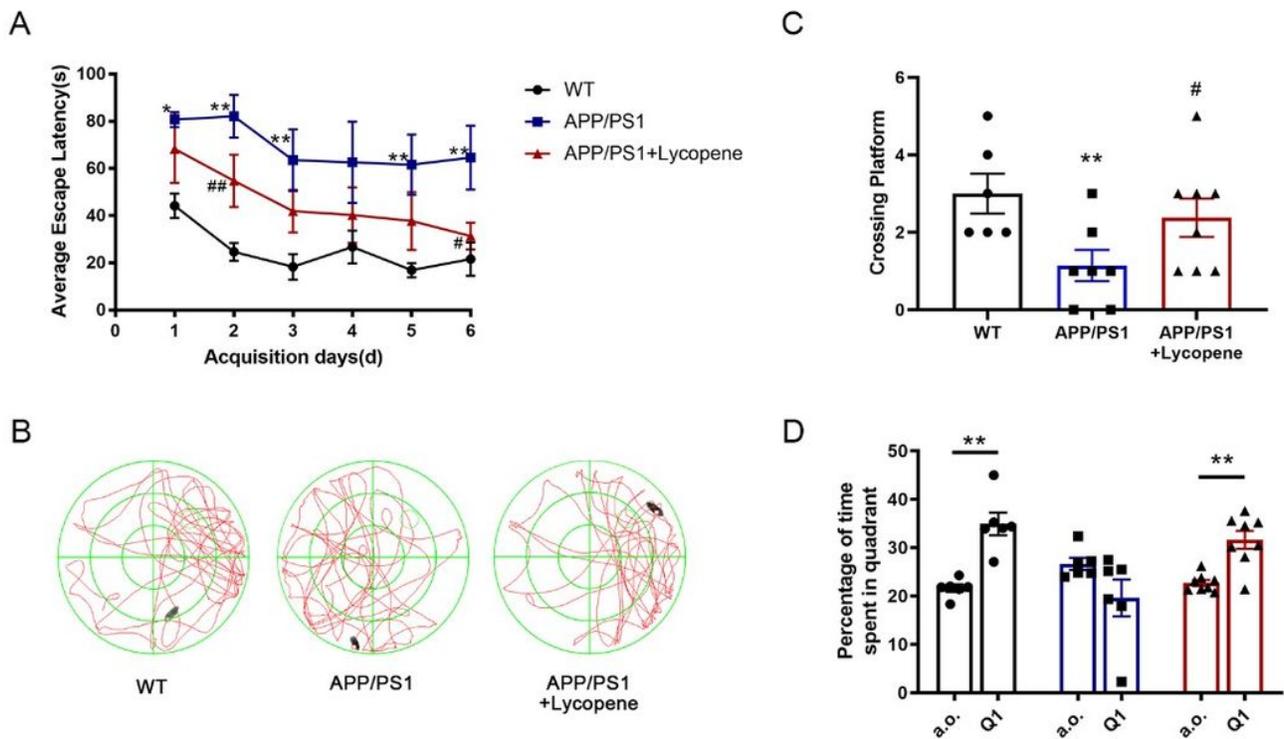


Figure 3

Lycopene attenuated cognitive deficits in the APP/PS1 mice. (A) The escape latency exhibited throughout the 6-days training phase in WT (n=6), APP/PS1 (n=7) and lycopene-treated mice (n=8) after lycopene treatment; (B, C) In the probe trial phase, the number of passes across the escape platform during the MWM test. (D) The average percentage of time spent in the target quadrant (Q1) and in all other (a.o.) quadrants. Q1 represents the platform location during days 1-6. Data is depicted in the terms of Mean \pm SEM. n=6-8, *P<0.05, **P<0.01, vs. control group; ##P<0.01, vs. APP/PS1 group. WT are wild type mice.

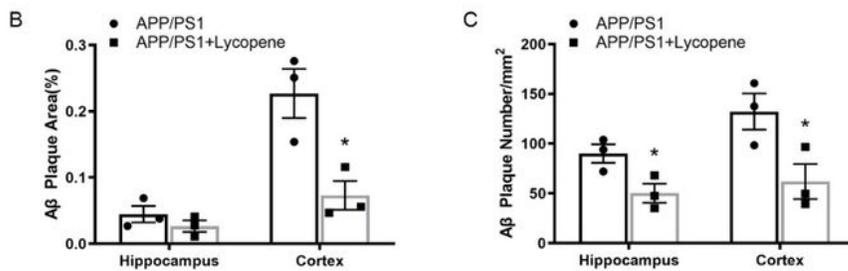
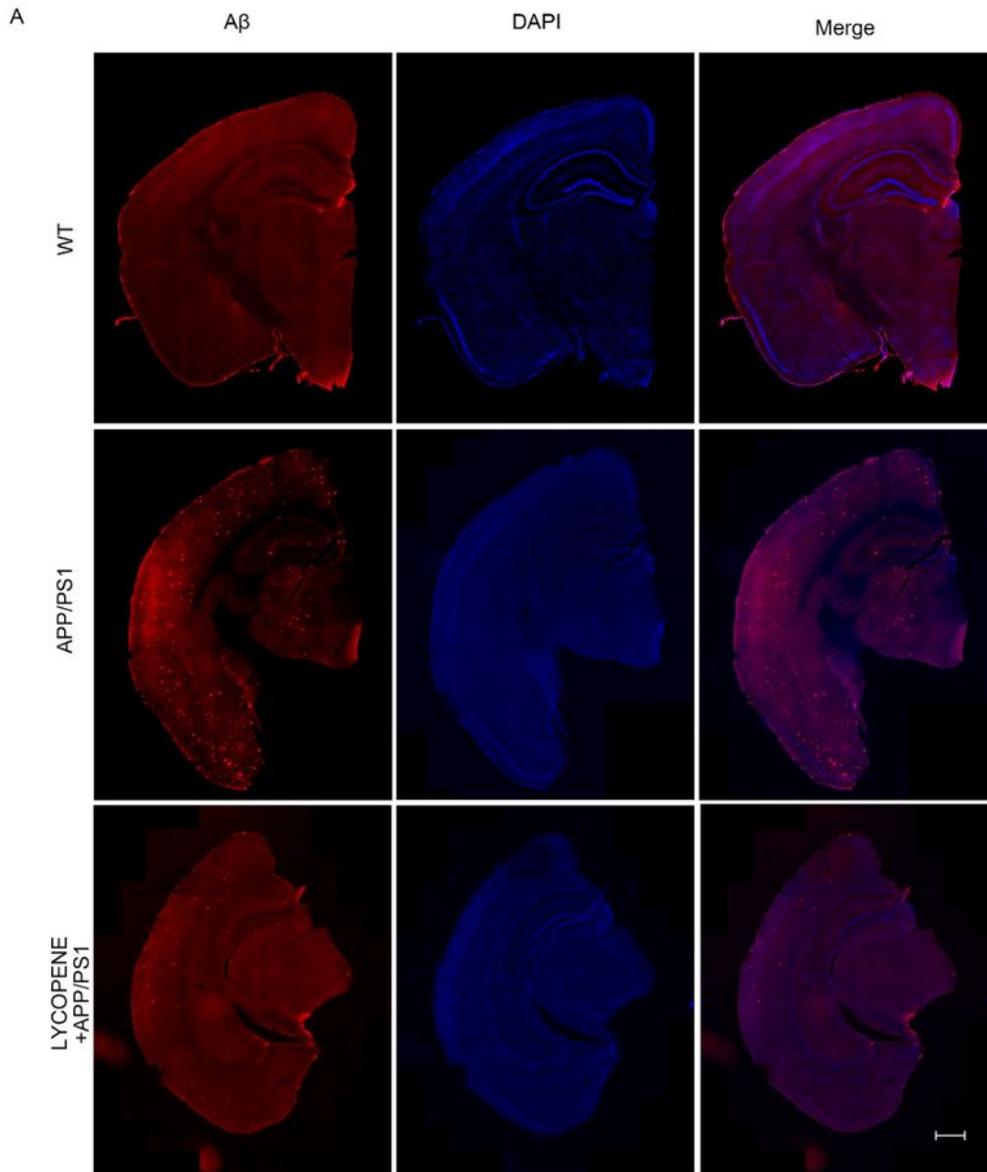


Figure 4

Lycopene alleviated A β deposition in the APP/PS1 mice. (A) Anti- A β antibody and DAPI staining of the hippocampus and cortex of APP/PS1 mice from three groups (3 for each group) (scale bar=200 μ m). (B, C) Hippocampus and cortex A β quantification in APP/PS1 mice. Data is depicted in the terms of Mean \pm SEM, *P<0.05, vs. APP/PS1 group.

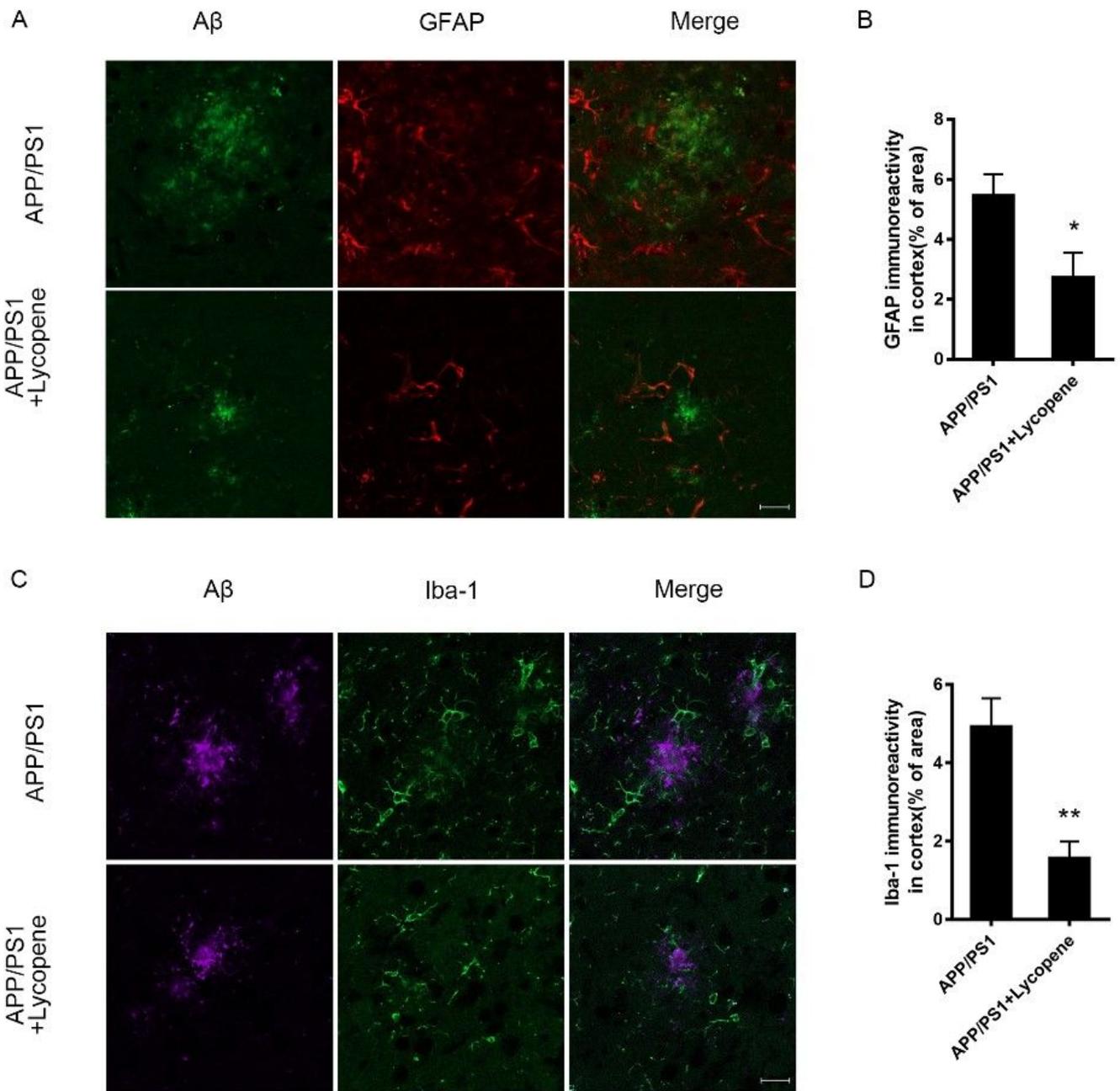


Figure 5

Lycopene ameliorated glial reactivity in cortex in APP/PS1 mice. (A) Anti- A β antibody, anti-GFAP antibody and DAPI staining cortical areas of APP/PS1 mice from two groups (scale bar=20 μ m). (B) Quantification of GFAP staining in cortex of mice. (C) Anti- A β antibody, anti-Iba-1 antibody and DAPI staining in cortical areas of APP/PS1 mice from two groups (scale bar=20 μ m). (D) Iba-1 staining quantification in mice cortices (scale bar=20 μ m). Data is depicted in terms of Mean \pm SEM, *P<0.05, **P<0.01, vs. APP/PS1 group.

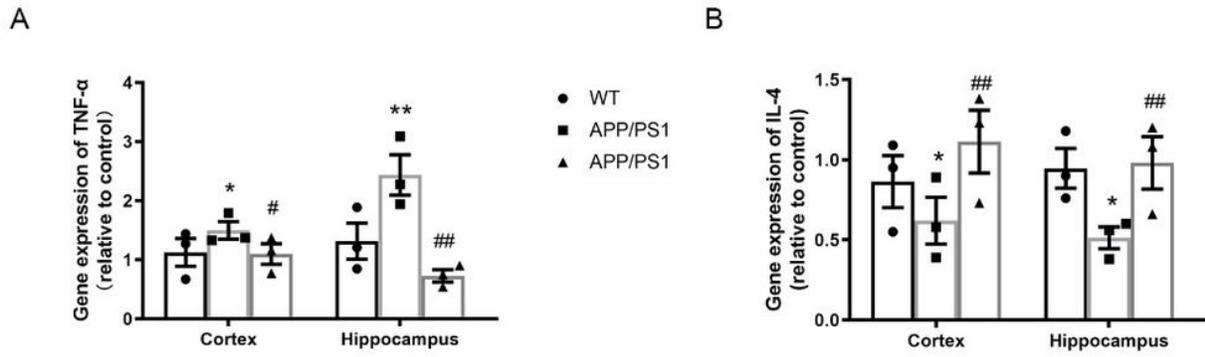


Figure 6

Lycopene suppressed TNF- α gene levels while augmenting IL-4 levels in the hippocampus and cortex of APP/PS1 mice. (A-B) The mRNA expression of two cytokines were measured by RT-PCR from WT, APP/PS1 and lycopene-treated mice (3 for each group). The results are all presented as the Mean \pm SEM, * $P < 0.05$, ** $P < 0.01$, vs. WT group; # $P < 0.05$, ## $P < 0.01$, vs. APP/PS1 group.

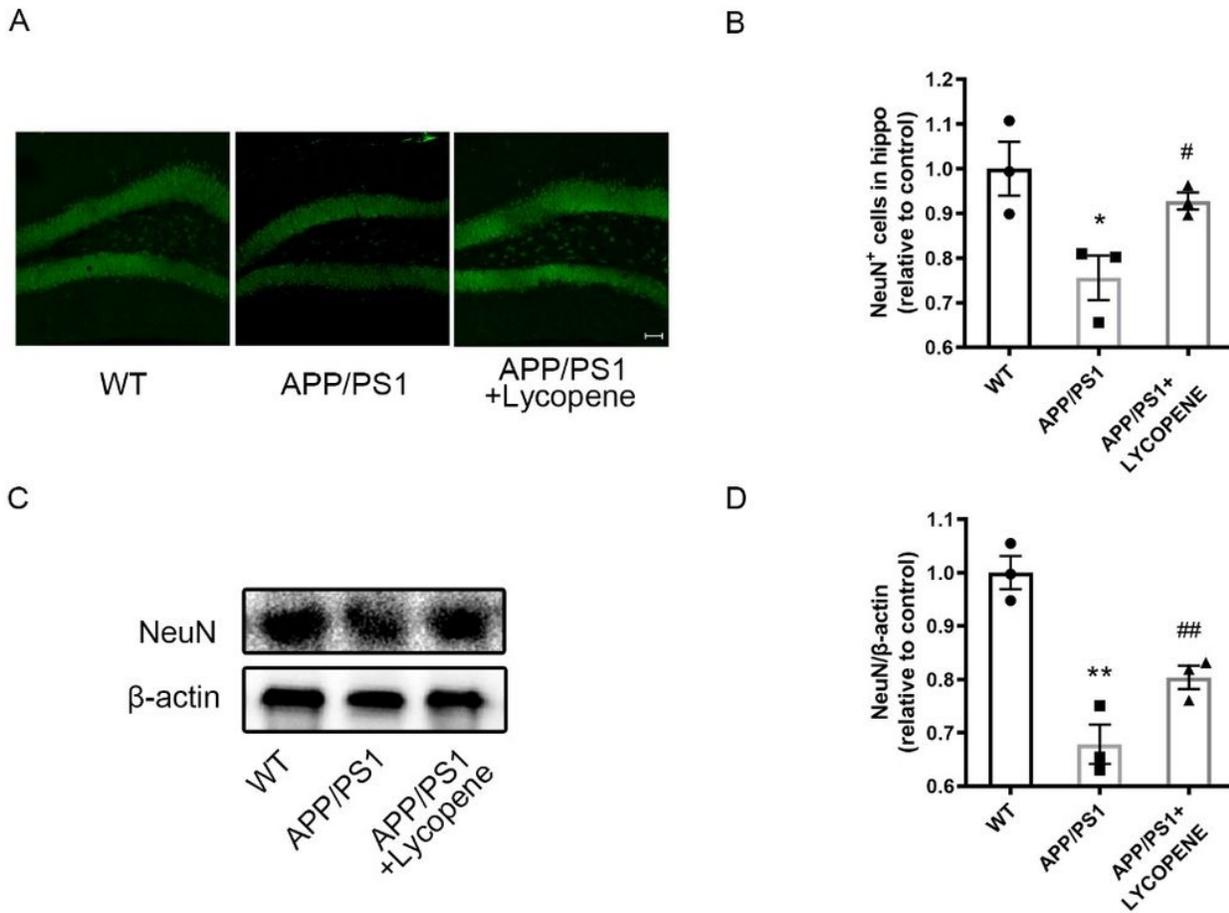


Figure 7

Lycopene prevented neuronal cell death in APP/PS1 mice. (A) Anti-NeuN antibody staining in hippocampal areas of mice from three groups, 3 for each group (scale bar=50 μ m). (B) Quantification of NeuN staining in hippocampus of mice. (C) The protein level of NeuN in hippocampus was detected by Western blot. (D) β -actin was used to normalize densitometric analysis of the NeuN protein. Data is depicted in terms of mean value \pm SEM, * P <0.05, ** P <0.01, vs. WT group; # P <0.05, ## P <0.01, vs. APP/PS1 group.

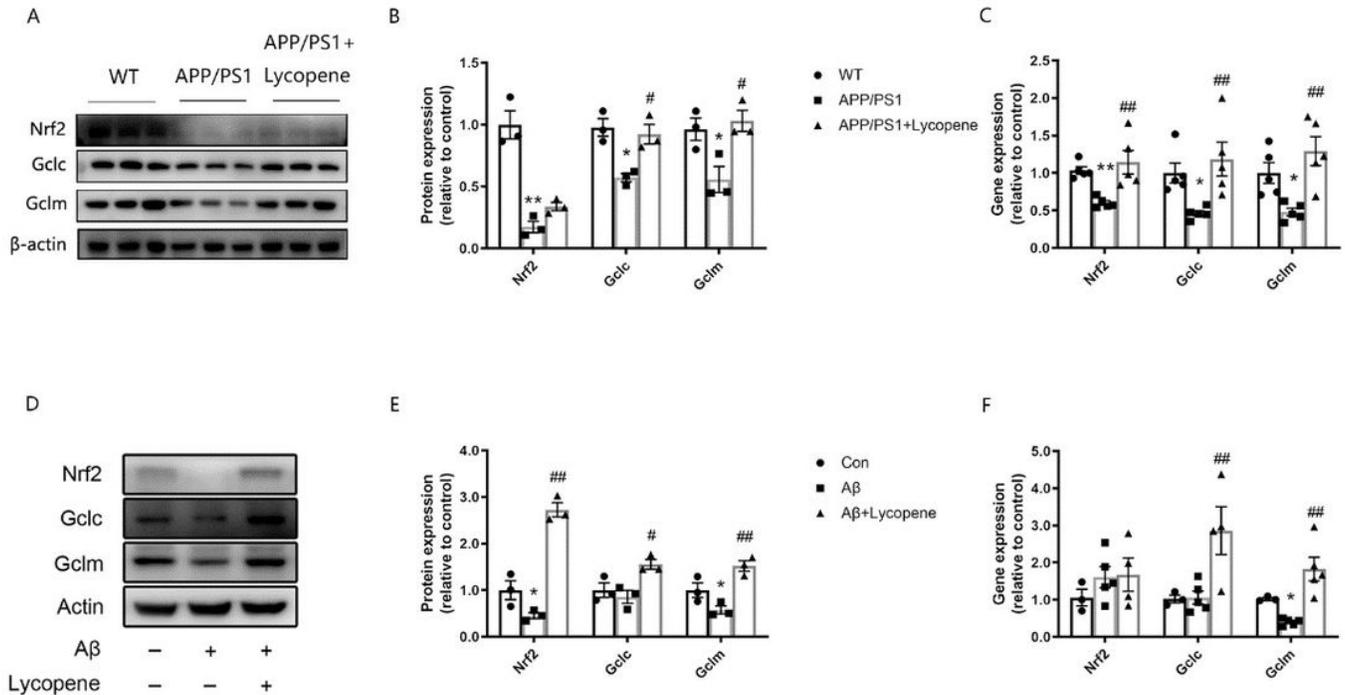


Figure 8

Lycopene activated Nrf2 signal pathway both in APP/PS1 mice and cell model of AD. (A) Nrf2, Gclc and Gclm protein levels in hippocampus of APP/PS1 was determined by Western Blot. (B) β -actin was used to normalize densitometric analysis of hippocampus protein. (D) Protein expression of Nrf2, Gclc and Gclm in SH-SY5Y cells were determined by Western Blot. (E) β -actin was used to normalize densitometric analysis of SH-SY5Y cells. (C, F) Nrf2, Gclc and Gclm mRNA expression in APP/PS1 mice and SH-SY5Y cells were determined by q-PCR. The results are all presented as the mean value \pm SEM, * P <0.05, ** P <0.01, vs. WT group or control group; # P <0.05, ## P <0.01, vs. APP/PS1 group or A β group.

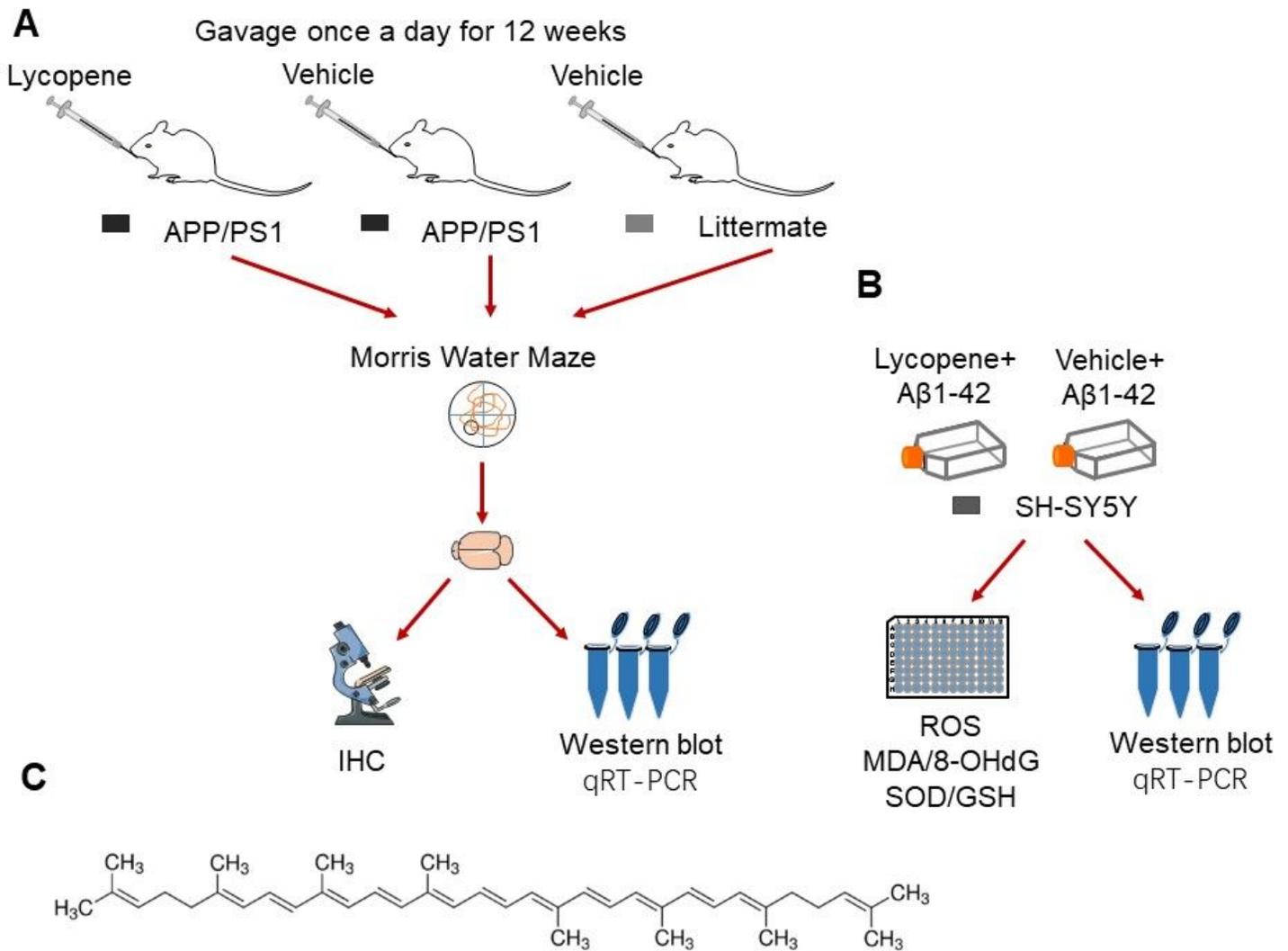


Figure 9

Experimental schematic protocol (A) Five-month-old APP/PS1 mice or age-matched WT littermates received lycopene treatment. The animals were orally gavaged with lycopene or corn oil once a day. After 12-weeks of treatment, memory and spatial learning abilities of the mice were assessed using the MWM. Both the cortex and hippocampus were dissected for further analysis. (B) SH-SY5Y cells were either exposed to lycopene or not for 2h before exposure to Aβ1-42 for 24h. (C) Chemical structure of lycopene.