Diammonium Glycyrrhizinate Attenuates A β _{1–42}-Induced Neuroinflammation and Regulates MAPK and NF- κ B Pathways *In Vitro* and *In Vivo*

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Keywords

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SUMMARY

Background and purpose: Beta-amyloid (A β)-mediated inflammation contributes to the progression and chronicity of Alzheimer's disease (AD), although the exact mechanism remains unclear. This study aimed to investigate whether diammonium glycyrrhizinate (DG) could inhibit A β -induced inflammation *in vitro* and *in vivo* and to explore the underlying mechanisms. **Methods:** A β _{1–42} was injected to bilateral hippocampus of mice to make the AD models *in vivo*. The levels of mRNA and protein of inflammatory cytokines were measured by real-time PCR and Western blotting, respectively. The viability of SH-SY5Y and HT-22 cells was determined by MTT. NF- κ B p65 translocation was analyzed by Western blotting and immunostaining. Phosphorylation of ERK, p38, and JNK was tested by Western blotting. **Results:** DG suppressed A β _{1–42}-induced activation of microglia and inflammation *in vitro* and *in vivo*. The media from A β _{1–42}-activated microglia decreased the viability of SH-SY5Y and HT-22 cells, but it was rescued when pretreated with DG. DG could inhibit the activation of MAPK and NF- κ B signaling pathways and attenuate the memory deficits in A β _{1–42}-induced AD mice. **Conclusions:** DG protects A β _{1–42}-induced AD models *in vitro* and *in vivo* through reducing activation of microglia and inflammation, which may be involved in MAPK and NF- κ B pathways.

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Introduction

Alzheimer's disease (AD), an age-related disorder with progressive cognitive decline and dementia, afflicts estimated 30 million people worldwide. AD is pathologically characterized by the senile plaques mainly composed of beta-amyloid (A β) and neurofibrillary tangles (NFTs) [1]. An increasing number of basic and clinical studies demonstrate that A β -mediated inflammation is a key pathological hallmark of AD, which may contribute to disease progression and chronicity [2,3]. In addition, epidemiological studies show that treatment with nonsteroidal antiinflammatory drugs (NSAIDs) could decrease the risk for developing AD, although more clinical evidences are needed [4].

Inflammatory components related to AD neuroinflammation include brain cells such as microglia, astrocytes, and cytokines. Once activated by A β , microglia and astrocytes release inflammatory factors such as interleukin-1 (IL-1), IL-6, inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumor necrosis factor- α (TNF- α), which results in neuron death [3]. Mitogen-activated protein kinase (MAPK) family includes three subtypes: p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK). Reports have indicated that MAPK signaling pathways are excessively activated in AD. ERK and/or p38 inhibitors could reduce the neurotoxicity by A β [5]. The NF- κ B pathway is also involved in the brain proinflammatory process of AD. Proinflammatory mediators TNF- α and IL-1 β , which are regu-

lated by NF- κ B, are increased in the brains of AD patients [6,7], and the activation of NF- κ B signaling pathway also occurs in A β -induced neuron death [7]. Suppression of NF- κ B pathway in microglia can ameliorate neurotoxicity [8]. COX-2, mainly regulated by NF- κ B, is notably upregulated in the brains of AD patients, which may be associated with the formation of A β plaque [9]. These results suggest that suppression of neuroinflammatory molecules could be developed as a therapeutic method of AD. However, no drugs have been clinically proven effective in the treatment of neuroinflammation of AD.

Diammonium glycyrrhizinate (DG), the salt form of glycyrrhizin acid (GA), has more stable, more significant bioactivities than GA. GA has a series of biological functions including antitumor, antiviral, and antiinflammatory effects [10–13]. DG was used for treatment of hepatitis in Asian countries mainly due to its antiinflammatory and antioxidative effects [14,15]. In addition, DG decreases the NF- κ B pathway and inflammatory injury in a rat model of ulcerative colitis induced by acetic acid [16]. Previous studies suggest that DG could improve focal cerebral ischemic–reperfusion injury in mice due to its antiinflammatory effect [17]. In this study, evidence was provided that DG played a critical role in suppressing A β _{1–42}-induced inflammation through regulating MAPK and NF- κ B pathways, suggesting that DG might be developed into a potential drug for treatment of inflammation-related neurodegeneration diseases, including AD.

Materials and Methods

A β _{1–42}-mediated AD Mice Model

A β _{1–42} (Millipore, CA, USA) was dissolved in 1% NH₃·H₂O at a concentration of 100 μ M and incubated at 37°C for 5 days to allow for fibril formation. DG was purchased from Jiangsu Chia-Tai Tianqing Pharmacy Company (Lianyungang, China). As described previously [18], A β _{1–42} (4 μ g) was injected to the bilateral hippocampus (anterior–posterior position -2.0 mm, medial–lateral position ± 1.6 mm, dorsoventral -1.5 mm from Bregma) of male ICR mice by infusion cannulae once. DG (10 mg/kg per day, i.p.) was injected intraperitoneally for 14 days. Control mice were injected with saline. Mice were divided into four groups, that is, A β _{1–42}-mediated AD mice with or without DG, and control mice with or without DG. All animal experiments were approved by the Animal Care Committee of Nanjing University and performed according to institutional guidelines. Every effort was made to minimize the number of mice used and their suffering.

Cell Culture

BV-2 (a murine microglia cell line) and SH-SY5Y (a neuroblastoma cell line) cells were obtained from American Type Culture Collection (ATCC). HT-22, a murine hippocampus neural cell line, was a kind gift of Dr. Jian Chen (Suzhou University, China). BV-2, SH-SY5Y, and HT-22 cells were maintained in DMEM containing 10% heat-inactivated fetal bovine serum (FBS, Gibco), 2 mM of L-glutamine, 100 U/mL of penicillin, and 100 μ g/mL of streptomycin at 37°C in a humidified 5% CO₂ incubator.

Real-time PCR

Real-time PCR was performed as described previously [18]. Briefly, total RNA was extracted by using the Trizol reagent (Takara, Dalian, China) and was reverse-transcribed into cDNA using a PrimeScript RT reagent kit (Takara) for Quantitative PCR (ABI 7500, Foster City, CA, USA) in the presence of a fluorescent dye (SYBR Green I; Takara). The primers are as follows:

iNOS: Forward, CAGCTGGGCTGTACAAACCTT, Reverse, CATTGGAAGTGAAGCGTTTCG;
COX-2: Forward, GATGACTGCCCAACTCCC, Reverse, AACCCAGGTCCTCGCTTA;
IL-1 β : Forward, AAGCCTCGTGCTGCGGACC, Reverse, TGAGGCCCAAGGCCACAGGT;
TNF- α : Forward, CAAGGGACAAGGCTGCCCG, Reverse, GCAGGGGCTCTTGACGGCAG;
IL-10: Forward, GGCATGAGGATCAGCAGGGGC, Reverse, TGGCTGAAGGCAGTCCGCAG;
GAPDH: Forward, GCCAAGGCTGTGGGCAAGGT, Reverse, TCTCCAGGCGGCACGTCAGA.

Western Blotting

Equal amounts of protein were separated by SDS-PAGE, electrophoretically transferred onto polyvinylidene fluoride membranes, and incubated overnight at 4°C with anti-COX-2 (1: 500, Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-iNOS (1: 1000, Cell Signaling, Danvers, MA, USA), anti-p-ERK (1: 1000, Cell Signaling), anti-ERK (1: 1000, Cell Signaling), anti-p-JNK (1: 1000, Cell Signaling), anti-JNK (1: 1000, Cell Signaling), anti-p-p38 (1: 1000, Cell Signaling), anti-p38 (1: 1000, Cell Signaling), anti-p65 (1: 500, Santa Cruz Biotechnology), and anti- β -actin (1: 5000, Bioworld, Louis Park, MN, USA) antibodies. Subsequently, the membranes were incubated with the corresponding secondary antibodies and visualized with chemiluminescence reagents provided with an ECL kit (Bioworld).

MTT Assay

BV-2 microglial cells were treated with A β _{1–42} (5 μ M) for 6 and 24 h in the presence and absence of DG (0.001 mg/mL). The culture media of BV-2 cells were collected as conditioned media. SH-SY5Y and HT-22 cells were treated with different groups of conditional media for 6 and 24 h, and cell viabilities were measured by MTT assay as described previously [18].

Nuclear Protein Extraction

The nuclear protein was extracted using the Nuclear and Cytoplasmic Protein Extraction Kit (Beyotime, Nantong, China) following the manufacturer's instruction. Hippocampal tissue was homogenized and resuspended in the cytoplasmic protein extraction reagent, followed by centrifugation at 14,000 *g* for 10 min at 4°C. The supernatant was the cytoplasmic protein, and the pellet was resuspended in the nuclear protein extraction reagent followed by centrifugation at 14,000 *g* for 15 min at 4°C. The supernatant was the nuclear protein. The cytoplasmic and nuclear protein was then subjected to Western blotting.

Immunostaining

BV-2 cells were seeded on cover slips after treatment cells were fixed in 4% paraformaldehyde for 20 min at room temperature. The cells were incubated overnight with anti-p65 (1: 100, Santa Cruz) at 4°C and then incubated with the secondary antibody (1: 200, Invitrogen, Carlsbad, CA, USA) for 45 min at room temperature. Fluorescent images were taken using a fluorescent microscope (Olympus, Japan).

Mice were anesthetized and transcardially perfused with 0.9% saline followed by 4% paraformaldehyde (PFA). The brains were cut into consecutive frozen sections and incubated overnight with antigenic fibrillary acidic protein (GFAP) (1: 100, BD) and anti-ionized calcium-binding adaptor molecule 1 (Iba1) (1: 400, Abcam, Cambridge, MA, USA) at 4°C and then incubated with the secondary antibody (1: 200, Invitrogen) for 45 min at room temperature. The immunoreactive cells to GFAP and Iba1 in about 100 areas of 100 $\times$ 100 μ m in 5 slices per brain were counted. All counting procedures were made by an expert in morphology blinded to the experiment.

Morris Water Maze Test

The Morris water maze test was performed as described previously [18]. Briefly, four training trials per day were conducted for four

consecutive days. In each trial, the latency to escape onto the platform was recorded up to 60 s. If the mouse found the platform, it was allowed to remain on the platform for 5 s, and then returned to the home cage. If the mouse was unable to find the platform within 60 s, it was gently guided to the platform and allowed to remain on the platform for 10 s and the latency was recorded for 60 s. On the fifth day, the probe trial was conducted for memory retention by removing the platform and each mouse was allowed to swim freely for 60 s. The time spent in each quadrant was recorded and analyzed. All data were recorded with a computerized video system.

Statistical Analysis

The data were expressed as means $\pm$ SEM and analyzed by SPSS 12.0 statistical analytical software (SPSS, Chicago, IL, USA). Group differences in the escape latency and searching distance during the MWM test were analyzed using two-way analysis of variance (ANOVA) with repeated measures followed by Bonferroni multiple comparison test with day and treatment as the sources of variation. Otherwise, statistical analysis among groups was performed with one-way ANOVA followed by Bonferroni's *post hoc* test. Values of $P < 0.05$ were considered statistically significant.

Figure 1 Diammonium glycyrrhizinate (DG) suppresses the inflammatory response in A β ₁₋₄₂-treated BV-2 cells. BV-2 cells were pretreated with DG (0.001 mg/mL) for 1 h and A β ₁₋₄₂ (5 μ M) for 3, 6, and 24 h. The mRNA levels of TNF- α (A), COX-2 (B), iNOS (C), IL-1 β (D), and IL-10 (E) were determined by real-time PCR. (F) The protein levels of COX-2 and iNOS were determined by Western blotting. The relative mRNA and protein levels of BV-2 cells were set as 1. *: $P < 0.05$ versus BV-2 cells; **: $P < 0.01$ versus BV-2 cells; #: $P < 0.05$ versus A β ₁₋₄₂-treated BV-2 cells; ##: $P < 0.01$ versus A β ₁₋₄₂-treated BV-2 cells.

Figure 2 Diammonium glycyrrhizinate (DG) reduces the inflammatory factors in A β_{1-42} -induced AD mice. The mRNA levels of TNF- α (A), COX-2 (B), iNOS (C), IL-1 β (D), and IL-10 (E) were determined by real-time PCR. (F) The protein levels of COX-2 and iNOS were determined by Western blotting. The relative mRNA and protein levels of normal mice were set as 1. Con: normal mice; DG: normal mice with DG (10 mg/kg per day, i.p. for 14 days); A β : A β_{1-42} -induced AD mice; A β +DG: A β_{1-42} -induced AD mice with DG (10 mg/kg per day, i.p. for 14 days). n = 4 mice per group. *: $P < 0.05$ versus Control; **: $P < 0.01$ versus Control; #: $P < 0.05$ versus A β_{1-42} -induced AD mice; ##: $P < 0.01$ versus A β_{1-42} -induced AD mice.

Results

DG Inhibits the Inflammatory Response in A β_{1-42} -treated BV-2 Cells

To investigate the effect of DG on the inflammatory response induced by A β_{1-42} *in vitro*, BV-2 cells were pretreated with DG (0.001 mg/mL) for 1 h and then with A β_{1-42} (5 μ M) for 3, 6, 12, and 24 h. As shown in Figure 1A–C, and 1D, the mRNA level of TNF- α , COX-2, iNOS, and IL-1 β was significantly increased at 3, 6, 12, and 24 h after A β_{1-42} treatment ($P < 0.01$), while it was greatly decreased in the presence of DG. In addition, A β_{1-42} treatment could significantly downregulate the expression of anti-inflammatory factor IL-10 ($P < 0.01$), while DG treatment could significantly increase the expression of IL-10 at 6 h ($P < 0.01$, Figure 1E). DG also attenuated the protein expression of COX-2 ($96.92 \pm 4.85\%$ at 3 h, $72.73 \pm 4.31\%$ at 6 h, and $76.78 \pm 6.16\%$ at 24 h) and iNOS ($63.34 \pm 2.77\%$ at 3 h, $56.82 \pm 3.08\%$ at 6 h, and $82.32 \pm 6.58\%$ at 24 h) in A β_{1-42} -treated BV-2 cells (Figure 1F).

DG Inhibits the Inflammatory Factors in A β_{1-42} -induced AD Mice

To further confirm inhibitory inflammation of DG *in vivo*, the relative mRNA levels of COX-2, iNOS, TNF- α , IL-1 β , and IL-10 and the relative protein levels of COX-2 and iNOS in the hippocampus of mice were determined. As shown in Figure 2A, the mRNA level

of COX-2 was increased 1.49 ± 0.07 -fold in A β_{1-42} -induced AD mice, while DG could significantly decrease the mRNA level of COX-2 ($77.43 \pm 3.07\%$, $P < 0.01$). Meanwhile, DG significantly decreased the mRNA level of TNF- α , iNOS, and IL-1 β ($66.43 \pm 4.44\%$, $24.05 \pm 5.93\%$, and $58.79 \pm 5.90\%$, $P < 0.01$, Figure 2B–D). In addition, the mRNA level of IL-10 was increased by 1.53 ± 0.07 -fold in A β_{1-42} -induced AD mice treated with DG (Figure 2E). The protein levels of COX-2 and iNOS in the hippocampus of AD mice were also attenuated after the treatment of DG ($52.89 \pm 4.15\%$ and $56.75 \pm 6.36\%$, Figure 2F).

DG Ameliorates the Microglia Activation and Attenuates Memory Deficits in A β_{1-42} -mediated AD Mice

Next, to reveal whether the inflammation was due to the activation of microglia and astrocyte by A β_{1-42} , Iba-1 and GFAP staining was performed. Iba-1 has specific affinity for microglial Ca²⁺-binding proteins, which are highly expressed in activated microglia, and GFAP is a marker of astrocyte activation. The results indicated that Iba-1 expression in the hippocampus of AD mice was significantly increased compared with that in control mice ($P < 0.01$), whereas it was significantly reduced to $57.14 \pm 7.41\%$ after the treatment of DG ($P < 0.01$). Likewise, the magnitude of astrocytosis was also significantly reduced to $52.17 \pm 4.55\%$ in the hippocampus of DG-treated AD mice ($P < 0.01$, Figure 3A).

Figure 3 Diammonium glycyrrhizate (DG) ameliorates the microglia activation and attenuates memory deficits in A β_{1-42} -induced AD mice. **(A)** Immunostaining for Iba-1 and GFAP in the hippocampus of normal mice and A β_{1-42} -induced AD mice treated with or without DG (10 mg/kg per day, i.p. for 14 days). $n = 3$ mice per group. **: $P < 0.01$ versus Control; #: $P < 0.01$ versus A β_{1-42} -induced AD mice. **(B)** Escape latency for escape to a submerged platform in the training trials. **(C)** Time spent in the target quadrant during the probe trial. **: $P < 0.01$ compared to control mice, #: $P < 0.05$ compared to A β_{1-42} -induced AD mice. $n = 10$ mice per group. Con: normal mice; DG: normal mice with DG (10 mg/kg per day, i.p. for 14 days); A β : A β_{1-42} -induced AD mice; A β +DG: A β_{1-42} -induced AD mice with DG (10 mg/kg per day, i.p. for 14 days).

To confirm that DG could attenuate memory impairment in A β_{1-42} -induced AD mice, Morris water maze test was performed. As shown in Figure 3B, the mean escape latency of AD mice was significantly increased compared with control mice ($P < 0.01$), while DG-treated AD mice showed significant improvements compared with AD mice after the training periods ($P < 0.05$). During the probe trial, A β_{1-42} -induced AD mice spent significantly less time in the target quadrant than control mice and DG treatment could reverse such effect (Figure 3C). These results demonstrated that DG treatment could ameliorate microglia activation and attenuate memory deficits in A β_{1-42} -induced AD mice.

Neuroprotective Effects of DG against Neuronal Cell Death by A β_{1-42} -activated Microglia in Conditioned Media System

To validate whether inhibition of the inflammatory response by DG in microglia could protect the neurons, conditional media of A β_{1-42} -treated BV-2 cells with or without DG were collected to treat HT22 and SH-SY5Y cells. As shown in Figure 4A, treatment of conditional media of A β_{1-42} -treated BV-2 cells without DG decreased cell viability of HT-22 cells to $41.18 \pm 2.76\%$ and $31.40 \pm 2.05\%$ at 6 and 24 h. However, cell viability, when treated with conditional media with DG, was markedly increased by

1.05 ± 0.11 -fold and 1.58 ± 0.10 -fold at 6 and 24 h. Similarly, results appeared in SH-SY5Y cells (Figure 4B), suggesting that neuroprotection of DG against A β_{1-42} -mediated toxicity may be partially related to inhibition of activated microglia.

DG Inhibits A β_{1-42} -induced Activation of NF- κ B p65 and MAPK Signaling Pathways *In Vivo* and *In Vitro*

To elucidate the mechanism of DG in suppression of inflammation by A β_{1-42} , NF- κ B p65 and MAPK signaling pathways, which contributed to the microglial activation and inflammation, were studied. As shown in Figure 5A,B, there was a low basal level of p65 in the nuclei of normal mice and A β_{1-42} -induced a translocation of p65 from the cytoplasm to the nucleus, while DG significantly attenuated the p65 translocation. These results were also confirmed by immunostaining of p65 in BV-2 cells (Figure 5C), which indicated that DG could attenuate A β_{1-42} -induced NF- κ B nuclear translocation.

The activation of MAPK pathways in the AD model was also tested. As shown in Figure 6, A β_{1-42} -activated phosphorylation of ERK, JNK, and p38 in the hippocampus of A β_{1-42} -induced AD mice, while DG significantly decreased the phosphorylation levels.

Figure 4 Neuroprotective effects of diammonium glycyrrhizinate (DG) against neuronal cell death by A β -activated microglia in conditioned media system. Conditional media of A β_{1-42} -treated BV-2 cells with or without DG were collected to treat HT22 and SH-SY5Y cells for 6 and 24 h. (A) Cell viability of SH-SY5Y cells was determined by MTT. (B) Cell viability of HT-22 cells was determined by MTT. *: $P < 0.05$ versus conditional media of A β_{1-42} -treated BV-2 cells, **: $P < 0.01$ versus conditional media of A β_{1-42} -treated BV-2 cells.

Figure 5 Diammonium glycyrrhizinate (DG) inhibits A β_{1-42} -induced translocation of NF- κ B p65 *in vivo* and *in vitro*. The expression of NF- κ B p65 in the nucleus (A) and cytoplasm (B) of hippocampus of normal mice and A β_{1-42} -induced AD mice treated with or without DG (10 mg/kg per day, i.p. for 14 days) was determined by Western blotting. $n = 4$ mice per group. **: $P < 0.01$ versus Control; #: $P < 0.05$ versus A β_{1-42} -induced AD mice. (C) BV-2 cells were pretreated with DG (0.001 mg/mL) for 1 h and A β_{1-42} (5 μ M) for 6 h and immunostaining for NF- κ B p65. Scale bar = 50 μ m.

Discussion

This study showed, for the first time, that DG could protect against A β_{1-42} -induced inflammation response *in vitro* and *in vivo*, and the underlying mechanism of this neuroprotection may be involved of downregulation of MAPK and NF- κ B pathways.

A β is the major component of senile plaques, which contribute to the pathogenesis of AD. Some studies have shown that there is an association between higher inflammatory levels and lower cognitive levels or higher risk of cognitive impairment

over time in populations of older adults living in the community. Specific circulating inflammatory markers have been associated with declines in cognitive function and worsening of brain structure [19]. A β could activate microglia and astrocytes, which produce a wide spectrum of proinflammatory factors such as IL-1 β , TNF- α , iNOS, COX-2, and so forth [8,19]. Current study indicated that A β_{1-42} did activate microglia, and the mRNA level of COX-2, iNOS, TNF- α , and IL-1 β was increased *in vivo* and *in vitro*. DG could reduce the release of A β_{1-42} -induced proinflammatory factors and suppress the activation of

Figure 6 DG inhibits A β_{1-42} -induced activation MAPK signaling pathways *in vivo*. (A) The expression of JNK, ERK and p38 MAPK signaling of hippocampus of normal mice and A β_{1-42} -induced AD mice treated with or without DG (10 mg/kg per day, i.p. for 14 days) was determined by western blotting. Quantitative analysis of the relative protein levels of p-JNK (B), p-ERK (C) and p-p38 (D) of (A). $n = 4$ mice per group. ** $P < 0.01$ versus control; ## $P < 0.01$ versus A β_{1-42} -induced AD mice.

microglia. Of great interest were the results showing that media from A β_{1-42} -treated BV-2 cells could decrease the cell viability of HT22 and SH-SY5Y cells. However, cell viability of these two cells was significantly increased after adding DG. These findings indicated that DG indeed protected neurons from activated microglia induced by A β_{1-42} . Given the fact that DG could directly protect the primary cortical neurons against A β_{1-42} -induced oxidative stress [18], it was likely that DG exerted protective effects by modulating the glial cells and neurons simultaneously. Furthermore, this study demonstrated that DG could ameliorate memory deficits partially through inhibiting brain inflammation in A β_{1-42} -mediated AD mouse models.

However, the mechanism on A β -induced neuroinflammation has not been fully elucidated. Researches have shown that NF- κ B pathway is activated, and both increased proinflammatory cytokines and activated glial cells are frequently around A β plaque in the brains of AD patients [20,21]. NF- κ B pathway has been regarded as one of the key mediators of aging and mediates immune and inflammatory responses. Without stimuli, NF- κ B is retained in the cytoplasm by binding to I κ B inhibitors. When activated, I κ B is phosphorylated by I κ B kinases and then degraded by the proteasome, which allows the translocation of p65/p50 from the cytoplasm to the nuclei [22]. The SIRT1 agonist resveratrol exerts neuroprotective effects by markedly suppression of NF- κ B signaling in microglia and decreasing of A β toxicity [9]. NF- κ B pathway also participates in the formation of amyloid- β precursor (APP) protein [22]. Moreover, NF- κ B can regulate the expression of COX-2 and control the production of proinflammatory mediators such as TNF- α and IL-1 β . In this study, our results demonstrated that DG significantly attenuated the NF- κ B p65 translocation induced by A β_{1-42} *in vivo* and *in vitro*.

In addition, MAPK pathways are associated with A β -induced inflammation. Aromatic turmerone protects the hippocampus from indirect neuronal toxicity induced by A β -stimulated microglia through inhibiting NF- κ B, JNK, and p38 MAPK signaling pathways [23]. Another study suggests that A β_{1-42} -induced acti-

vation of MEK/ERK pathway leads to the release of inflammatory factors such as IL-1 β and TNF- α , which might be toxic to neuroblastoma SK-N-SH cells and lead to the onset of AD [24]. In A β -stimulated SH-SY5Y cells, Xylocoside G exerts neuroprotection by inhibition of JNK and NF- κ B signaling and reduction of A β -induced inflammation and apoptosis [25]. This study demonstrated that DG significantly decreased the phosphorylation of ERK, JNK, and p38 in the hippocampus of A β_{1-42} -induced AD mice, which was also supported by reports that ERK and p38 inhibitors attenuated memory deficit in A β -treated rats [26], and inhibition of JNK phosphorylation reversed memory deficit in A β -mediated rats [27].

In conclusion, the present study demonstrates that administration of DG provides neuroprotection against A β -induced inflammation *in vitro* and *in vivo*, and the mechanisms may be related with downregulation of MAPK and NF- κ B pathways. The findings contribute to the understanding of pathological effects of A β -activated microglia in the inflammatory process of AD brain and point to an unidentified role of DG as a neuroprotective agent for AD.

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Conflict of Interest

The authors declare no conflict of interest.

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