JEBHM Journal of Evidence-Based Herbal Medicine

Article type: Research Article

The effect of genkwanin on amyloid β aggregation

Eunbi Cho[1], Dong Hyun Kim[2], Hye-Sun Lim[3*], Gunhyuk Park[3,4*]

[1] Department of Neural Development and Disease, Korea Brain Research Institute (KBRI), 61, Cheomdan-ro, Dong-gu 41068, Republic of Korea
[2] Department of Pharmacology, College of Medicine, Konkuk University, 286 Chungju-si 27478, Republic of Korea
[3] Herbal Medicine Resources Research Center, Korea Institute of Oriental Medicine, 111 Geonjae-ro, Naju-si, Jeollanam-do, 58245, Republic of Korea
[4] Korean Convergence Medicine Major, Campus of Korea Institute of Oriental Medicine, University of Science & Technology (UST), Daejeon 34113, Republic of Korea

* Correspondence: gp1015@kiom.re.kr and gpark@kiom.re.kr

Abstract: Objective: This study investigates the potential of genkwanin as a therapeutic agent for Alzheimer's disease (AD), specifically targeting its effects on amyloid β (Aβ) aggregation, dissociation, cytotoxicity, and Aβ-induced memory impairment. **Methods**: A series of *in vitro* and *in vivo* experiments were conducted to assess the effects of genkwanin on Aβ pathology. *In vitro* assays evaluated the ability of genkwanin to inhibit Aβ aggregation and disaggregate pre-formed Aβ fibrils, measured through Thioflavin T (ThT) fluorescence and MTT cytotoxicity assays in Neuro2a cells. *In vivo*, a passive avoidance test was conducted in an Aβ-injected mouse model to determine the effect of genkwanin on memory retention. **Results:** Genkwanin demonstrated a significant concentration-dependent inhibition of Aβ aggregation and effectively reduced the cytotoxicity of Aβ aggregates in Neuro2a cells when co-incubated with Aβ. In contrast, pre-formed Aβ aggregates treated with genkwanin showed disaggregation efficacy and partial cytotoxicity mitigation. In the passive avoidance test, genkwanin-treated mice exhibited significantly improved latency times in test trials, indicating a protective effect against Aβ-induced memory impairment. **Conclusion**: Genkwanin exhibits promising potential as an AD therapeutic by inhibiting Aβ aggregation, reducing Aβ-related cytotoxicity, and mitigating Aβ-induced cognitive decline. These findings support further exploration of genkwanin as a multifunctional small-molecule treatment for AD.

Keywords: Genkwanin, Alzheimer's disease, Amyloid β, Memory.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that primarily affects older adults, leading to a gradual decline in cognitive functions such as memory, reasoning, and problem-solving [1]. A prominent pathological hallmark of AD is the accumulation of Amyloid β (Aβ) peptides in the brain, forming insoluble plaques. These plaques, along with neurofibrillary tangles composed of hyperphosphorylated tau protein, contribute to neuronal death, synaptic dysfunction, and widespread neuroinflammation, which are regarded as primary factors in AD pathology [2]. Due to the critical role of Aβ in AD progression, considerable research has focused on preventing Aβ aggregation, promoting its clearance, and mitigating its neurotoxic effects [3].

Recently, antibody-based therapies targeting Aβ have attracted significant interest in AD treatment development. However, the large molecular structure of antibodies poses challenges in crossing the blood-brain barrier (BBB) and may result in adverse side effects, including systemic immune reactions, cerebral edema, and microhemorrhages [4, 5]. Additionally, antibody-based treatments have high production costs and complex administration requirements, limiting their practicality for widespread clinical application [6]. These challenges underscore the need for alternative approaches, particularly small-molecule drugs, in effective AD therapy. Small molecules can more easily penetrate the BBB, typically have fewer side effects, and are often more cost-effective to produce, offering a more accessible therapeutic option for patients [7].

Flavonoids, a class of polyphenolic compounds derived from plants, have shown potential as neuroprotective agents due to their anti-inflammatory, antioxidant, and anti-apoptotic properties [8, 9]. Among these compounds, genkwanin (apigenin-7-methyl ether), a naturally occurring O-methylated flavonoid, has attracted attention for its wide range of bioactivities, including anti-inflammatory and anticancer effects [6]. Recent studies suggest that genkwanin may exhibit

protective effects against neurodegenerative processes, supporting its potential as a therapeutic candidate for AD [10, 11]. However, the specific effects of genkwanin on AD-related pathologies, such as its ability to inhibit Aβ aggregation, facilitate Aβ clearance, and protect neurons from Aβ-induced toxicity, remain underexplored.

This study aims to investigate the therapeutic potential of genkwanin in AD by examining its effects on multiple aspects of Aβ pathology. Specifically, we assess whether genkwanin can inhibit Aβ aggregation, promote the clearance of aggregated Aβ, and reduce Aβ-induced cytotoxicity in neuro2a cells. Additionally, we evaluate the efficacy of genkwanin in preventing Aβ-induced memory impairment in an ICR mouse model to determine its utility as multifunctional small-molecule therapeutics.

2. Materials and Methods

2.1. Animals

For experiments involving Aβ-induced memory impairment, male ICR mice (6 weeks old, weighing 25–30 g) were obtained from Samtako (Osan, Korea). The mice were acclimated to the laboratory environment for one week prior to beginning the experiments, which started with 7-week-old mice. Before Aβ injection, the 60 mice were randomly assigned to six groups (n = 10 per group): Normal, Aβ + vehicle, Aβ + genkwanin 10 mg/kg, Aβ + genkwanin 30 mg/kg, Aβ + genkwanin 100 mg/kg, and Aβ + curcumin 10 mg/kg. All mice were housed with free access to food and water, under a 12/12 h light/dark cycle. The animals were cared for in accordance with National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978), and all procedures were approved by the Institutional Animal Care and Use Committee of Dong-A University (DIACUC-approve-19–1).

2.2. Materials

Genkwanin, thioflavin T (ThT) and 3-(4,5-dimethylthiazol-2-yl) (MTT) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Aβ1–42 was supplied by Anaspec (Fremont, CA, USA). All other materials were sourced from standard commercial suppliers and were of the highest available purity.

2.3. ThT assay

ThT assay was performed to assess the aggregation of $A\beta_{1-42}$ peptides and evaluate the inhibitory effects of genkwanin on this aggregation and dissociation processes [12]. First, lyophilized Aβ peptides were dissolved in 100% dimethyl sulfoxide (DMSO) to prepare a 5 mM stock solution, which was then aliquoted and stored at -80°C to prevent degradation from repeated freeze-thaw cycles. For the assay, the stock solution was diluted in phosphate-buffered saline (PBS, pH 7.4) to a working concentration of 25 μM and incubated at 37°C for 24 hours to promote aggregation. Freshly aggregated Aβ was prepared before each experiment to maintain consistency. The ThT solution was prepared separately by dissolving ThT powder in PBS to create a 1 mM stock solution, which was then filtered through a 0.22 μm filter to remove any particulates. This ThT stock was further diluted in PBS to a final concentration of 20 μM immediately before the assay to prevent potential photobleaching, which could reduce assay sensitivity. For the experimental setup, ThT solution and pre-aggregated Aβ1–42 solution were mixed in a black 96-well plate (designed to minimize background fluorescence), resulting in a final concentration of 10 μM for Aβ and 20 μM for ThT in each well. Control wells containing only ThT were included to measure baseline fluorescence and confirm ThT specificity in binding to Aβ aggregates. In wells evaluating inhibitory effects, test compounds (e.g., Genkwanin) were added at varying concentrations, with care taken to maintain a consistent final volume across all wells. After plate preparation, it was incubated in the dark at room temperature for 15 minutes to allow ThT to fully interact with Aβ aggregates. Fluorescence was then measured using a microplate reader set to an excitation wavelength of 440 nm and an emission wavelength of 485 nm, as ThT fluorescence increases upon binding to β-sheet-rich structures in Aβ fibrils. Each measurement was performed in triplicate to ensure reliability.

To assess the dissociation effect of genkwanin on pre-formed Aβ aggregates, a two-step incubation procedure followed by a Thioflavin T (ThT) assay was performed. Initially, Aβ peptides were dissolved in 100% dimethyl sulfoxide (DMSO) to prepare a stock solution, which was then diluted to a working concentration of 25 μM in phosphate-buffered saline

(PBS, pH 7.4). This Aβ solution was incubated at 37°C for 24 hours to promote aggregation. After the initial 24-hour aggregation period, genkwanin was added to the pre-formed Aβ aggregates at varying concentrations (e.g., 10, 30, and 100 μM), and the mixture was incubated for an additional 24 hours at 37°C.

2.4. MTT assay with neuro2a cell

The MTT assay was utilized to measure cell viability in Neuro2a cells treated with Aβ aggregates and to examine the protective effect of genkwanin on cellular survival [12]. Neuro2a cells, a murine neuroblastoma cell line, were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained in a humidified environment at 37°C with 5% CO_2. For the experiment, cells were seeded in 96-well plates at a density of 1 × 10^4 cells per well and allowed to attach overnight. The next day, the culture medium was exchanged with fresh DMEM containing Aβ aggregates (Aβ + genkwanin co-incubation for anti-aggregation, Aβ aggregates + genkwanin co-incubation for dissociation) at a concentration identified in preliminary studies to induce cellular stress without excessive mortality. After 24 hours of incubation with Aβ and/or test compounds, the medium in each well was replaced with 100 μL of DMEM containing 0.5 mg/mL of MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The plate was incubated at 37°C for an additional 4 hours to allow viable cells to convert MTT into purple formazan crystals. Following this, the medium was carefully removed, and 100 μL of DMSO was added to each well to dissolve the formazan, resulting in a uniform purple solution. Absorbance was measured at 570 nm using a microplate reader, with readings at 630 nm subtracted as background correction. All samples were measured in triplicate.

2.5. Intracerebroventricular injection of Aβ aggregates

The administration of Aβ aggregates into the third ventricle of ICR mice was conducted to investigate the effects of Aβ on memory and cognitive function. Before surgery, mice were anesthetized using an intraperitoneal injection of a ketamine-xylazine mixture (ketamine: 100 mg/kg, xylazine: 10 mg/kg) to ensure deep anesthesia. Each animal was then placed on a stereotaxic apparatus, and the head was secured to maintain a flat skull position, with the bregma and lambda aligned horizontally. Betadine and 70% ethanol were applied to the scalp for antisepsis, and a small incision was made along the midline to expose the skull. The third ventricle was targeted based on stereotaxic coordinates relative to the bregma: anterior-posterior (AP) = -1.8 mm, medial-lateral (ML) = 0 mm, and dorsal-ventral (DV) = -5.0 mm. A small hole was drilled at the designated point, taking care to avoid excessive damage to the surrounding tissue. Aβ peptide, previously prepared in sterile artificial cerebrospinal fluid (aCSF) and aggregated by incubation at 37°C for 24 hours, was injected at a volume of 3 μL with a Hamilton microsyringe. The injection was performed slowly over a 5-minute period to prevent backflow and minimize tissue damage. Following injection, the needle was left in place for an additional 2 minutes to allow diffusion of the solution. After injection, the needle was carefully withdrawn, and the scalp was sutured. Mice were given analgesic treatment (e.g., meloxicam, 2 mg/kg, subcutaneously) to minimize post-surgical pain and monitored until full recovery. The animals were returned to their home cages and closely observed for signs of distress or infection.To verify the placement of injections, a subset of mice was injected with trypan blue dye and examined post-mortem to confirm the accuracy of the third ventricle targeting. 1 h after the injection genkwanin was administered genkwanin (10, 30 or 100 mg/kg) was administered orally once daily for 6 days, beginning 1 hour after the Aβ injection.

2.6. Passive avoidance test

The passive avoidance test was conducted to assess memory retention and cognitive function in ICR mice following Aβ administration. This test evaluates the animal's ability to remember and avoid an aversive stimulus in a specific context. A two-chamber passive avoidance apparatus was used, consisting of a brightly lit compartment and a dark compartment, separated by a guillotine door. On the first day (training phase), each mouse was placed individually in the brightly lit compartment. After a 30-second acclimatization period, the door to the dark compartment was opened. Due to their natural preference for dark spaces, the mice typically moved into the dark compartment within a few seconds. Once the animal fully entered the dark compartment (defined by all four paws crossing the threshold), the door was closed, and an electric foot shock (0.5 mA, 2 seconds) was administered through the grid floor. The animal was then removed from the apparatus and returned to its home cage. The retention test was conducted 24 hours after the training session. Each mouse was again placed in the brightly lit compartment, and after a 30-second delay, the door to the dark compartment was opened. The latency time to enter the dark compartment was recorded, with a maximum cutoff time of 300 seconds.

Mice that remembered the aversive stimulus from the training session typically delayed or avoided entering the dark compartment.

2.7. Statistics

All experimental data were analyzed using one-way analysis of variance (ANOVA) to determine the statistical significance of differences among groups. Post hoc tests were conducted using Tukey's multiple comparison test to identify specific group differences where ANOVA indicated significance. Data are presented as mean ± SEM, and a p-value of less than 0.05 ($p < 0.05$) was considered statistically significant. Statistical analyses were performed using GraphPad Prism (version 9.5.1) software. All experiments were conducted in triplicate to ensure data reliability and reproducibility.

3. Results

3.1. The effect of genkwanin on Aβ aggregation and Aβ aggregates dissociation

The effects of genkwanin on Aβ aggregation and Aβ aggregates dissociation were evaluated across various concentrations (0, 1, 3, 10, 30, and 100 μM), demonstrating that genkwanin significantly inhibited Aβ aggregation at higher concentrations. Specifically, at 30 μM and 100 μM, genkwanin led to a notable reduction in Aβ aggregation, while at 100 μM, it also exhibited dissociation effects on preformed Aβ aggregates. The positive control, curcumin, displayed both inhibition of Aβ aggregation and dissociation of Aβ aggregates at a lower concentration of 10 μM, indicating a similar effect at a reduced concentration. These findings suggest that genkwanin is capable of concentration-dependent inhibition and disaggregation of Aβ aggregates, with significant effects observed at higher concentrations.

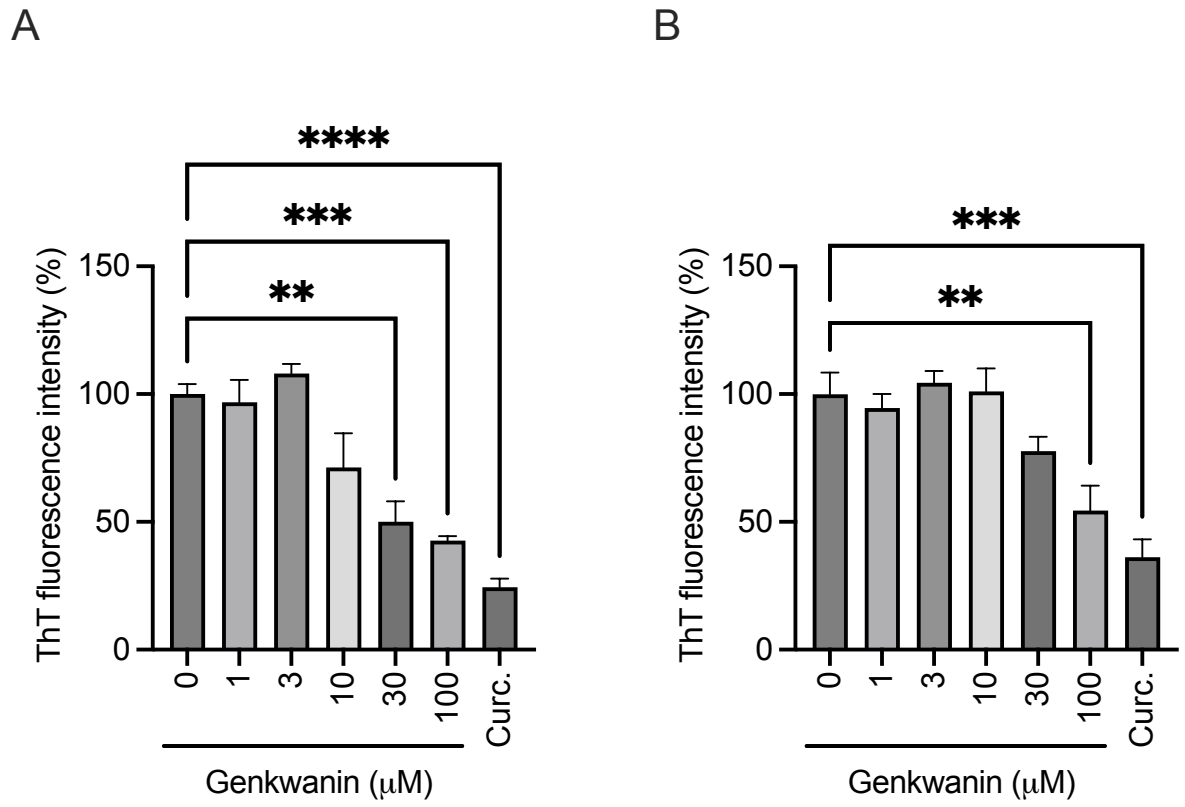

Figure 1. Effects of genkwanin on Aβ aggregation and dissociation. (A) Inhibitory effect of genkwanin on Aβ aggregation. The extent of Aβ aggregation was measured in the presence of varying concentrations of genkwanin (1, 3, 10, 30 or 100 μM), with curcumin (10 μM)as the positive control. Lower fluorescence intensity indicates reduced aggregation. (B) Dissociation effect of genkwanin on pre-formed Aβ aggregates. Genkwanin was added to pre-aggregated Aβ, and the degree of disaggregation was assessed. Curcumin

was included as a positive control. Data represent mean ± SEM for each group, with statistical significance indicated for comparisons between treated and control groups. **$p < 0.01$. ***$p < 0.001$. ****$p < 0.0001$. Curc., curcumin.

3.2. The effect of genkwanin on Aβ-induced neuro2a cell death

To determine whether the Aβ aggregates inhibited or disaggregated by genkwanin were broken down sufficiently to eliminate cytotoxicity, a toxicity assay was conducted using Neuro2a cells. When Aβ (10 μM) was co-aggregated with genkwanin (100 μM) for 24 hours prior to being applied to Neuro2a cells, cell death was reduced compared to Neuro2a cells exposed to Aβ alone that had been pre-aggregated for 24 hours. This suggests a protective effect of genkwanin when simultaneously incubated with Aβ. However, when pre-aggregated Aβ (after 24 hours) was further incubated with genkwanin for an additional 24 hours before being applied to Neuro2a cells, there was no significant reduction in cell death, indicating that genkwanin did not reverse toxicity in pre-formed Aβ aggregates. In contrast, the positive control, curcumin (10 μM), demonstrated protective effects in both conditions, reducing cell death in Neuro2a cells regardless of whether it was co-incubated with Aβ from the beginning or added after Aβ had already aggregated. These findings suggest that genkwanin's protective effects against Aβ-induced cytotoxicity may be limited to early stages of aggregation, whereas curcumin displays broader protective capabilities in both early and late stages of Aβ aggregation.

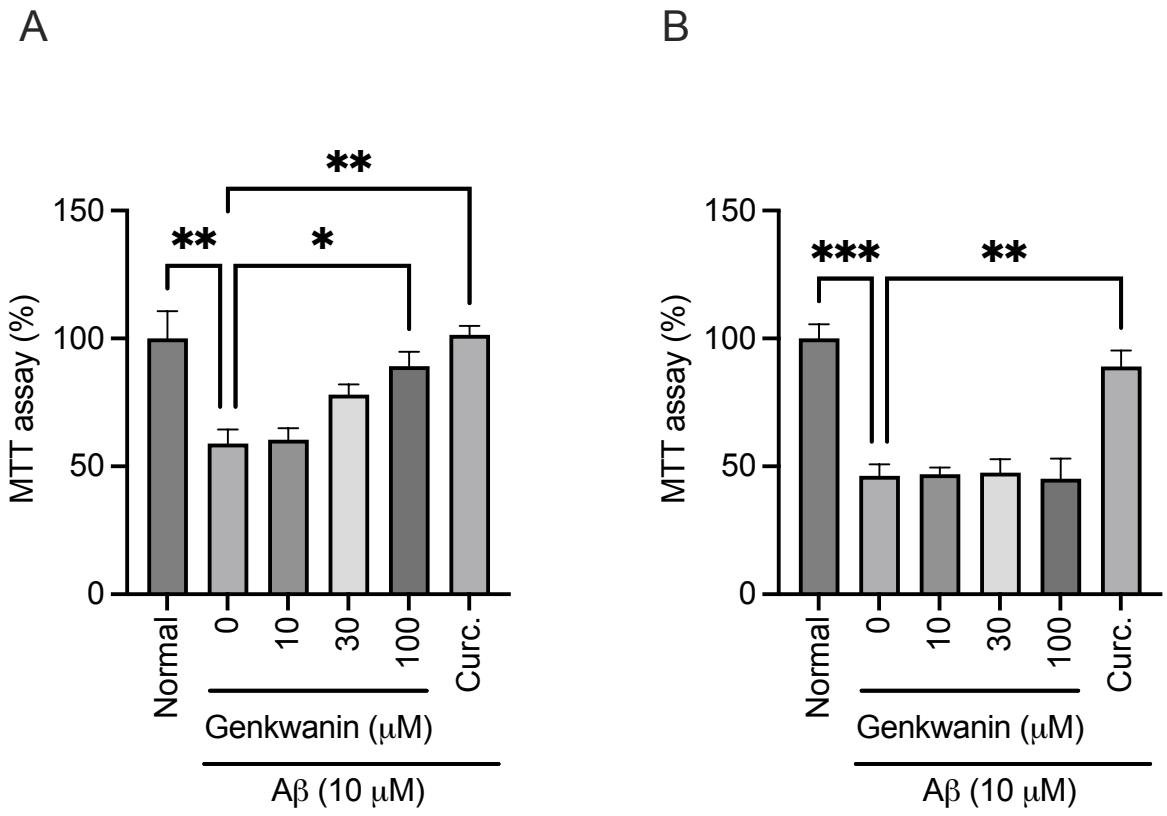

Figure 2. Cytotoxicity of Aβ aggregates treated with genkwanin in Neuro2a cells. (A) Neurotoxicity of Aβ aggregates co-incubated with genkwanin for 24 hours, as assessed by MTT assay in Neuro2a cells. This panel shows the effect of genkwanin on the cytotoxicity of Aβ when aggregated in the presence of the compound. (B) Neurotoxicity of Aβ aggregates that were pre-formed over 24 hours, followed by an additional 24-hour incubation with genkwanin, evaluated by MTT assay in Neuro2a cells. Data represent mean ± SEM for each group, with statistical significance indicated for comparisons between treated and control groups. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$. Curc., curcumin.

3.3. The effect of genkwanin on Aβ-induced memory impairment

Following the administration of Aβ aggregates into the third ventricle of male mice, genkwanin was given orally once daily for 7 days, starting 1 hour post-Aβ injection. In the training phase, there were no significant difference in latency time between groups (Fig. 3A) In the subsequent test trial of passive avoidance test, the Aβ-only group exhibited a significantly lower latency time compared to the control group, indicating impaired memory retention (Fig. 3B). In contrast, the genkwanin (100 mg/kg)-treated group demonstrated a significantly higher latency time, suggesting a protective effect of genkwanin against Aβ-induced cognitive decline (Fig. 3B). Similarly, the positive control group treated with curcumin (10 mg/kg) also showed a significant increase in latency time compared to the Aβ-only group (Fig. 3B). These results suggest that genkwanin, like curcumin, effectively mitigates Aβ-induced memory impairment in mice.

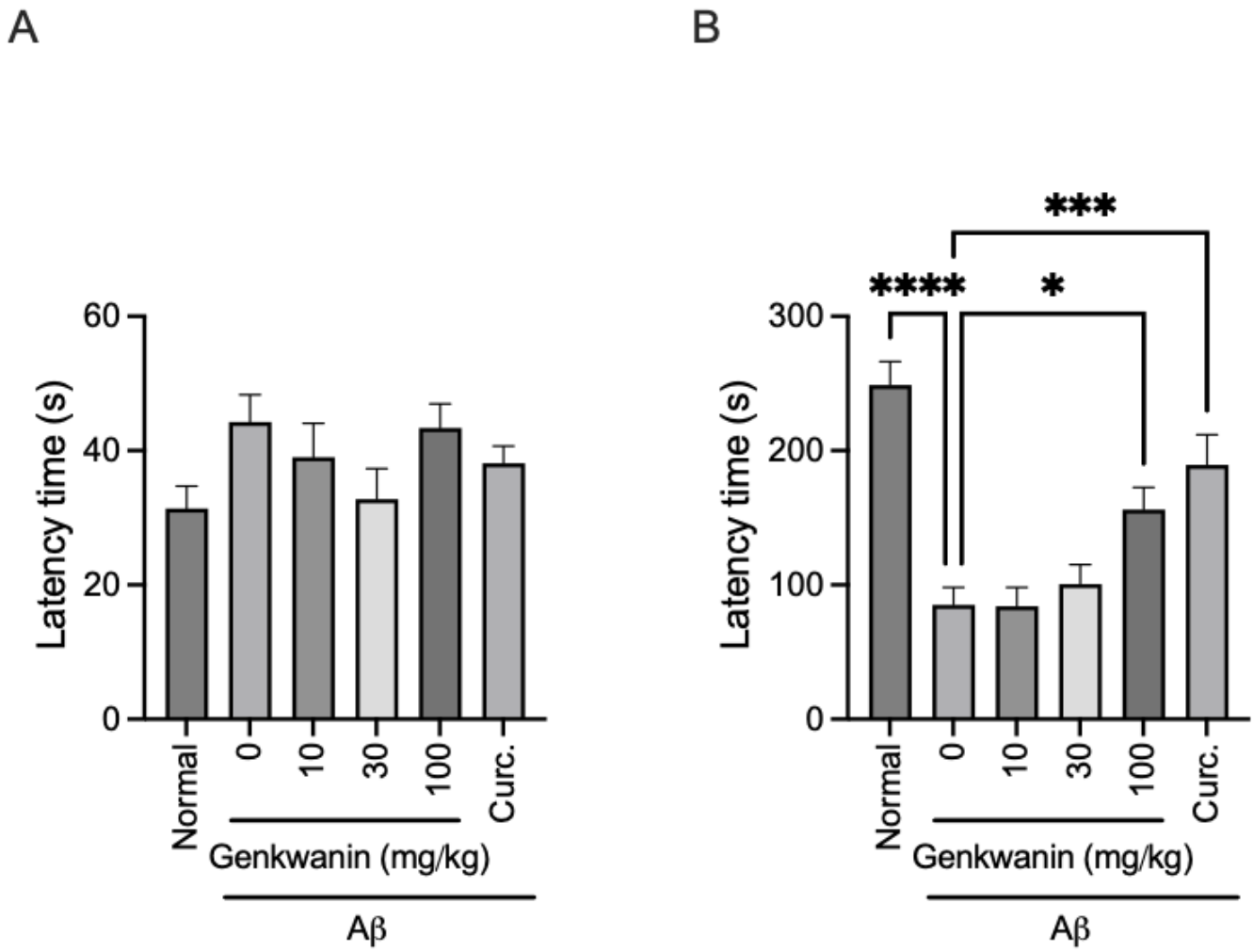

Figure 3. Effect of genkwanin on Aβ-induced memory impairment in the passive avoidance test. (A) Latency time during the training phase. (B) Latency time in the test trial, assessing memory retention post-treatment to evaluate the potential of genkwanin in counteracting Aβ-induced memory impairment. Data represent mean ± SEM for each group, with statistical significance indicated for comparisons between treated and control groups. *$p < 0.05$. ***$p < 0.001$. ****$p < 0.0001$. Curc., curcumin.

4. Discussion

This study examined the effects of genkwanin on Aβ aggregation, Aβ-induced cytotoxicity in Neuro2a cells, and cognitive decline in an Aβ-injected mouse model. The findings suggest that genkwanin holds potential as a therapeutic agent targeting key mechanisms associated with AD pathology.

Genkwanin demonstrated a concentration-dependent inhibition of Aβ aggregation, with significant effects at concentrations of 30 μM and 100 μM, effectively preventing fibril formation. Furthermore, at 100 μM, genkwanin showed a disaggregation effect on pre-formed Aβ fibrils, suggesting its ability to not only interfere with initial aggregation but also destabilize established fibrillar structures [13, 14]. However, curcumin, used as a positive control, displayed similar effects at a lower concentration of 10 μM, indicating that while genkwanin possesses promising anti-amyloidogenic properties, it may require higher concentrations to achieve similar efficacy compared to curcumin [15].

In cell viability assays using Neuro2a cells, genkwanin exhibited a protective effect when co-incubated with Aβ for 24 hours, reducing cell death compared to cells exposed only to Aβ. However, when genkwanin was added to pre-aggregated Aβ (after 24 hours of aggregation), it did not significantly reduce Aβ-induced cytotoxicity. In contrast, curcumin provided significant cellular protection under both conditions, showing a broad protective effect against Aβ toxicity regardless of the aggregation stage [16]. The protective effects of genkwanin against Aβ-induced cytotoxicity were significant when co-incubated during the early stages of Aβ aggregation but not with pre-aggregated Aβ. This suggests that genkwanin may inhibit the formation of toxic Aβ oligomers, reducing early-stage cytotoxicity. Additionally, genkwanin's antioxidant properties likely help mitigate Aβ-induced oxidative stress, although these effects may be insufficient to disrupt mature aggregates. In contrast, curcumin's ability to bind directly to Aβ fibrils and reduce toxicity at both early and late aggregation stages demonstrates its broader protective mechanism [16]. Therefore, genkwanin appears to be more effective in early intervention, while curcumin shows versatility across different aggregation stages. Further studies are needed to clarify the specific molecular interactions involved. These findings indicate that genkwanin's protective effects are more effective during the early stages of Aβ aggregation, whereas curcumin exhibits versatility, providing cellular protection even in the presence of mature Aβ aggregates.

Previous studies have reported possible neuroprotective effects of genkwanin on the brain. For instance, genkwanin has been shown to exhibit anti-inflammatory and antioxidant activity, thereby reducing oxidative stress in brain tissues [10, 17]. The present study's findings align with these previous studies, particularly strengthening evidence for genkwanin's neuroprotective mechanism related to Aβ aggregation inhibition. Genkwanin's ability to mitigate Aβ-induced neurotoxicity and protect cognitive function at specific concentrations provides valuable support for its potential as a candidate in AD treatment and as a drug that could target multiple mechanisms of neurodegeneration.

In behavioral tests, genkwanin demonstrated cognitive protection in Aβ-injected male mice. After administering genkwanin orally once daily for 7 days (beginning 1 hour post-Aβ injection), the passive avoidance test showed that genkwanin-treated mice had significantly longer latency times than the Aβ-only group, suggesting a protective effect on memory retention. This indicates that genkwanin may be effective in alleviating Aβ-induced memory impairment, possibly due to its ability to inhibit Aβ aggregation and provide early-stage cellular protection. Curcumin also significantly increased latency time, confirming its known cognitive protective effects against Aβ toxicity [18, 19]. While curcumin was effective at a lower concentration than genkwanin, both compounds demonstrated meaningful effects in reducing Aβ-induced cognitive decline.

In conclusion, genkwanin shows potential as an anti-amyloid and neuroprotective agent based on both molecular and behavioral outcomes. However, its efficacy in reversing the toxicity of pre-existing Aβ aggregates appears to be limited compared to curcumin, suggesting that higher concentrations or combination therapies may be needed for optimal effectiveness. Future research should explore the optimal administration methods, long-term effects, and more detailed mechanisms of genkwanin to better establish its therapeutic utility for AD.

Author Contributions: E.J. conducted the experiments, organized the data, and prepared the initial manuscript draft. D.H.K. designed the experiments and provided the resources. H.S.L. and G.P. was responsible for experimental design, overall supervision of the experiments, securing funding, and preparing the final manuscript.

Acknowledgments: National Research Foundation of Korea (NRF) grant funded by the Korea government (RS-2024-00352796) and Development of Sustainable Application for Standard Herbal Resources (KSN1822320) from the Korea Institute of Oriental Medicine, Republic of Korea.

Conflicts of Interest: There is no conflict of interest.

References

[1] H.W. Querfurth, F.M. LaFerla, Alzheimer's disease, N Engl J Med 362(4) (2010) 329-44.

[2] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problems on the road to therapeutics, Science 297(5580) (2002) 353-6.

[3] C. Haass, D.J. Selkoe, Soluble protein oligomers in neurodegeneration: lessons from the Alzheimer's amyloid beta-peptide, Nat Rev Mol Cell Biol 8(2) (2007) 101-12.

[4] J. Sevigny, P. Chiao, T. Bussiere, P.H. Weinreb, L. Williams, M. Maier, R. Dunstan, S. Salloway, T. Chen, Y. Ling, J. O'Gorman, F. Qian, M. Arastu, M. Li, S. Chollate, M.S. Brennan, O. Quintero-Monzon, R.H. Scannevin, H.M. Arnold, T. Engber, K. Rhodes, J. Ferrero, Y. Hang, A. Mikulskis, J. Grimm, C. Hock, R.M. Nitsch, A. Sandrock, The antibody aducanumab reduces Abeta plaques in Alzheimer's disease, Nature 537(7618) (2016) 50-6.

[5] F. Panza, M. Lozupone, G. Logroscino, B.P. Imbimbo, A critical appraisal of amyloid-beta-targeting therapies for Alzheimer disease, Nat Rev Neurol 15(2) (2019) 73-88.

[6] L.X. Yi, E.K. Tan, Z.D. Zhou, Passive immunotherapy for Alzheimer's disease: challenges & future directions, J Transl Med 22(1) (2024) 430.

[7] W.M. Pardridge, Drug transport across the blood-brain barrier, J Cereb Blood Flow Metab 32(11) (2012) 1959-72.

[8] J.P. Spencer, The impact of fruit flavonoids on memory and cognition, Br J Nutr 104 Suppl 3 (2010) S40-7.

[9] D. Vauzour, K. Vafeiadou, A. Rodriguez-Mateos, C. Rendeiro, J.P. Spencer, The neuroprotective potential of flavonoids: a multiplicity of effects, Genes Nutr 3(3-4) (2008) 115-26.

[10] Q. Li, P. Zhang, Y. Cai, Genkwanin suppresses MPP(+)-induced cytotoxicity by inhibiting TLR4/MyD88/NLRP3 inflammasome pathway in a cellular model of Parkinson's disease, Neurotoxicology 87 (2021) 62-69.

[11] N. El Menyiy, S. Aboulaghras, S. Bakrim, R. Moubachir, D. Taha, A. Khalid, A.N. Abdalla, A.S. Algarni, A. Hermansyah, L.C. Ming, M.E. Rusu, A. Bouyahya, Genkwanin: An emerging natural compound with multifaceted pharmacological effects, Biomed Pharmacother 165 (2023) 115159.

[12] E. Cho, K. Youn, H. Kwon, J. Jeon, W.S. Cho, S.J. Park, S.H. Son, D.S. Jang, C.Y. Shin, M. Moon, M. Jun, N.J. Kim, D.H. Kim, Eugenitol ameliorates memory impairments in 5XFAD mice by reducing Abeta plaques and neuroinflammation, Biomed Pharmacother 148 (2022) 112763.

[13] M.S. Uddin, M.T. Kabir, K. Niaz, P. Jeandet, C. Clement, B. Mathew, A. Rauf, K.R.R. Rengasamy, E. Sobarzo-Sanchez, G.M. Ashraf, L. Aleya, Molecular Insight into the Therapeutic Promise of Flavonoids against Alzheimer's Disease, Molecules 25(6) (2020).

[14] E. Cho, S.J. Jeon, J. Jeon, J.H. Yi, H. Kwon, H.J. Kwon, K.J. Kwon, M. Moon, C.Y. Shin, D.H. Kim, Phyllodulcin improves hippocampal long-term potentiation in 5XFAD mice, Biomed Pharmacother 161 (2023) 114511.

[15] F. Yang, G.P. Lim, A.N. Begum, O.J. Ubeda, M.R. Simmons, S.S. Ambegaokar, P.P. Chen, R. Kayed, C.G. Glabe, S.A. Frautschy, G.M. Cole, Curcumin inhibits formation of amyloid beta oligomers and fibrils, binds plaques, and reduces amyloid in vivo, J Biol Chem 280(7) (2005) 5892-901.

[16] M. Singh, M. Arseneault, T. Sanderson, V. Murthy, C. Ramassamy, Challenges for research on polyphenols from foods in Alzheimer's disease: bioavailability, metabolism, and cellular and molecular mechanisms, J Agric Food Chem 56(13) (2008) 4855-73.

[17] M.U. Ijaz, A. Ishtiaq, A. Tahir, M.A. Alvi, A. Rafique, P. Wang, G.P. Zhu, Antioxidant, anti-inflammatory, and anti-apoptotic effects of genkwanin against aflatoxin B(1)-induced testicular toxicity, Toxicol Appl Pharmacol 481 (2023) 116750.

[18] G.P. Kumar, F. Khanum, Neuroprotective potential of phytochemicals, Pharmacogn Rev 6(12) (2012) 81-90.

[19] G.P. Lim, T. Chu, F. Yang, W. Beech, S.A. Frautschy, G.M. Cole, The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse, J Neurosci 21(21) (2001) 8370-7.