Melittin

Subjects: Neurosciences

Contributor: Gihyun Lee

Melittin, a 26-amino acid peptide, is the main component of the venom of four honeybee species and exhibits neuroprotective actions. Melittin alleviates HT22 in vivo and in vitro oxidative stress injury induced by Aβ25–35; For the first time, melittin has been reported to enhance cognitive function in a learning memory-deficit model; Melittin ameliorating mechanisms were observed via the nuclear translocation of Nrf2, stimulating 1-HO production and the regulation of the TrkB/CREB/BDNF pathway.

bee venom BDNF beta amyloid oxidative stress neurodegeneration melittin

1. Introduction

For centuries, bee venom therapy has been used to manage acute and chronic human diseases; recently, this focus has steadily gained more interest from researchers and practitioners for bee venom's anti-neurodegeneration ability [1]. Melittin, a 26-amino acid peptide, is the dominant component in the venom of four most common honeybee species—*Apis dorsata*, *Apis mellifera*, *Apis florea*, and *Apis cerena*, which contain 95.8 ± 3.2%, 76.5 ± 1.9%, 66.3 ± 8.6%, and 56.8 ± 1.8% melittin, respectively [2][3].

Regarding neuroprotective effects, as evidenced by previous studies, melittin exhibits suppressive effects on the proinflammatory, and potentially pro-oxidative, response of BV2 microglia including the ability to reduce nitric oxide (NO) and inducible nitric oxide synthase (iNOS) by blocking LPS-induced activation of NF-κB7, suppressing the expression of COX-2/PGE2, resulting in anti-inflammatory properties [4]. Another study revealed that melittin stabilized pros and anti-apoptosis factor in SY5Y human neuroblastoma cells under stress induced by H_2O_2 [5].

As the primary component of honeybee venoms, melittin potential is high, and more research needs to be conducted on this compound to further explore its role in combating neurodegeneration. Neuro-oxidative stress ignited by beta amyloid (Aβ) in the human brain is a main trigger for the progression of neural disorder [6][7], and melittin's protective capacity and mechanisms in this aspect are still unclear. Further, at an in-vivo level, the ability of melittin to convalesce deficit cognitive function remains elusive.

In previous cardiology and renal studies, melittin was reported to regulate the nuclear translocation of Nrf2, a key transcription factor that upregulates HO-1 expression [8][9]. HO-1 is a critical cellular antioxidant enzyme that normalizes redox balance in various disorders including in neurodegenerative oxidative stress [10]. A step from this, in an anti-neurodegeneration study, the TrkB/CREB/BDNF loop was also a companionable route that inter-connects and is commonly studied alongside cellular antioxidant aspects [10][11]. Therefore, we hypothesized that

melittin also protects neuron cells such as HT22 against neurodegeneration induced by oxidative stress by regulating the Nrf2/HO-1, and TrkB/CREB/BDNF pathways; consequently, this substance has a high chance of exhibiting protection effects in a neurodegenerative cognitive induced in vivo model.

2. Protective Effect of Melittin on Oxidative Stress-Induced Apoptosis in HT-22 Cells

To determine the therapeutic window, we first identified the maximum safety concentrations; we tested melittin concentrations of 0.1–30 μM on HT22 cells and found that a concentration of 3 μM was the maximum safe concentration for our experiment (**Figure 1**A). Next, for protection effect against 7-μM $A\beta_{25–35}$-induced stress, melittin (0.3–3 μM) exhibited observable dose-dependent effects; therefore, these concentrations were selected for further study (**Figure 1**B).

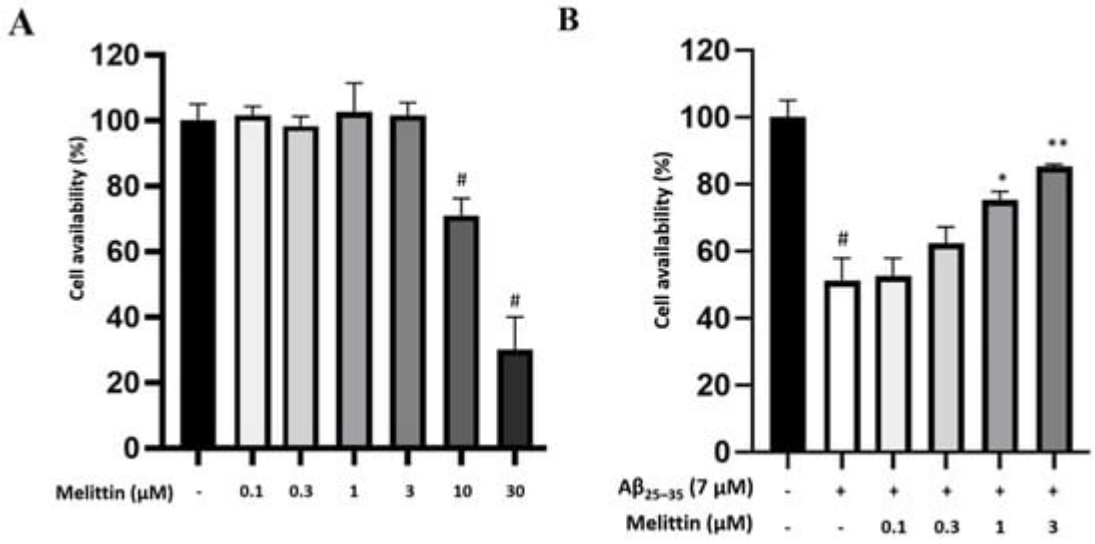

Figure 1. Screening for melittin maximum safety dosage and melittin protective effect against $A\beta_{25–35}$ stress induced in HT22 cells. (**A**) Screening for maximum melittin safety concentration. The result indicated that 3 μM was the highest safety concentration of melittin to be studied. To conduct this experiment: Cells were seeded in 96-well plates; after incubation for 24 h, Melittin at different concentrations was introduced, and cell availability was examined after 24 h of incubation. (**B**) Protective effect of melittin against $A\beta_{25–35}$ stress. The result indicated that 0.3 to 3 μM melittin was found to dosage-dependently ameliorate HT22 cell availability. To conduct this experiment: Cells were seeded in 96-well plates; after incubation for 24 h, melittin from 0.1 to 3 μM was introduced 1 h before $A\beta_{25–35}$ (7 μM) challenge, and cell availability was examined after 24 h of incubation. Data are presented as the mean ± standard deviation values of triple determinations. # $p < 0.01$ vs. control * $p < 0.05$ and ** $p < 0.01$ vs. $A\beta_{25–35}$ only-treated group.

Further experiments were conducted to elucidate the protective effect of melittin against HT22 cell apoptosis. Results revealed that 0.1 μM melittin did not exhibit statistically significant efficacy; however, at 1 and 3 μM, melittin markedly reduced the apoptosis level in HT22 cells by 3- and 4-fold, respectively (**Figure 2**A). Western blot results

indicated that the Bax/Bcl-2 protein ratio was increased 2.5-fold once cells were introduced with $A\beta_{25–35}$, this parameter was dosage dependently normalized by melittin. A step from that, other apoptosis-closely-associated proteins such as AIF, Calpain, CytoC, and CleaCas3 levels were all simultaneously normalized by melittin (**Figure 2**B) [11][12]. These results are indicators confirming the effect of melittin in protecting HT22 cells against $A\beta_{25–35}$ stress-induced neuronal apoptosis.

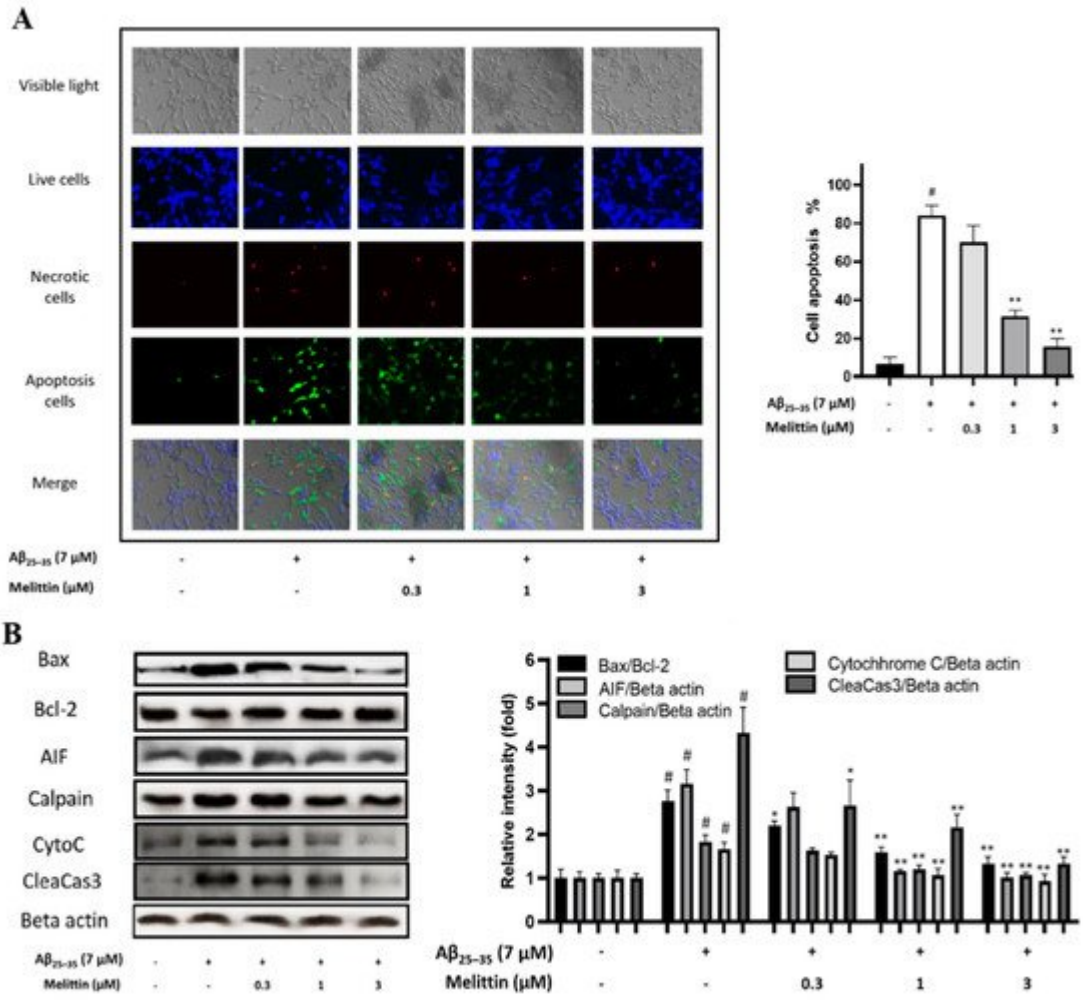

Figure 2. Apoptosis-inhibitory effects of melittin on $A\beta_{25–35}$ induced HT-22 cells. (**A**) Immunofluorescence analysis to determine the cellular apoptosis rate. The result indicated that melittin at 0.3 to 3 μM dosage-dependently ameliorated HT22 cell apoptosis under $A\beta_{25–35}$ (7 μM) stress-induced conditions. To conduct this experiment: Seven hours after $A\beta_{25–35}$ (7 μM) challenge in 96-well plates, immunofluorescence staining was carried out to exhibit live cells (blue, stained by CytoCalcein Violet 450), necrotic cells (red, indicated by 7-AAD staining), and apoptotic cells (green, Apopxin Green Indicator). (**B**) Western blot analysis of key apoptosis proteins. The result indicated that melittin at 0.3 to 3 μM dosage-dependently normalized the expression of pro and anti-apoptosis protein under $A\beta_{25–35}$ stress challenge. To conduct this experiment: Seven hours after $A\beta_{25–35}$ (7 μM) challenge in 6-well plates, cells were lysed, and western blot was performed. Data are presented as mean ± standard deviation values of triple determinations. # $p < 0.01$ vs. control * $p < 0.05$ and ** $p < 0.01$ vs. $A\beta_{25–35}$ only-treated group.

3. Effects of Melittin on $A\beta_{25–35}$-Induced Oxidative Stress

Melittin has previously been reported to possess antioxidant activity [2][8][9]. This suggests that the antioxidant properties of melittin can reduce the accumulation of intracellular ROS under $A\beta_{25–35}$ oxidative stress-induced conditions [13]. We found that melittin, 1 and 3 μM, significantly reduced the cellular ROS levels (**Figure 3**A) by 2- and 3-fold, respectively. This further confirmed that 0.3 to 3 μM melittin dosage-dependently ameliorated the leakage of the key oxidative stress markers MDA and LDH. These excessive destructive cellular free radical sources can modify structure scaffolds and functional apparatus, which eventually increase protein carbonylation [14]. Protein carbonyl is a specific oxidative stress marker that is associated with various disease, including Alzheimer's disease [15]. Our result indicated that 0.3 to 3 μM melittin exhibited a significant effect in down-regulating cellular protein carbonyl levels (**Figure 3**B).

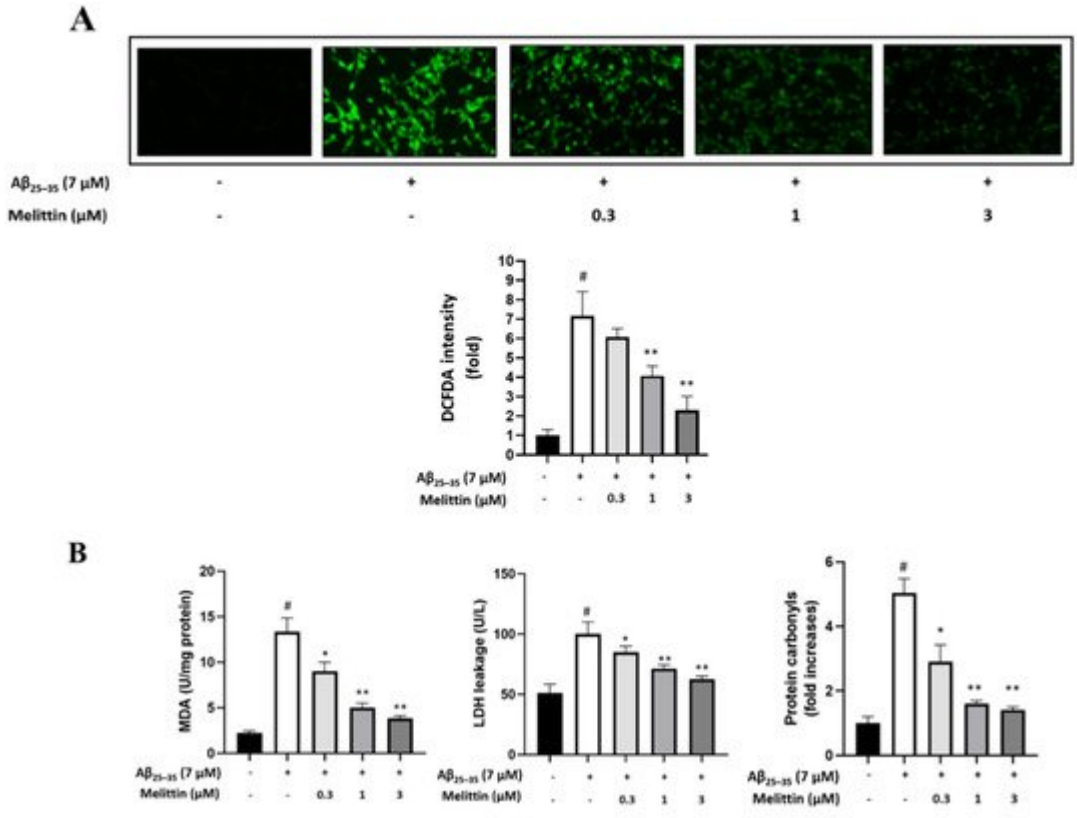

Figure 3. Melittin regulates cellular oxidative stress induced by $A\beta_{25–35}$ in HT22 cells. (**A**) Immunofluorescence analysis to determine the cellular ROS rate. The result indicated that melittin at 0.3 to 3 μM dosage-dependently normalized the expression of pro and anti-apoptosis proteins under $A\beta_{25–35}$ stress challenge. To conduct this experiment: Seven hours after $A\beta_{25–35}$ (7 μM) challenge in 96-well plates, immunofluorescence analysis by DCFDA staining was carried out. (**B**) Cellular MDA, LDH, and protein carbonyls levels. The result indicated that melittin at 0.3 to 3 μM dosage-dependently down-regulated MDA, LDH, and protein carbonyl parameters under $A\beta_{25–35}$ stress challenge. To conduct this experiment: Seven hours after $A\beta_{25–35}$ (7 μM) challenge in 6-well plates, kits measuring MDA, LDH, and protein carbonyls were used to determine the parameters. Data are presented as mean ± standard

deviation values of triple determinations. # $p < 0.01$ vs. control * $p < 0.05$ and ** $p < 0.01$ vs. $A\beta_{25–35}$ only-treated group.

4. Discussion

Neurodegenerative diseases, including Alzheimer which results in a decrease of human cognitive function, have become the main obstacles that degrade human life span and the overall wellbeing [16].

Through aging, Aβ is a product that naturally emerges and accumulates in the brain. Accumulated Aβ gradually forms aggregates with beta-sheet structures and has the capacity to alter the intercellular redox balance of neurons cells. This is considered a major cause of the onset of cognitive dysfunction [17][18][19]. Among Aβ variances, the $A\beta_{25–35}$ fragment is the shortest segment of full-length $A\beta_{1–42}$that can form a beta-sheet; this fragment was proven to have similar oxidative neurotoxicity output compared with the full-length $A\beta_{1–42}$ [20]. With good solubility and stress-induced efficiency, interest in this undecapeptide $A\beta_{25–35}$ has grown over the last decade [21].

In detail, both full-length and short Aβs were proven to form ion-like channels in cell membranes that promote Ca^{2+} influx, destabilize intercellular balance, and produce cellular oxidative stress [22][23][24]. Another source of oxidative stress arises as $A\beta_{25–35}$ and $A\beta_{1–42}$were also both found to cause mitochondrial abnormalities via the deactivation of mitochondrial complex IV [25][26][27][28]. In neurodegenerative progression, ROS overproduction from these important events causes damage to the cellular structure, increases MDA, LDH, and protein carbonyl levels, eventually initiates neuron cell death mechanisms, and promotes neuro-cognitive impairments [10][29][30]. Explaining this similar effect of $A\beta_{25–35}$ and $A\beta_{1-42}$ is still a topic of debate, but the single methionine-35 located in both Aβs was mentioned as its redox states are significantly important for Aβ-correlated free radical oxidative stress and neurotoxicity causes [27][31][32][33].

In the in vitro study, our results demonstrated that $A\beta_{25–35}$ induced injury in HT-22 cells, including massive ROS release, resulting in MDA and LDH leakage and increased protein carbonyl levels, which substantially reduced HT-22 cell viability. Melittin dramatically mitigated $A\beta_{25–35}$-induced oxidative stress injury by reducing ROS, MDA, SOD, and eventually, the protein carbonyl levels. To further explain these phenomena, we examined the melittin effect on the generation of HO-1, an important component of the cellular antioxidant system. This protein expression is inducible and has been demonstrated to shield cells against oxidative damage [34]. HO-1 expression is regulated by Nrf2. In the primitive stage, Nrf2 is retained in the Nrf2-Keap1 complex, and activation of cellular protection mechanisms leads to the separation of Nrf2 from the complex and transfer to the nucleus. Nrf2 acts as a transcription factor in the antioxidant response element (ARE) gene region, which in turn upregulates the expression of the HO-1 gene [10][14]. Our results showed that under cellular stress-induced conditions, melittin markedly upregulated the nuclear translocation of Nrf2 and enhanced the overall production of antioxidant enzyme activity, suggesting that the beneficial effect of melittin on $A\beta_{25–35}$-induced HT22 cell injury was attributed to its antioxidant properties. This is in line with the findings of previous studies indicating that melittin enhances Nrf2 nuclear translocation and subsequently upregulates the expression of important antioxidant genes such as HO-1 [8][9].

One other mechanism of melittin can be related to the TrkB/CREB/BDNF pathway, which is a commonly studied direction along with antioxidant aspects [11][12][35][36]. The activation of TrkB can lead to downstream enhancement of both cellular antioxidant defensive and brain-derived neurotrophic factor neuro-proliferative shields [10]. In our experiment, $A\beta_{25–35}$ presence significantly depleted the TrkB/CREB/BDNF pathway. Remarkably, melittin induced p-TrkB activation, increased the amount of p-CREB transcription factor, and in turn upregulated the expression of BDNF [37]. Moreover, BDNF was examined to stimulate subsets of Trk receptors and can further protect neuronal cells from oxidative stress-induced cell death [38][39]. The grounds above suggested the mechanism on how melittin demonstrated the ability to protect neuron cell HT22 apoptosis induced by $A\beta_{25–35}$, an protective effect which was confirmed by the normalization of Bax/Bcl-2 ration, apoptosis-inducing factor, Calpain, CytoC, and CleaCas3.

For in vivo research, the injection of $A\beta_{25–35}$ into mice brains was a utilized as a mean to induce oxidative stress, initiating synaptic loss, suppressing neurogenesis, and resulting in cognitive impairments in animal experiments [21][40][41][42]. This model elicits a considerable degree of Alzheimer's progression signs and neurodegeneration conditions in general [41].

In this study, melittin significantly enhanced the memory and learning abilities of cognitive impairment-induced animals, a novel finding. The neuroprocessing ability of mice is closely related to hippocampal physiology [43]. Among the many anatomical parts of the hippocampus, the dentate gyrus is commonly studied and plays a key role in the formation, recall, and discrimination of episodic memory [44]. The significant increase in neurogenesis in this region has proven the effect of melittin at an anatomical scale.

To further explore the antioxidant property of melittin, we measured ROS and MDA levels in the hippocampus and serum; the levels of these parameters were significantly increase due to $A\beta_{25–35}$ ICV., and melittin treatment clearly decreased these oxidative stress markers. Our results showed melittin reduced the amount of NO accumulation and iNOS protein expression in the hippocampus, which indicates melittin's effect in lowering neuronal-derived nitric oxide—a key element stimulating neural diseases [45][46].

Maintenance of the balance of the cholinergic system is necessary for normal memory function [47]. Previous studies have revealed that patients with Alzheimer's disease have downregulated expression of mAChR 1; hence, it is an important neuroreceptor to study in the cholinergic system [47]. In the synaptic cleft, ACh binds to postsynaptic mAChRs, and the synaptic signal communicates sequentially with the cyclic adenosine monophosphate/protein kinase A/CREB signaling pathway via G-coupled protein receptors. This bridge reflects the reality that the cholinergic signaling system and intercellular neurotrophic factors are two reciprocal entities. The disruption of one can cause a negative effect on the other and they both synergistically facilitate the grounds for neuronal grow and brain normal cognitive functions [48]. In our study, downregulation of mAChR 1 expression by $A\beta_{25–35}$ in hippocampal tissues was significantly normalized by melittin pretreatment. Excessive AChE activity leads to a decrease in the Ach level in hippocampal cholinergic synapses. $A\beta_{25–35}$ ICV. injection augmented AChE activity by 1.5-fold in hippocampal tissue, whereas pretreatment with melittin completely attenuated the excessive activation of AChE and increased ACh levels. Regarding intercellular neurotrophic factors, the transcription factor CREB, which plays a key role in BDNF synthesis, is essential for memory and synaptic plasticity [49]. In line with

the in vitro experiments, our in vivo results showed that melittin-treated mice also had increased hippocampal p-CREB and BDNF levels.

Therefore, through both in vivo and in vitro experiments, melittin had proved itself to be a drug candidate combating neurovegetative disease.

The drug administration route of melittin is often subcutaneous, which can cause adverse effects if over-dosed [50]. Although melittin exhibited the ability to recover cognitive function in neurodegenerative-induced models, its irritation properties should be alleviated, and an optimal dosage for humans should be determined. In other disease research with melittin, up-to-date recombinant technology and computational bioinformatics modified the specific amino acid sequences and created a specialized-engineered-melittin. This produced effective augmentation and enhanced drug delivery, which enabled melittin to event be intravenously injected and to target a specific group of malarian cells [51]. Such advances can alleviate the side effects of melittin and further increase the popularity of melittin treatment.

References

1. Hwang, D.S.; Kim, S.K.; Bae, H. Therapeutic effects of bee venom on immunological and neurological diseases. Toxins 2015, 7, 2413–2421.
2. Frangieh, J.; Salma, Y.; Haddad, K.; Mattei, C.; Legros, C.; Fajloun, Z.; Obeid, D. El First characterization of the venom from apis mellifera syriaca, a honeybee from the middle east region. Toxins 2019, 11, 191.
3. Somwongin, S.; Chantawannakul, P.; Chaiyana, W. Antioxidant activity and irritation property of venoms from Apis species. Toxicon 2018, 145, 32–39.
4. Moon, D.O.; Park, S.Y.; Lee, K.J.; Heo, M.S.; Kim, K.C.; Kim, M.O.; Lee, J.D.; Choi, Y.H.; Kim, G.Y. Bee venom and melittin reduce proinflammatory mediators in lipopolysaccharide-stimulated BV2 microglia. Int. Immunopharmacol. 2007, 7, 1092–1101.
5. Han, S.M.; Kim, J.M.; Park, K.K.; Chang, Y.C.; Pak, S.C. Neuroprotective effects of melittin on hydrogen peroxide-induced apoptotic cell death in neuroblastoma SH-SY5Y cells. BMC Complement. Altern. Med. 2014, 14, 1–8.
6. Atlante, A.; Bobba, A.; Petragallo, V.A.; Marra, E. Alzheimer’s proteins, oxidative stress, and mitochondrial dysfunction interplay in a neuronal model of Alzheimer’s disease. Int. J. Alzheimers. Dis. 2010, 2010.
7. Butterfield, D.A.; Swomley, A.M.; Sultana, R. Amyloid β-Peptide (1-42)-induced oxidative stress in alzheimer disease: Importance in disease pathogenesis and progression. Antioxid. Redox Signal. 2013, 19, 823–835.

8. Wang, T.; Zhang, J.; Xiao, A.; Liu, W.; Shang, Y.; An, J. Melittin ameliorates CVB3-induced myocarditis via activation of the HDAC2-mediated GSK-3β/Nrf2/ARE signaling pathway. Biochem. Biophys. Res. Commun. 2016, 480, 126–131.
9. Kim, J.Y.; Leem, J.; Hong, H.L. Melittin Ameliorates Endotoxin-Induced Acute Kidney Injury by Inhibiting Inflammation, Oxidative Stress, and Cell Death in Mice. Oxid. Med. Cell. Longev. 2021, 2021, 8843051.
10. Hannan, M.A.; Dash, R.; Sohag, A.A.M.; Haque, M.N.; Moon, I.S. Neuroprotection Against Oxidative Stress: Phytochemicals Targeting TrkB Signaling and the Nrf2-ARE Antioxidant System. Front. Mol. Neurosci. 2020, 13, 116.
11. Yoo, J.M.; Lee, B.D.; Sok, D.E.; Ma, J.Y.; Kim, M.R. Neuroprotective action of N-acetyl serotonin in oxidative stress-induced apoptosis through the activation of both TrkB/CREB/BDNF pathway and Akt/Nrf2/Antioxidant enzyme in neuronal cells. Redox Biol. 2017, 11, 592–599.
12. Cells, N.; Lee, B.D.; Yoo, J.; Baek, S.Y.; Li, F.Y.; Sok, D.; Kim, M.R. Antioxidant Enzyme Formation via TrkB/Akt Pathway Activation for Neuroprotection against Oxidative Stress-Induced Apoptosis in Hippocampal. Antioxidants 2019, 9, 3.
13. Seo, J.Y.; Pyo, E.; An, J.P.; Kim, J.; Sung, S.H.; Oh, W.K. Andrographolide Activates Keap1/Nrf2/ARE/HO-1 Pathway in HT22 Cells and Suppresses Microglial Activation by A β 42 through Nrf2-Related Inflammatory Response. Mediat. Inflamm. 2017, 2017.
14. Gutierrez-Merino, C.; Lopez-Sanchez, C.; Lagoa, R.K.; Samhan-Arias, A.; Bueno, C.; Garcia-Martinez, V. Neuroprotective Actions of Flavonoids. Curr. Med. Chem. 2011, 18, 1195–1212.
15. Dalle-Donne, I.; Giustarini, D.; Colombo, R.; Rossi, R.; Milzani, A. Protein carbonylation in human diseases. Trends Mol. Med. 2003, 9, 169–176.
16. Hou, Y.; Dan, X.; Babbar, M.; Wei, Y.; Hasselbalch, S.G.; Croteau, D.L.; Bohr, V.A. Ageing as a risk factor for neurodegenerative disease. Nat. Rev. Neurol. 2019, 15, 565–581.
17. Koike, H.; Iguchi, Y.; Sahashi, K.; Katsuno, M. Significance of Oligomeric and Fibrillar Species in Amyloidosis : Insights into Pathophysiology and Treatment. Molecules 2021, 26, 5091.
18. Koike, H.; Katsuno, M. The ultrastructure of tissue damage by amyloid fibrils. Molecules 2021, 26, 4611.
19. Cioffi, F.; Adam, R.H.I.; Broersen, K. Molecular Mechanisms and Genetics of Oxidative Stress in Alzheimer’s Disease. J. Alzheimer’s Dis. 2019, 72, 981–1017.
20. Pike, C.J.; Walencewicz-Wasserman, A.J.; Kosmoski, J.; Cribbs, D.H.; Glabe, C.G.; Cotman, C.W. Structure-Activity Analyses of β-Amyloid Peptides: Contributions of the β25–35 Region to Aggregation and Neurotoxicity. J. Neurochem. 1995, 64, 253–265.

21. Zussy, C.; Brureau, A.; Keller, E.; Marchal, S.; Blayo, C.; Delair, B.; Ixart, G.; Maurice, T.; Givalois, L. Alzheimer's Disease Related Markers, Cellular Toxicity and Behavioral Deficits Induced Six Weeks after Oligomeric Amyloid-β Peptide Injection in Rats. PLoS ONE 2013, 8, e0053117.
22. Lee, J.; Kim, Y.H.; Arce, F.; Gillman, A.L.; Jang, H.; Kagan, B.L.; Nussinov, R.; Yang, J.; Lal, R. Amyloid β Ion Channels in a Membrane Comprising Brain Total Lipid Extracts. ACS Chem. Neurosci. 2017, 8, 1348–1357.
23. Zaretsky, D.V.; Zaretskaia, M.V. Flow cytometry method to quantify the formation of beta-amyloid membrane ion channels. Biochim. Biophys. Acta Biomembr. 2021, 1863, 183506.
24. Ekinci, F.J.; Linsley, M.D.; Shea, T.B. β-Amyloid-induced calcium influx induces apoptosis in culture by oxidative stress rather than tau phosphorylation. Mol. Brain Res. 2000, 76, 389–395.
25. Canevari, L.; Clark, J.B.; Bates, T.E. β-Amyloid fragment 25-35 selectively decreases complex IV activity in isolated mitochondria. FEBS Lett. 1999, 457, 131–134.
26. Lahmy, V.; Long, R.; Morin, D.; Villard, V.; Maurice, T. Mitochondrial protection by the mixed muscarinic/σ1 ligand ANAVEX2-73, a tetrahydrofuran derivative, in Aβ25–35 peptide-injected mice, a nontransgenic Alzheimer's disease model. Front. Cell. Neurosci. 2015, 8, 463.
27. Clementi, M.E.; Marini, S.; Coletta, M.; Orsini, F.; Giardina, B.; Misiti, F. Aβ(31-35) and Aβ(25-35) fragments of amyloid beta-protein induce cellular death through apoptotic signals: Role of the redox state of methionine-35. FEBS Lett. 2005, 579, 2913–2918.
28. Luque-Contreras, D.; Carvajal, K.; Toral-Rios, D.; Franco-Bocanegra, D.; Campos-Peña, V. Oxidative stress and metabolic syndrome: Cause or consequence of Alzheimer's disease? Oxid. Med. Cell. Longev. 2014, 2014, 497802.
29. Weitzel, K.; Chemie, F.; Rev, M.S.; Introduction, I.; Reference, C. Protein Carbonylation as a Major Hallmark of Oxidative Damage: Update of Analytical Strategies Maria. WHO Libr. Cat. Data 2014, 33, 79–97.
30. Hung, C.H.L.; Cheng, S.S.Y.; Cheung, Y.T.; Wuwongse, S.; Zhang, N.Q.; Ho, Y.S.; Lee, S.M.Y.; Chang, R.C.C. A reciprocal relationship between reactive oxygen species and mitochondrial dynamics in neurodegeneration. Redox Biol. 2018, 14, 7–19.
31. Misiti, F.; Martorana, G.E.; Nocca, G.; Di Stasio, E.; Giardina, B.; Clementi, M.E. Methionine 35 oxidation reduces toxic and pro-apoptotic effects of the amyloid β-protein fragment (31-35) on isolated brain mitochondria. Neuroscience 2004, 126, 297–303.
32. Butterfield, D.A.; Kanski, J. Methionine residue 35 is critical for the oxidative stress and neurotoxic properties of Alzheimer's amyloid β-peptide 1-42. Peptides 2002, 23, 1299–1309.
33. Clementi, M.E.; Martorana, G.E.; Pezzotti, M.; Giardina, B.; Misiti, F. Methionine 35 oxidation reduces toxic effects of the amyloid β-protein fragment (31-35) on human red blood cell. Int. J.

Biochem. Cell Biol. 2004, 36, 2066–2076.

34. Chen, N.; Wang, J.; He, Y.; Xu, Y.; Zhang, Y.; Gong, Q.; Yu, C.; Gao, J. Trilobatin Protects Against Aβ25–35-Induced Hippocampal HT22 Cells Apoptosis Through Mediating ROS/p38/Caspase 3-Dependent Pathway. Front. Pharmacol. 2020, 11, 1–12.
35. Kim, H.J.; Baek, S.Y.; Sok, D.E.; Lee, K.J.; Kim, Y.J.; Kim, M.R. Neuroprotective activity of polyphenol-rich ribes diacanthum pall against oxidative stress in glutamate-stimulated ht-22 cells and a scopolamine-induced amnesia animal model. Antioxidants 2020, 9, 895.
36. Baek, S.Y.; Kim, M.R. Neuroprotective effect of carotenoid-rich enteromorpha prolifera extract via TrkB/Akt pathway against oxidative stress in hippocampal neuronal cells. Mar. Drugs 2020, 18, 372.
37. Wang, H.; Xu, J.; Lazarovici, P.; Quirion, R.; Zheng, W. cAMP Response Element-Binding Protein (CREB): A Possible Signaling Molecule Link in the Pathophysiology of Schizophrenia. Front. Mol. Neurosci. 2018, 11, 255.
38. Francis, P.T. The interplay of neurotransmitters in Alzheimer’s disease. CNS Spectr. 2005, 10, 6–9.
39. Bartkowska, K.; Paquin, A.; Gauthier, A.S.; Kaplan, D.R.; Miller, F.D. Trk signaling regulates neural precursor cell proliferation and differentiation during cortical development. Development 2007, 134, 4369–4380.
40. Stepanichev, M.Y.; Zdobnova, I.M.; Zarubenko, I.I.; Moiseeva, Y.V.; Lazareva, N.A.; Onufriev, M.V.; Gulyaeva, N.V. Amyloid-β(25-35)-induced memory impairments correlate with cell loss in rat hippocampus. Physiol. Behav. 2004, 80, 647–655.
41. Fang, F.; Liu, G.T. Protective effects of compound FLZ on β-amyloid peptide-(25-35)- induced mouse hippocampal injury and learning and memory impairment. Acta Pharmacol. Sin. 2006, 27, 651–658.
42. Mantha, A.K.; Moorthy, K.; Cowsik, S.M.; Baquer, N.Z. Neuroprotective role of Neurokinin B (NKB) on β-amyloid (25-35) induced toxicity in aging rat brain synaptosomes: Involvement in oxidative stress and excitotoxicity. Biogerontology 2006, 7, 1–17.
43. Vorhees, C.V.; Williams, M.T. Morris water maze: Procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 2006, 1, 848–858.
44. Bernier, B.E.; Lacagnina, A.F.; Ayoub, A.; Shue, F.; Zemelman, B.V.; Krasne, F.B.; Drew, M.R. Dentate gyrus contributes to retrieval as well as encoding: Evidence from context fear conditioning, recall, and extinction. J. Neurosci. 2017, 37, 6359–6371.
45. Ledo, A.; Frade, J.; Barbosa, R.M.; Laranjinha, J. Nitric oxide in brain: Diffusion, targets and concentration dynamics in hippocampal subregions. Mol. Aspects Med. 2004, 25, 75–89.

46. Ledo, A.; Lourenço, C.F.; Cadenas, E.; Barbosa, R.M.; Laranjinha, J. The bioactivity of neuronal-derived nitric oxide in aging and neurodegeneration: Switching signaling to degeneration. Free Radic. Biol. Med. 2021, 162, 500–513.
47. Lee, J.S.; Kim, H.G.; Lee, H.W.; Han, J.M.; Lee, S.K.; Kim, D.W.; Saravanakumar, A.; Son, C.G. Hippocampal memory enhancing activity of pine needle extract against scopolamine-induced amnesia in a mouse model. Sci. Rep. 2015, 5, 9651.
48. Hu, H.; Zhang, R.; Zhang, Y.; Xia, Z.; Hu, Y. Role of CREB in the regulatory action of sarsasapogenin on muscarinic M1 receptor density during cell aging. FEBS Lett. 2010, 584, 1549–1552.
49. Bramham, C.R.; Messaoudi, E. BDNF function in adult synaptic plasticity: The synaptic consolidation hypothesis. Prog. Neurobiol. 2005, 76, 99–125.
50. Hossen, M.S.; Gan, S.H.; Khalil, M.I. Melittin, a Potential Natural Toxin of Crude Bee Venom: Probable Future Arsenal in the Treatment of Diabetes Mellitus. J. Chem. 2017, 2017.
51. Duffy, C.; Sorolla, A.; Wang, E.; Golden, E.; Woodward, E.; Davern, K.; Ho, D.; Johnstone, E.; Pfleger, K.; Redfern, A.; et al. Honeybee venom and melittin suppress growth factor receptor activation in HER2-enriched and triple-negative breast cancer. npj Precis. Oncol. 2020, 4, 1–16.