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Original article

# Inhibitory effects of *Tabernaemontana divaricata* root extract on oxidative stress and neuronal loss induced by amyloid $\beta_{25-35}$ peptide in mice

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ABSTRACT

In Alzheimer's disease, there are numerous amyloid plaques, neurofibrillary tangles, and neuronal loss in several brain areas. Oxidative stress is involved in the mechanisms of Aβ-peptide induced neurotoxicity by the generation of free radical oxidative stress that may lead to neurodegeneration. *Tabernaemontana divaricata* has various medical properties in Thai folklore medicine including prevent forgetfulness or improve memory. The present study aimed to investigate the effects of *T. divaricata* root extract (TDE) on $A\beta_{25-35}$ peptides induced neuronal loss and oxidative stress in mice. Male ICR mice were administered with vehicle or TDE (250, 500, and 1000 mg/kg b.w., p.o.) for 28 consecutive days. Then, these mice were given a single intracerebroventricular (i.c.v.) injection of $A\beta_{25-35}$ or phosphate buffer saline (PBS) (10 μg/mouse). The novel object recognition (NOR) test was used to determine memory disturbance. In addition, the neuronal cells in the cerebral cortex and hippocampus were measured by using crystal violet staining and lipid peroxidation was determined by measuring the formation of thiobarbituric acid reactive substances. An i.c.v. injection of $A\beta_{25-35}$ peptides could significantly induce memory impairment, increase level of lipid peroxidation including the neuronal loss in CA3 of hippocampus. However, the mice pretreated with TDE could prevent the memory loss, neuronal loss and decrease lipid peroxidation. These results suggest the potential therapeutic value in dementia of TDE through its antioxidant property.



## 1. Introduction

Alzheimer's disease (AD) is the most common form of dementia in the elderly and characterized by a progressive and gradual neurodegeneration resulting in cognitive impairment, neuropsychiatric and behavioral disturbances, and restrictions in activities of daily living (ADLs).[1,2] Hallmark neuropathological features of AD are neuronal cell loss, extracellular deposits of amyloid beta (Aβ)-peptide and neurofibrillary tangles (NFT).[2,3] Numerous evidences suggested that the presence of extensive oxidative stress correlate with the accumulation of beta-amyloid.[4–6] Oxidative stress mediates Aβ-induced neurotoxicity and enhances Aβ-peptide production, forming a vicious cycle Alzheimer affected brains.[7–9] So, it is considered as one of the important factors contributing to the initiation and progression of AD pathogenesis.[9,10] Moreover, *in vitro* study suggested that oxidative stress is involved in the mechanism of Aβ peptides-induced synaptic impairment and neuronal loss by activation of N-methyl-D-aspartate (NMDA) receptors and increase in calcium concentrations in the neuronal cell.[11] These processes initiate the generation of reactive oxygen species (ROS)[11] which can cause oxidative damage to lipid, protein or DNA, eventually leading to cellular dysfunction and neuronal death.[12,13] Recently, treatment or prevention of AD by an oral administration of dietary supplements of natural antioxidants obtained from medicinal herbs or marine animals has become a popular intervention.[14,15] Therefore, the present study aims to determine the effect of TDE on cognition,

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neuronal density and oxidative stress in the mice model of amnesia induced by $A\beta_{25-35}$ peptides.

*Tabernaemontana divaricata* (L, TD), a common garden plant in tropical countries, has been used in traditional rejuvenation remedies such as preventing forgetfulness and improving memory.[16] Several potential effects of TD extract from various parts (flowers, leaves, stem, root) were reported such as anti-convulsant,[17] anti-inflammation,[18] anti-diabetic[19] including Anti-acethylcholinesterase (AChE) activity.[20,21] In our previous study, we demonstrated that subchronic administration of TD root extract (TDE) decreased AChE activity in cerebral cortex and hippocampus resulting in the improvement of memory impairment-induced by $A\beta_{25-35}$ peptides.[22] No toxicity was observed in acute and subchronic treatment of TD in animal models.[22,23] Interestingly, there is also a report about the *in vitro* anti-oxidant potential of TD extract from the leaves.[24] However, there is no reported research that examines the anti-oxidative effects of TDE in the mice model of amnesia. Therefore, the present work aims to study the inhibitory effects of TDE on oxidative stress to improve memory dysfunction and neuronal loss induced by $A\beta_{25-35}$ peptides.

## 2. Materials and methods

### 2.1. Plant material and extract preparation

TD was collected from Phitsanulok, Thailand. The voucher specimen (collection no. Changwijit 001) was deposited at a PBM herbarium, Faculty of Pharmaceutical Sciences, Mahidol University. TDE preparation was mentioned previously.[22] Briefly, the dried TD root was macerated with 95% ethanol and then dried out by evaporating. The HPLC fingerprint of TDE was recorded. The HPLC system used in the analysis consisted of Shimadzu LC-2OAT pump, rheodyne injector with 20 μl loop and a SPD-20A UV/VIS Detector set at 280 nm. A reversed phase C18 column (Phenomenex Luna 150 × 4.60 mm 5 μm) was used together with a C18 guard column (Phenomenex). The isocratic mobile phase was acetonitrile:phosphate buffer solution (pH 7.40) 60:40 v/v at a flow rate of 1 mL/min.

Solution of TDE was prepared by dissolving known quantities of TDE in propyleneglycol (PG), and the solutions were administered at concentrations of 250, 500 and 1000 mg/kg BW per day.

### 2.2. Animals and experimental groups

Male ICR mice (weighing 25–30 g) were obtained from National Animal Center, Thailand. The animals were housed in a group of five and kept in the animal room with a standard light-dark cycle, constant humidity, and controlled temperature (25 ± 2 °C). After a seven-day acclimatizing period, the mice were randomly divided into five groups: PG-PBS, PG-Aβ, TDE250-Aβ, TDE500-Aβ and TDE1000-Aβ (n = 10/group). The PG-PBS and PG-Aβ group were fed with PG and the TDE250-Aβ, TDE500-Aβ and TDE1000-Aβ group were orally administered with TDE at the dose of 250, 500 and 1,000 mg/kg respectively for 28 consecutive days. After a 28 days treatment with TDE, the mice were given an i.c.v. injection of phosphate buffer saline (PBS) or $A\beta_{25-35}$ peptides, respectively. All of the TDE treated groups were given an i.c.v. injection of $A\beta_{25-35}$ peptides. Seven days after the i.c.v. injection, the memory behavioral test for these animals was determined by using Novel object recognition test. Then they were sacrificed, and the lipid peroxidation measured, as well as the assessment of morphological changes. The experimental protocols were approved by the Ethical Committee for the Use of Animals, the Naresuan University (license no. 50040007).

### 2.3. Intracerebroventricular (i.c.v.) injection of $A\beta_{25-35}$ peptides

The experimental $A\beta_{25-35}$ peptide-induced AD mice model was described previously.[22] Briefly, $A\beta_{25-35}$ peptides was dissolved in PBS (1 mg/mL) and incubated at 37 °C for 4 days for aggregation. An i.c.v. administration of aggregated $A\beta_{25-35}$ peptides or PBS (10 μL) was performed into the right lateral ventricle in anesthetized mice.

### 2.4. The novel object recognition (NOR) test

The NOR test was performed by using a chamber ($37 \times 51 \times 20$ $cm^3$). The objects used were plastic toys of different shapes and colors but similar size and were placed centrally at 15 cm apart. Before training, the mice were first allowed to acclimatize to the testing environment in an empty chamber for 10 min. After acclimatization sessions, they were ready for the training stage. At this stage, object A and B were placed in a symmetric position. Each mouse was allowed to explore in the box for a total of 10 min and was considered to be exploring the object when its nose pointed toward the object at a distance $\leq 1$ cm.[25] The amount of time spent exploring object A and B ($T_A$ and $T_B$) were recorded and calculated as a preference index $[(T_A \times 100)/(T_A + T_B)]$. Following the training period, it was removed from the environment for a delay period (24 h). Then it was returned to the box where one of the original objects (B) was replaced by a new object (C). A recognition index of each animal was calculated as the ratio $[(T_C \times 100)/(T_A + T_C)]$, where $T_A$ and $T_C$ are the time spent exploring the objects A and C respectively.

### 2.5. Measurement of lipid peroxide

Lipid peroxidation in the brain was determined by measuring the formation of thiobarbituric acid reactive substances (TBARS) according to the method of Jainkang et al.[26] Briefly, the level of products of lipid peroxidation, malondialdehyde (MDA), was usually assayed by reaction with 2-thiobarbituric acid (TBA) which forms a chromogen adduct at acid pH under elevated reaction temperatures.[27,28] The reaction mixture in tubes contained 100 μL of brain homogenate or standard (1,1′, 3,3′ tetramethoxy propane: TMP), 200 μL of 8% sodiumdodecyl sulphate (SDS), 1.5 mL of 20% acetic acid solution (pH 3.5) and 1.5 mL of 0.81% TBA. The mixtures were heated at 90 °C for 60 min, cooled by immersion in tap water for 10 min to stop the reaction and then centrifuged at 2,500 g for 20 min. Next, the absorbance of supernatant was measured at 532 nm by using spectrophotometer (Model CE1010, Cecil Instruments Ltd, England). The protein content was also determined by using the bicinchoninic acid (BCA) protein assay reagent kit (Pierce, Thermo Fisher Scientific Inc., U.S.A.) and expressed as μg/mL. MDA standard was diluted with ultrapure water to produce the final concentration of 0.0001, 0.001, 0.01, 0.1, 1, and 10 μmol/mL to get the standard curve for the estimation of total MDA. The standard curve was linear response for TMP concentration (with a regression line corresponding to $y = 240.91x + 0.0324$, $r^2 = 0.9964$). Level of MDA was calculated from standard curve prepared from TMP and expressed as μmol MDA/mg of protein for the brain tissue.

### 2.6. Histological study

The brains were fixed in 10% neutral buffered formalin until tissue processing. After embedding, each paraffin block of brain tissues was coronal sectioned by using a rotary microtome. Five coronal 5-μm-thick sections, with 250 μm between sections, were taken from each brain at the level of the medial prefrontal cortex (mPFC) (approximately 1.70–1.98 from bregma) and CA1 and CA3 area of hippocampus (approximately −1.22 to −2.30 from bregma). Detection of amyloid plaques and neuronal density were

performed by using Crystal violet staining. To measure the neuronal density, undamaged neurons were identified as the cell with round-shaped, violet nuclei and clear perinuclear cytoplasm. Damaged neurons were characterized as the cells with changed nuclei (pyknosis, karyorrhexis, and karyolysis).[29,30] The number of undamaged pyramidal neurons per square area 5,000 $\mu m^2$ was counted under 40× magnifications. All images were captured with a digital photo camera attached to the Olympus BX50 microscope and counting was performed by using the Image pro plus program.

### 2.7. Statistics

All data were expressed as mean values with standard error of the mean (mean ± S.E.M.) and were analyzed using a statistical package (SPSS 11.5, IBM, Chicago, IL, USA). Comparisons between the groups were performed by one-way analysis of variance (ANOVA) with *post-hoc* Duncan's multiple test. A $P < 0.05$ was the criterion for statistical significance.

## 3. Results

### 3.1. Effect of TDE on object recognition memory

During the training session of novel object recognition (NOR) test, the preference index were not significantly different between the five groups (Fig. 1, the NOR test). This indicated that there was no biased exploratory preference of each group without affecting the total exploring time in the object exploration. However, the data shown that there was significantly difference in the recognition index. The PG-Aβ group (53.7 ± 2.13) was significantly lower recognition index than the PG-PBS group (75.1 ± 2.28). Additionally, the TDE-pretreated mice that received an i.c.v. injection of $A\beta_{25-35}$ peptides showed significantly higher percentage of recognition index when compared to the control amnesic (PG-Aβ) group. As shown in Fig. 1(B), these percentages of TDE250-Aβ, TDE500-Aβ and TDE1000-Aβ group were 65.7 ± 2.60, 71.1 ± 2.06 and 69.1 ± 2.11, respectively.

### 3.2. Effect of TDE on oxidative stress

Both cortical and hippocampal MDA concentrations of PG-Aβ groups were significantly higher than that of PG-PBS groups. In addition, MDA concentrations in the cortex and hippocampus of PG-Aβ and PG-PBS group showed 4.7 ± 0.82 and 2.1 ± 0.42 μmol/mg protein and 5.6 ± 1.58 and 1.6 ± 0.56 μmol/mg protein, respectively. Interestingly, the Aβ peptides-induced increase in the concentrations of MDA was remarkably low in the TDE-treated groups. The cortical MDA levels of Aβ peptides-induced amnesic mice pretreated with TDE at the doses of 250, 500, and 1,000 mg/kg measured 2.5 ± 0.31, 2.3 ± 0.22, and 1.8 ± 0.28 μmol/mg protein

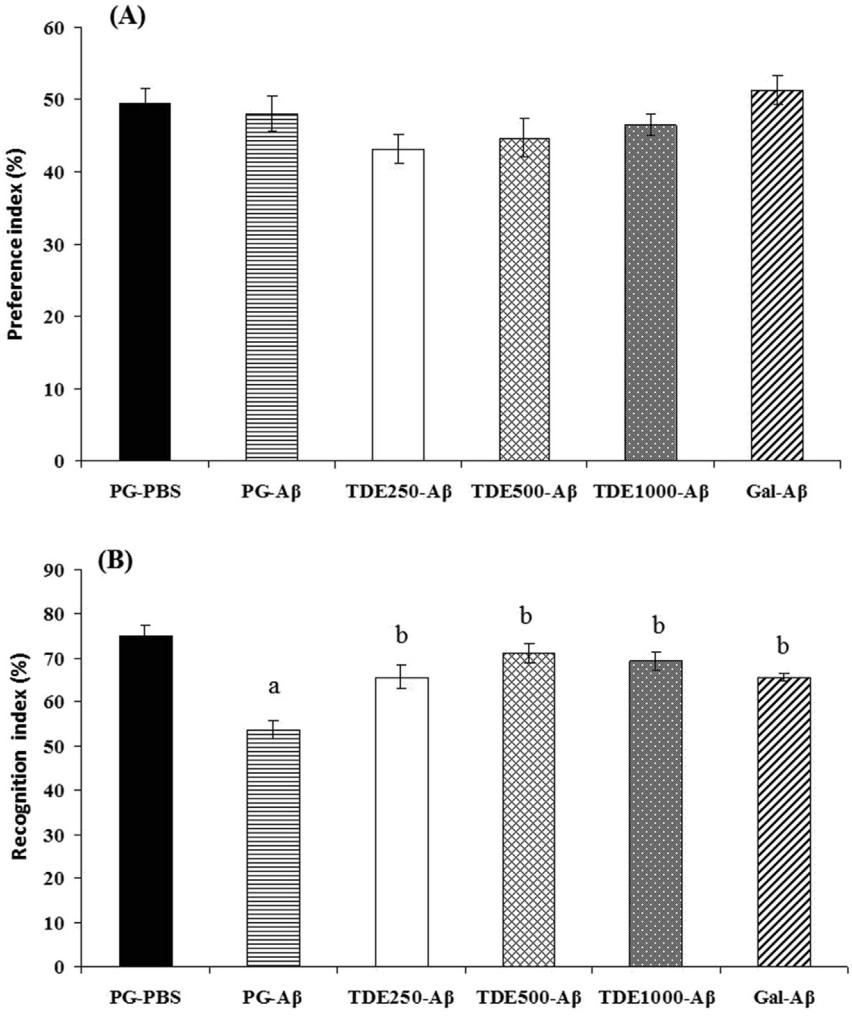


**Fig. 1.** The NOR test. The preference index (A) and the recognition index (B). Each column is mean ± SEM for 10 mice in each group. Columns that have a different letter (a, b) differ significantly with each other ($p < 0.05$). (a) $P < 0.5$ compared to PG-PBS group, (b) $P < 0.05$ compared to PG-Aβ group

while the hippocampal MDA concentrations measured 1.9 ± 0.58, 1.2 ± 0.29, and 1.6 ± 0.30 μmol/mg protein, respectively. So, there were significant differences in both MDA levels of the control (PG-Aβ) groups versus the TDE-treated groups ($P < 0.05$; ANOVA) (Fig. 2, the MDA concentration).

### 3.3. Effect of TDE on neuronal density

In the PFC, CA1 and CA3 regions, marked reductions of the neuronal density of PG-Aβ groups were observed as shown in Fig. 3. Especially in the CA3 region, the number of neurons of the PG-Aβ group was significantly decreased compared to PG-PBS group whereas mice pretreated with TDE had significantly higher density when compared to PG-Aβ group. The number of undamaged pyramidal neurons of PG-PBS, PG-Aβ, TDE250-Aβ, TDE500-Aβ, and TDE1000-Aβ group were 3837.5 ± 110.63, 3162.5 ± 185.26, 3800 ± 86.6, 3887.5 ± 255.25, and 3750 ± 113.65 number of cells/mm$^2$ in this area, respectively. Similar effects of TDE in PFC and CA1regions were also revealed as the TDE-treated groups showed higher densities than that of the PG-Aβ groups.

## 4. Discussion

An i.c.v. injection of $A\beta_{25-35}$ peptides induced amnesia has been extensively employed as the animal model for screening the potential effects of drugs or natural products on learning and memory impairment.[31,32] In this study, NOR was used to evaluate the recognition memory that consisted of familiarity and recollection component. Our study demonstrated that $A\beta_{25-35}$ peptides exposure causes impairment to discriminate between new and familiar objects. The PG-Aβ group showed significantly lower percentage of recognition index when compared to PG-PBS group in the NOR task. Additionally, $A\beta_{25-35}$ peptides-injection didn't affect in motivation and movement as evidenced by the preference index during the training session was not significantly different between all groups. Therefore, the low percentage of recognition index of PG-Aβ group is due to learning and memory deficit. These findings agree with the previous reports.[33,34] Moreover, the result from this task (visual recognition memory) is correlated with the result of other behavioral test in our previous study[22] by using Morris water maze (spatial memory) and step-down avoidance (associative fear memory) test.

The Aβ-peptides accumulation has been reported to be critical events during the neuropathogenesis of AD.[35] It stimulates glial cell activation leading to the generation of ROS and inflammatory cytokines; which seems to be responsible for neuronal dysfunction in AD.[9] Aβ peptides-induced lipid peroxidation is an early event in the oxidative stress that results in the loss of membrane integrity leading to cellular and mitochondrial dysfunction, such as loss of $Ca^{2+}$ homeostasis, disruption of signal pathways, and activation of nuclear transcription factors and apoptotic pathways, which further causes cell death.[14,36,37] Thus antioxidant properties may enable delayed occurrence of neuronal death.[38] Our results showed that i.c.v. $A\beta_{25-35}$ peptides could induce the production of lipid

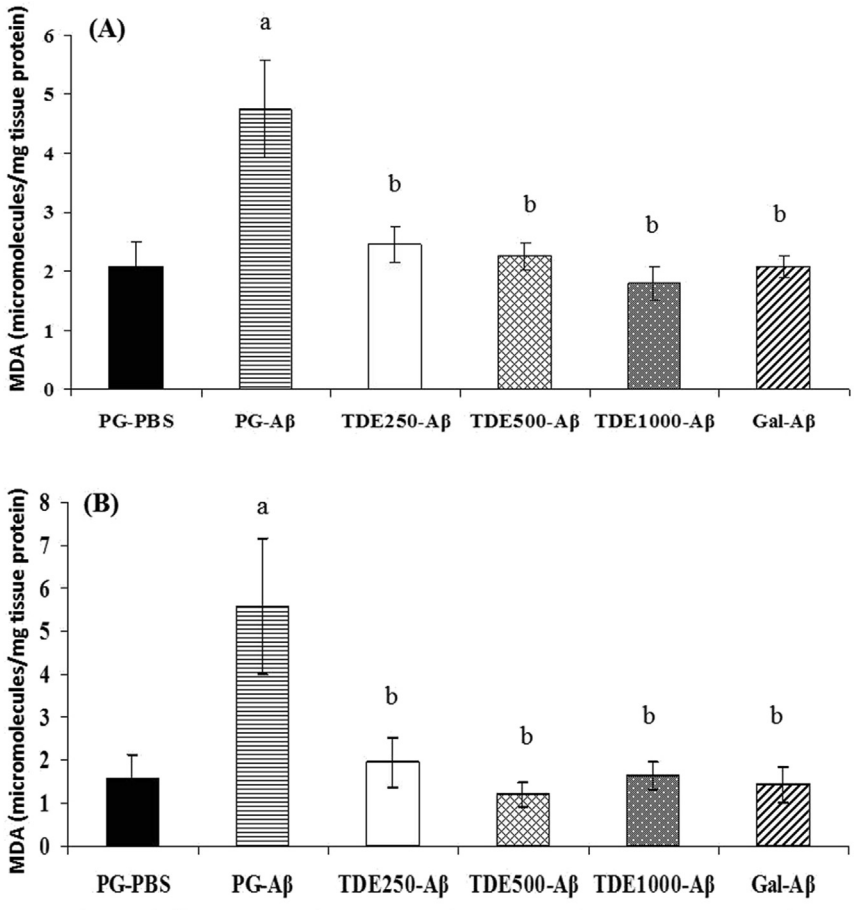


**Fig. 2.** The MDA concentration. Effect of TDE on TBARS production in cerebral cortex (A) and hippocampus (B) of $A\beta_{25-35}$ peptides induced mice. Each column is mean ± SEM for 6 mice in each group. Columns that have a different letter (a, b) differ significantly with each other ($p < 0.05$). (a) $P < 0.5$ compared to PG-PBS group, (b) $P < 0.05$ compared to PG-Aβ group.

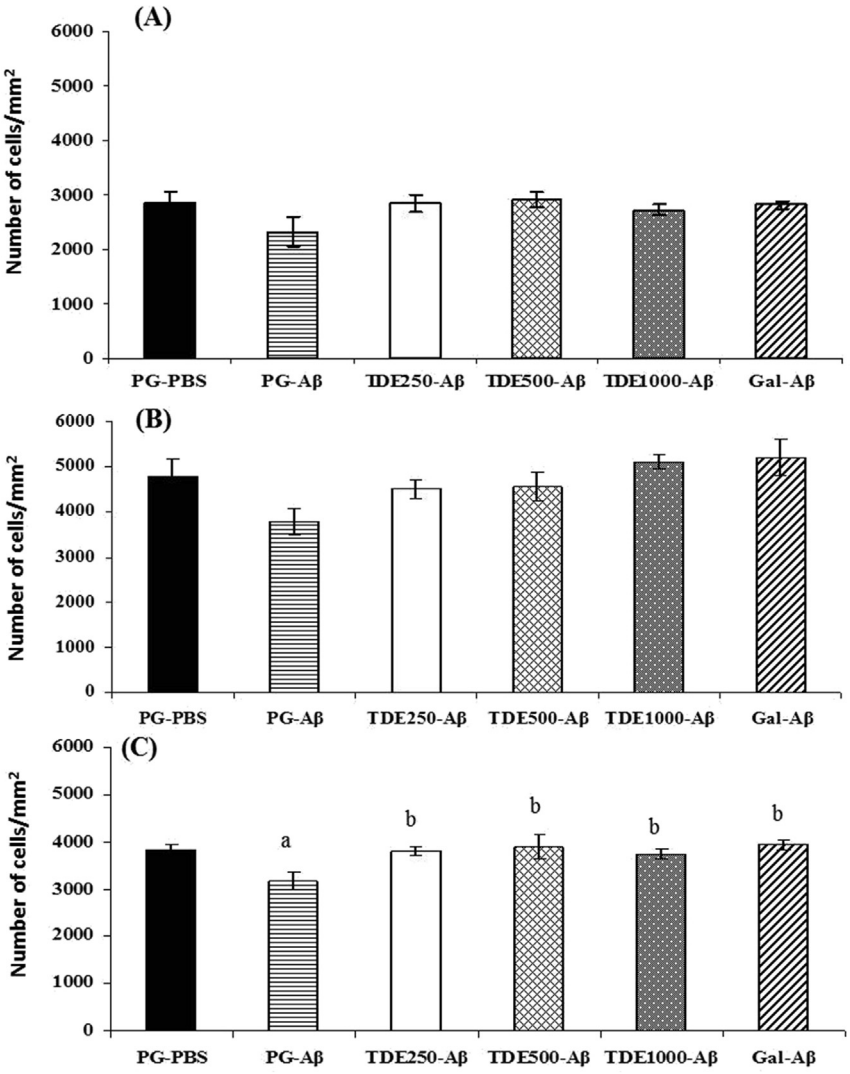


**Fig. 3.** Histology. Effect of TDE on neuronal densities in prefrontal cortex (A) and hippocampal CA1 (B) and CA3 (C) areas of $A\beta_{25-35}$ peptides induced mice. Each column is mean ± SEM for 4 mice in each group. Columns that have a different letter (a, b) differ significantly with each other ($p < 0.05$). (a) $P < 0.5$ compared to PG-PBS group, (b) $P < 0.05$ compared to PG-Aβ group.

peroxide both in the cerebral cortex and hippocampus as indicated by an increase of MDA level in $A\beta_{25-35}$ peptides-injected mice. These findings were in agreement with the previous studies demonstrating similar results in mice and rats.[35,39–41] For the histological studies, we found that the neuronal density of CA3 area was significantly decreased following i.c.v. injection of $A\beta_{25-35}$ peptides. This suggests the occurrence of neuronal cell damage when the mice were induced by $A\beta_{25-35}$ peptides.

The present study has demonstrated that TDE significantly improves memory deficit together with the decreased oxidative stress and increased neuronal density in hippocampus. Pretreatment with TDE for 28 days could restore the cognitive impairment in the NOR test induced by $A\beta_{25-35}$ peptides in accordance with our previous study,[22] that TDE showed the protective effects on $A\beta_{25-35}$ peptides-induced spatial reference memory impairment as tested in Morris water maze. Additionally TDE could attenuate the increase of lipid peroxidation induced by $A\beta_{25-35}$ peptides in the cerebral cortex and hippocampus. This was in accordance with the study of Rumzhum et al. and Gupta, et al.[24,42] An *in vitro* study revealed the antioxidant property of TD leave extract in assay of nitric oxide scavenging activity and reducing power test.[24] In addition, previous *in vivo* study has reported that TD extract possesses hepatoprotective effect by decreasing lipid peroxidation and increasing the level of anti-oxidant agents such as glutathione, superoxide dismutase, and catalase.[42] These agents play the important role in defense mechanisms to protect against free radical-induced cell damage. The imbalance between oxidant production and antioxidant defense system as the generation of oxidant exceeds the scavenging capacity lead to the occurrence of oxidative stress in pathogenesis of AD.[14] Furthermore, sub-chronic treatment of TDE exhibited neuroprotective effects on hippocampal neurons against damages induced by $A\beta_{25-35}$ peptides. This neuroprotection is correlated with behavioral alterations that TDE could prevent the memory impairment caused by $A\beta_{25-35}$ peptides. From our previous study, we found that TDE could decrease acetylcholinesterase (AChE) activity resulting in the increased acetylcholine (ACh) level in neuronal synaptic cleft and bring to the improvement of memory deficits induced by $A\beta_{25-35}$ peptides in behavioral testing.[22] Previous study showed that $A\beta_{25-35}$ peptides enhanced the level of AChE activity in the cerebral cortex and hippocampus probably resulting in memory impairment.[22] This enhancement of AChE activity induced by $A\beta_{25-35}$ peptides is

mediated by oxidative stress[43] which results in the occurrence of lipid peroxidation leading to the cellular dysfunction and neuronal death.[13,37] Based on these findings, we did suggest that the decrease of lipid peroxidation level might be due to enhanced ACh concentration and restore neuronal density and function.

## 5. Conclusion

It can be concluded that subchronic administration of TDE improves cognitive deficits induced by $A\beta_{25-35}$ peptides. This effect might be mediated by its antioxidant property and amelioration of neuronal damage.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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## References

1. Perry RJ, Hodges JR. Attention and executive deficits in Alzheimer's disease. *Brain*. 1999;122:383–404.
2. Querfurth HW, LaFerla FM. Alzheimer's disease. *N. Engl J Med*. 2010;362:329–344. http://dx.doi.org/10.1056/NEJMra0909142.
3. Goedert M, Spillantini MGA. Century of Alzheimer's disease. *Science*. 2006;314:777–781. http://dx.doi.org/10.1126/science.1132814.
4. Smith SA, Hirai K, Hsiao K, et al. Amyloid-β deposition in Alzheimer transgenic mice is associated with oxidative stress. *J Neurochem*. 1998;70:2212–2215.
5. Matsuoka Y, Picciano M, La Francois J, Duff K. Fibrillar β-amyloid evokes oxidative damage in a transgenic mouse model of Alzheimer's disease. *Neuroscience*. 2001;104:609–613.
6. Mohmmad Abdul H, Sultana R, Keller JN, St Clair DK, Markesbery WR, Butterfield DA. Mutations in amyloid precursor protein and presenilin-1 genes increase the basal oxidative stress in murine neuronal cells and lead to increased sensitivity to oxidative stress mediated by amyloid beta-peptide (1-42), HO and kainic acid: implications for Alzheimer's disease. *J Neurochem*. 2006;96:1322–1335. http://dx.doi.org/10.1111/j.1471-4159.2005.03647.x.
7. Li F, Calingasan NY, Yu F, et al. Increased plaque burden in brains of APP mutant MnSOD heterozygous knockout mice. *J Neurochem*. 2004;89:1308–1312.
8. Nishida Y, Yokota T, Takahashi T, Uchihara T, Jishage K, Mizusawa H, et al. Deletion of vitamin E enhances phenotype of Alzheimer disease model mouse. *Biochem Biophys Res Commun*. 2006;350:530–536.
9. Zhao Y, Zhao B. Oxidative stress and the pathogenesis of Alzheimer's disease. *Oxidative Med Cell Longev*. 2013;2013.
10. Praticò D. Oxidative stress hypothesis in Alzheimer's disease: a reappraisal. *Trends Pharmacol Sci*. 2008;29:609–615.
11. De Felice FG, Velasco PT, Lambert MP, et al. Aβ oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent mechanism that is blocked by the Alzheimer drug memantine. *J Biol Chem*. 2007;282:11590–11601.
12. Butterfield DA, Hensley K, Harris M, Mattson M, Carney J. β-amyloid peptide free radical fragments initiate synaptosomal lipoperoxidation in a sequence-specific fashion: implications to Alzheimer' s disease. *Biochem Biophys Res Commun*. 1994;200:710–715.
13. Uttara B, Singh AV, Zamboni P, Mahajan R. Oxidative stress and neurodegenerative diseases: a review of upstream and downstream antioxidant therapeutic options. *Curr Neuropharmacol*. 2009;7:65.
14. Zhao Y, Zhao B. Natural antioxidants in prevention and management of Alzheimer's disease. *Front Biosci (Elite Ed)*. 2011;4:794–808.
15. Barros MP, Poppe SC, Bondan EF. Neuroprotective properties of the marine carotenoid astaxanthin and omega-3 fatty acids, and perspectives for the natural combination of both in krill oil. *Nutrients*. 2014;6:1293–1317.
16. Ingkaninan K, Temkitthawon P, Chuenchom K, Yuyaem T, Thongnoi W. Screening for acetylcholinesterase inhibitory activity in plants used in Thai traditional rejuvenating and neurotonic remedies. *J Ethnopharmacol*. 2003;89:261–264.
17. Basavaraj P, Shivakumar SB, Shivakumar H, Manjunath VJ. Evaluation of anticonvulsant activity of Tabernaemontana divaricata (Linn) R. BR. Flower extract. *Int J Pharm Pharm Sci*. 2011;3:310–315.
18. Qamruzzamaa, J. A. A, M. S. Analgesic and anti-inflammatory effect of ethanolic extract of Tabernaemontana divaricata L. Flowers in rats. *Der Pharm Lett*. 2012;4:1518–1522.
19. Rahman M, Sayeed MA, Biplab KP, Siddique SA. Antidiabetic and cytotoxic activities of methanolic extract of Tabernaemontana divaricata (L.) leaves in alloxan induced mice. *Asian J Pharm Clin Res*. 2012;5:49–52.
20. Ingkaninan K, Changwijit K, Suwanborirux K. Vobasinyl-iboga bisindole alkaloids, potent acetylcholinesterase inhibitors from Tabernaemontana divaricata root. *J Pharm Pharmacol*. 2006;58:847–852.
21. Chattipakorn S, Pongpanparadorn A, Pratchayasakul W, Pongchaidacha A, Ingkaninan K, Chattipakorn N. Tabernaemontana divaricata extract inhibits neuronal acetylcholinesterase activity in rats. *J Ethnopharmacol*. 2007;110:61–68.
22. Nakdook W, Khongsombat O, Taepavarapruk P, Taepavarapruk N, Ingkaninan K. The effects of Tabernaemontana divaricata root extract on amyloid β-peptide 25–35 peptides induced cognitive deficits in mice. *J Ethnopharmacol*. 2010;130:122–126.
23. Henriques A, Melo AA, Moreno PR, Ene LL, Henriques JA, Schapoval EE. Ervatamia coronaria: chemical constituents and some pharmacological activities. *J Ethnopharmacol*. 1996;50:19–25.
24. Rumzhum NN, Rahman MM, Kazal MK. Antioxidant and cytotoxic potential of methanol extract of Tabernaemontana divaricata leaves. *Int Curr Pharm J*. 2012;1:27–31.
25. Dodart J-C, Bales KR, Gannon KS, et al. Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model. *Nat Neurosci*. 2002;5:452–457.
26. Liu J, Edamatsu R, Kabuto H, Mori A. Antioxidant action of Guilingji in the brain of rats with FeCl 3-induced epilepsy. *Free Radic Biol Med*. 1990;9:451–454.
27. Buege JA, Aust SD. [30] Microsomal lipid peroxidation. *Methods Enzym*. 1978;52:302–310.
28. de Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage: applications in experimental animals and in humans. *Free Radic Biol Med*. 1999;26:202–226.
29. Trump BF, Berezesky IK, Sato T, Laiho KU, Phelps PC, DeClaris N. Cell calcium, cell injury and cell death. *Environ health Perspect*. 1984;57:281.
30. Stepanichev MY, Zdobnova IM, Zarubenko II, et al. Amyloid-β (25–35)-induced memory impairments correlate with cell loss in rat hippocampus. *Physiol Behav*. 2004;80:647–655.
31. Maurice T, Su T-P, Privat A. Sigma 1 (σ 1) receptor agonists and neurosteroids attenuate β 25–35-amyloid peptide-induced amnesia in mice through a common mechanism. *Neuroscience*. 1998;83:413–428.
32. Alkam T, Nitta A, Mizoguchi H, Itoh A, Nabeshima T. A natural scavenger of peroxynitrites, rosmarinic acid, protects against impairment of memory induced by Aβ 25–35. *Behav Brain Res*. 2007;180:139–145.
33. Wang D, Noda Y, Zhou Y, et al. The allosteric potentiation of nicotinic acetylcholine receptors by galantamine ameliorates the cognitive dysfunction in beta amyloid25–35 icv-injected mice: involvement of dopaminergic systems. *Neuropsychopharmacology*. 2007;32:1261–1271.
34. Tsunekawa H, Noda Y, Mouri A, Yoneda F, Nabeshima T. Synergistic effects of selegiline and donepezil on cognitive impairment induced by amyloid beta (25–35). *Behav Brain Res*. 2008;190:224–232.
35. Ianiski FR, Alves CB, Souza AC, et al. Protective effect of meloxicam-loaded nanocapsules against amyloid-β peptide-induced damage in mice. *Behav Brain Res*. 2012;230:100–107.
36. Butterfield DA, Lauderback CM. Lipid peroxidation and protein oxidation in Alzheimer's disease brain: potential causes and consequences involving amyloid β-peptide-associated free radical oxidative stress 1, 2. *Free Radic Biol Med*. 2002;32:1050–1060.
37. Mattson MP. Pathways towards and away from Alzheimer's disease. *Nature*. 2004;430:631–639. http://dx.doi.org/10.1038/nature02621.
38. Zhang L, Yu H, Zhao X, et al. Neuroprotective effects of salidroside against beta-amyloid-induced oxidative stress in SH-SY5Y human neuroblastoma cells. *Neurochem Int*. 2010;57:547–555.
39. Yatin SM, Varadarajan S, Butterfield DA. Vitamin E prevents Alzheimer's amyloid ß-Peptide (1-42)-induced neuronal protein oxidation and reactive oxygen species production. *J Alzheimer's Dis*. 2000;2:123–131.
40. Um M-Y, Choi W-H, Aan J-Y, Kim S-R, Ha T-Y. Protective effect of Polygonum multiflorum Thunb on amyloid β-peptide 25-35 induced cognitive deficits in mice. *J Ethnopharmacol*. 2006;104:144–148.
41. Fu A-L, Dong Z-H, Sun M-J. Protective effect of N-acetyl-L-cysteine on amyloid β-peptide-induced learning and memory deficits in mice. *Brain Res*. 2006;1109:201–206.
42. Gupta M, Mazumder U, Kumar RS, Sivakumar T, Gomathi P. Antioxidant and protective effects of Ervatamia coronaria Stapf., leaves against carbon tetrachloride-induced liver injury. *Eur Bull Drug Res*. 2004;12:13–22.
43. Melo JB, Agostinho P, Oliveira CR. Involvement of oxidative stress in the enhancement of acetylcholinesterase activity induced by amyloid beta-peptide. *Neurosci Res*. 2003;45:117–127.