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Synergistic Effects of Probiotic Strains and *Abelmoschus esculentus* (L.) Moench (Okra) on Learning and Memory Impairment in Amnestic Rat Models

Nawapon Chaima[1], Thanyaphon Photi[2], Kotchakorn Klinprathap[3], Narawadee Choompoo[4], Thanyarat Lekchaoum[5], Supita Tanasawet[6], Suthkamol Suttikul[7], Pennapa Chonpathompikunlert[5] and Onrawee Khongsombat[1,8,*]

[1]*Department of Physiology, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000, Thailand*
[2]*Faculty of Medicine, Western University, Pathum Thani 12150, Thailand*
[3]*Faculty of Medicine, Vongchavalitkul University, Nakhon Ratchasima 30000, Thailand*
[4]*Department of Anatomy, Faculty of Medical Sciences, Naresuan University, Phitsanulok 65000, Thailand*
[5]*Pre-Clinical and Clinical Research Service Unit, Biodiversity Research Centre, Thailand Institute of Scientific and Technological Research, Pathum Thani 12120, Thailand*
[6]*Division of Health and Applied Sciences, Faculty of Science, Prince of Songkla University, Songkhla 90110, Thailand*
[7]*Expert Centre of Innovative Clean Energy and Environment, Thailand Institute of Scientific and Technological Research, Pathum Thani 12120, Thailand*
[8]*The Center of Excellence for Innovation in Chemistry, Faculty of Science, Mahidol University, Bangkok 10400, Thailand*

([*]Corresponding author's e-mail: onraweek@nu.ac.th)

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Abstract

This study investigated the neuroprotective effects of probiotics and okra on learning, memory, and antioxidant activity in a rat model of $A\beta_{25-35}$ induced cognitive impairment. Male Sprague Dawley rats divided into 8 groups: Control group, sham group, amyloid beta (Aβ) group, Aβ with low or high doses of probiotics, Aβ with okra, Aβ with okra and low or high doses of probiotics. Rats were orally gavage of probiotics, okra, or their combinations for 6 weeks and injected intracerebroventricularly with $A\beta_{25-35}$to induce Alzheimer's disease-like symptoms. Rats in the Aβ-treated group showed significant impairments in both spatial and recognition memory, as evidenced by decreased retention time in the Morris's water maze and reduced recognition index in the novel object recognition tests. These behavioral deficits were accompanied by elevated malondialdehyde (MDA) and superoxide dismutase (SOD) levels, and decreased catalase (CAT) activities, indicating increased oxidative stress. Treatment with probiotics, okra, or their combination significantly improved performance in both behavioral tests. Notably, the combined high-dose probiotic and okra group showed the most pronounced improvements. This group also exhibited the greatest reduction in MDA level and SOD activity and the most substantial restoration of CAT activity. These findings suggest that probiotics and okra, particularly in combination, exert the neuroprotective effects against $A\beta_{25-35}$-induced cognitive deficits, potentially through modulation of oxidative stress pathways. This combined therapy may represent a promising dietary intervention for the prevention or management of neurodegenerative disorders such as Alzheimer's disease.

Keywords: Okra, $A\beta_{25-35}$, Learning and memory, Alzheimer's disease, Probiotic, Oxidative stress, Antioxidant activity

Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, marked by memory loss, cognitive impairment, and behavioral changes [1,2]. The pathology of AD involves complex mechanisms, including synaptic dysfunction, neuronal death, neuroinflammation, and oxidative stress [3]. Amyloid-β (Aβ) accumulation is a major contributor to oxidative damage, as its insertion into neuronal membranes promotes excessive reactive oxygen species (ROS) generation, leading to lipid peroxidation, mitochondrial dysfunction, membrane disruption, and ultimately neuronal apoptosis with impaired synaptic plasticity [4,5]. This oxidative burden further disturbs antioxidant defenses; for example, superoxide dismutase (SOD) normally converts superoxide radicals into hydrogen peroxide, which is then detoxified by catalase (CAT) to water and oxygen, but dysregulation of this cascade amplifies AD pathology [6,7].

Among the various Aβ isoforms, the $A\beta_{25-35}$ fragment is widely used to model early AD pathology. This peptide contains the neurotoxic core of full-length Aβ, enabling rapid aggregation into β-sheet fibrils that induce oxidative stress, mitochondrial dysfunction, synaptic impairment, and neuronal death [8]. Thus, the $A\beta_{25-35}$ model reliably mimics early pathological features of AD and serves as a useful tool for evaluating potential neuroprotective agents [8-10].

Recent research also highlights the influence of gut microbiota on brain health and its potential role in neurodegenerative diseases such as AD [11,12]. An imbalance in the gut microbial community has been linked to increased systemic inflammation and cognitive impairment. In contrast, the administration of probiotics has shown promise in restoring microbial balance, reducing oxidative stress, and improving cognitive functions such as learning and memory [13-16]. Meanwhile, *Abelmoschus esculentus* (L.) Moench (commonly known as okra) is a functional food traditionally used in various medicinal systems. It contains bioactive compounds such as flavonoids, polysaccharides, and antioxidants, and has demonstrated potential in reducing oxidative stress and improving cognitive performance [17,18].

The synergistic effects of probiotics and okra on cognitive health remain underexplored. Investigating their combined efficacy in animal models can provide valuable insights into their potential role in managing learning and memory impairments. Therefore, in this study, the researchers aim to compare the efficacy of combined probiotics containing *Bifidobacterium longum* TISTR 2893 (*B. longum*) and *Lacticaseibacillus rhamnosus* TISTR 2716 (*L. rhamnosus*) with okra powder in improving learning, memory, and antioxidant activity in the brain. The study uses an amnestic rat model induced by amyloid-beta peptides, a widely used model that exhibits structural, chemical, and behavioral changes resembling those observed in AD patients.

Materials and methods

Preparation of probiotics powder and okra powder

Probiotics and okra powder were provided by the Thailand Institute of Scientific and Technological Research (TISTR). *Lacticaseibacillus rhamnosus* TISTR 2716 and *Bifidobacterium longum* TISTR 2893 were obtained from the TISTR culture collection, Thailand. Both strains were characterized and evaluated as probiotic candidates by the Biodiversity Research Centre, TISTR. The mixed probiotic powder was produced and supplied by the Innovative Center for Production of Industrially Used Microorganisms, Biodiversity Research Centre. For preparation, the 2 strains were cultured separately in de Man, Rogosa, and Sharpe broth (Himedia, India) for 8 - 10 h at 37 °C in a 5-L bioreactor under anaerobic conditions (no oxygen input, uncontrolled dissolved oxygen). Cultures were centrifuged at 9,000 rpm, 4 °C for 10 min. The cell pellets were washed twice with phosphate-buffered saline (PBS) and resuspended in 10% (w/v) cryoprotective agent to achieve 10^{10} CFU/mL, then freeze-dried. The freeze-dried powders of both strains were homogenized to ensure even distribution. The mixed probiotic powder was dissolved in distilled water and administered daily to rats by oral gavage at concentrations of 10^6 and 10^9 CFU/mL.

Okra was collected from the Nakhon Pathom province, Thailand, and was authenticated by the Bangkok herbarium: BK No. 171158 (Collector P. Chonpathompikunlert, No. 02), Department of Agriculture, Chatuchak, Bangkok, Thailand. Firstly, fresh okra fruits (*Abelmoschus Esculentus* L.) were

blanched in 0.1% NaCl solution. Then, it was dried in a hot air oven (UM 400, Memmert, Germany) at 55 °C for 24 h. The dried okra was ground into powder using a blender (HR2115/02, Philips, Thailand). Next, to extract the mucilage of dried okra, the okra powder was mixed with 85% ethanol solution, yielding 9.58% (w/w) of extract. The final extract was kept away from light and moisture for further use. High-performance liquid chromatography analysis was consequently applied to examine the okra contents of Quercetin-3-O-glucoside [19]. Okra extract was re-dissolved by distilled water to obtain concentration for use.

Reagent

Amyloid β-Protein Fragment 25 - 35 (A4559), 1,1,3,3-tetramethoxypropene (TMP, Cat. No. 108383), sodium dodecyl sulfate (SDS, Cat. No. 71729), thiobarbituric acid (TBA, Cat. No. T5500), and pyrogallol (Cat. No. P0381) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hydrogen peroxide (H_2O_2, Cat. No. 7722841) was obtained from Merck Millipore (Darmstadt, Germany).

Animals

Male Sprague Dawley rats weighing 200 - 250 g were purchased from the Nomura Siam International Co., Ltd., Bangkok, Thailand. The rats were kept in a room temperature 22 ± 1 °C, 55 ± 10% humidity, and exposed to 12:12 h light/dark cycle. Rats were provided with ad libitum access to food and tap water. All animal studies were reviewed under the ethical guidelines and policies of Naresuan University and were approved by the Ethics Committee of the Centre for Animal Research of Naresuan University (NU-AE620409).

The rats were divided into eight groups **(**n = 7**)** as follows: 1) A control group receiving a normal diet with water, 2) a sham group receiving a normal diet with intracerebroventricular injection (i.c.v.) of PBS (15 µL), 3) a amyloid beta (Aβ) group fed normally and given ICV amyloid- $\beta_{25\text{-}35}$ ($A\beta_{25\text{-}35}$) 10 nmol (15 µL), 4) a low-concentration probiotics (PL) group receiving $A\beta_{25\text{-}35}$ and normal food containing probiotic 1×10^6 CFU/mL, 5) a high-concentration probiotics (PH) group receiving $A\beta_{25\text{-}35}$ with normal food containing probiotic 1×10^9 CFU/mL, 6) an okra (OK) group receiving $A\beta_{25\text{-}35}$ and normal diet with okra (60 mg/kg BW.) 7) a low-concentration probiotics with okra (PLO) group receiving $A\beta_{25\text{-}35}$ and normal diet with probiotic 1×10^6 CFU/mL and okra (60 mg/kg), and finally 8) a high-concentration probiotics with okra (PHO) group receiving $A\beta_{25\text{-}35}$ and normal food with probiotic 1×10^9 CFU/mL and okra (60 mg/kg). Rats in groups 1 - 3 received an identical oral gavage of distilled water with the same volume and schedule as the treatment groups.

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

The experimental $A\beta_{25\text{-}35}$ peptide-induced rat was described previously [20]. $A\beta_{25\text{-}35}$ peptides were be dissolved in PBS (1 mg/mL) and incubated at 37 °C for 5 days for aggregation. $A\beta_{25\text{-}35}$ aggregation was observed under a light microscope to check the structures and globular aggregations. In week 4, the animals received an intraventricular injection of aggregated $A\beta_{25\text{-}35}$ peptide into the right lateral ventricle under anesthesia. The rat was anesthetized with isoflurane. Induction was performed in an induction chamber using 4% - 5% isoflurane in oxygen at a flow rate of 1 - 3 L/min. During surgery, anesthesia was maintained with 1% - 2% isoflurane in oxygen at a flow rate of 0.5 - 1 L/min. The rat was placed in a stereotaxic apparatus to identify the reference coordinates for intracerebroventricular (ICV) injection of $A\beta_{25\text{-}35}$. Ten microliters of PBS or Aβ was injected into the lateral ventricle of the brain in the co-ordinates (AP, − 0.8 mm; ML, 1.8 mm; DV, − 3.6 mm) [21].

Experimental design

Following a habituation period, rats received oral gavage of probiotics and okra powder for 6 weeks. Locomotor activity testing was conducted after 3 weeks, followed by Morris's water maze (MWM) training for 7 consecutive days. In the fourth week, $A\beta_{25\text{-}35}$ or PBS was administered via intraventricular injection. Subsequently, MWM testing and novel object recognition (NOR) assessments were performed over a 2-week period after $A\beta_{25\text{-}35}$ injections (**Figure 1**). At the end of the experiment, the animals were euthanized, and brain samples were collected for molecular analyses.

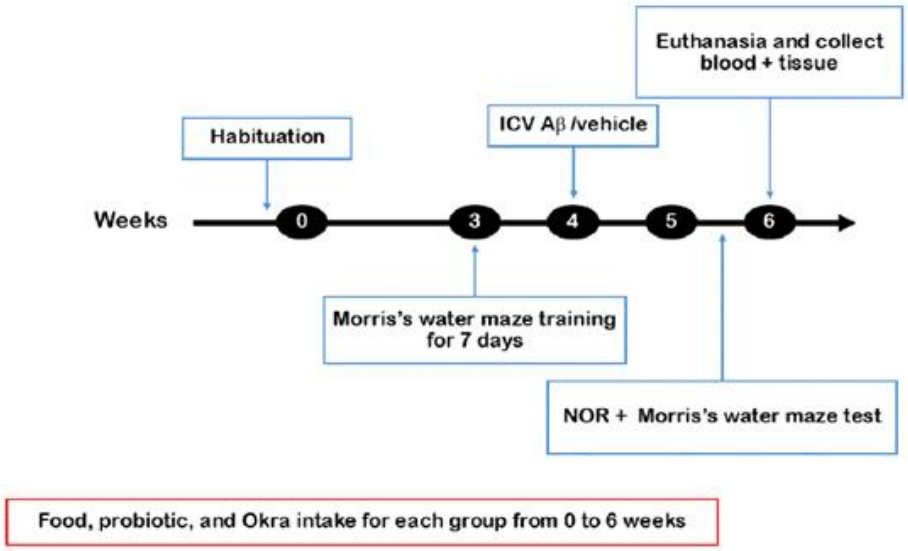

Figure 1 shows the experimental design diagram.

Morris water maze test

The Morris Water Maze (MWM) was conducted in a circular pool measuring 100 cm in diameter and 60 cm in height, filled with water maintained at 26 ± 2 °C. Inside the pool, there is a fixed-position platform with a diameter of 20 cm located in the center of 1 quadrant. The water level in the pool was set 2 cm higher than the platform level. The pool was divided into 4 quadrants labeled as A, B, C, and the probe quadrant, each marked with distinct cues. To obscure the platform from the rat's view, cornstarch was added to the water, giving it a semi-opaque appearance. During each experiment, rats were placed in the water facing the pool wall. Daily training sessions were conducted over a period of 7 days, each mouse was placed in the pool, allowed 60 s to find the hidden platform, and permitted to stay there for 10 s [22]. During training sessions, the time to find the hidden platform (escape latency) of 3 trials per day was recorded. In the test phase the next day, rats were placed in the pool without a platform, and their movements were recorded by a video camera. The recorded actions were then analyzed using Smart Junior 3.0 video tracking software from Panlab, Spain. The time spent in the quadrant where the platform was previously located was termed as retention time in seconds.

Novel object recognition

The NOR test was performed by using a 100×100×40 cm^3 square box. The objects used were white wooden objects of different shapes but similar sizes, placed centrally and 20 cm apart. Before testing, rats were allowed to acclimate in the empty box for 10 min. After acclimatization, they are ready for the training phase. During the familiarization phase, the rat spent 10 min in an open field with 2 objects: Object A and object B. During the test phase, the rat was returned to the box with a modification: Object B was replaced with a novel object C to evaluate recognition memory. Following each phase, the rat was returned to its cage for 10 min. The duration of object exploration was recorded when the rat's nose was within ≤ 1 cm of an object [22]. The time spent exploring objects A and C (TA and TC) was recorded and calculated as the percentage recognition index using the formula: (TA×100)/(TA + TC).

Tissue preparation

The prefrontal cortex and hippocampus were dissected from rat brains, immediately stored at –80 °C, and subsequently used for biochemical assays. Each tissue sample was homogenized in 0.1X phosphate-buffered saline (PBS, pH 7.4) and centrifuged at 9,000× g for 20 min at 4 °C. The resulting supernatant was collected for further biochemical analyses [22].

Oxidative stress

Malondialdehyde (MDA), the end product of lipid peroxidation, was measured using an acid reagent assay. Standard solutions of thiobarbituric acid reactive

substances (TBARS) and 1,1,3,3 tetramethoxypropene (TMP) at concentrations of 2, 4, 8, 16, 32, and 64 nmol/mL were prepared. The supernatant was combined with a reaction mixture containing 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid (pH adjusted to 3.5), and 0.8% thiobarbituric acid (TBA), followed by incubation at 95 °C for 60 min. MDA levels were then assessed at 532 nm and reported as nmol MDA per mg of protein according to Khongsombat *et al.* [23].

Antioxidant activity

The superoxide dismutase (SOD) activity was measured using an assay based on the enzyme's ability to inhibit the autooxidation of pyrogallol [24]. The reaction mixture comprised of 50 mM Tris EDTA (pH 8.2), 0.2 mM pyrogallol in 50 mM Tris HCl (pH 7.4), and the sample. The reaction kinetics were monitored by measuring the change in absorbance at 420 nm for 5 min at 25 °C. The percentage of inhibition was determined by comparing the results with a blank assay system. One unit of SOD activity was defined as the amount of SOD in the sample to inhibit pyrogallol oxidation by 50%, and the results were expressed as U/mg protein.

The Catalase (CAT) activity was assessed by evaluating the decomposition reaction of hydrogen peroxide (H_2O_2) into water and oxygen, following the method described by Beers and Sizer [25]. The reaction mixture contained 0.05 M sodium phosphate buffer (pH 7), 0.059 M H_2O_2 in the buffer, and the sample. The reaction kinetics were assessed by monitoring the changes in absorbance at 240 nm for 5 min at 25 °C. CAT activity was determined using the molar absorbance coefficient of H_2O_2 (43.6) and expressed as U/mg protein.

Protein quantification

Protein concentration in the brain tissue supernatant was quantified to normalize MDA, SOD, and CAT activities relative to protein content (mg protein). The analysis was carried out using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Briefly, the working reagent was combined with the supernatant, using bovine serum albumin as the calibration standard and 0.1 M PBS as the blank. The mixtures were shaken on a plate shaker for 30 s and then incubated at 37 °C for 30 min in a heat chamber. Absorbance was subsequently recorded at 562 nm with a microplate reader. This procedure follows the method originally reported by Smith *et al.* [26].

Statistical analysis

Statistical analysis was conducted using GraphPad Prism version 9 (GraphPad Software, Inc.). Results were displayed as mean ± standard error of the mean (SEM). Data compared a 1-way ANOVA with a post-hoc Bonferroni test. A significant level of $p < 0.05$ was considered to indicate a statistically significant difference.

Results and discussion

Effects of probiotics and Okra on Aβ$_{25-35}$-induced learning and memory impairment in the Morris water maze (MWM) test

We investigated the effects of probiotics and Okra on spatial learning and memory in rodents using the MWM test. During the training phase, all groups exhibited a progressive reduction in average escape latency time over repeated trials, indicating that all rats were able to remember the location of the platform before being induced into a state of memory impairment through Aβ$_{25-35}$-injection (**Figure 2(A)**).

In the testing phase (probe trial), the amyloid beta (AB) group spent significantly less time in the target quadrant compared to the sham control group, suggesting that Aβ$_{25-35}$-injection induced spatial memory deficits (**Figure 1(B)**). Notably, treatment with probiotics alone (PL and PH), okra alone (OK), or their combinations (PLO and PHO) significantly increased the time spent in the platform zone compared to the AB-injected group. These findings suggest that both probiotics and okra, whether administered alone or in combination, effectively reverse Aβ$_{25-35}$-induced spatial memory impairments (**Figure 2(B)**).

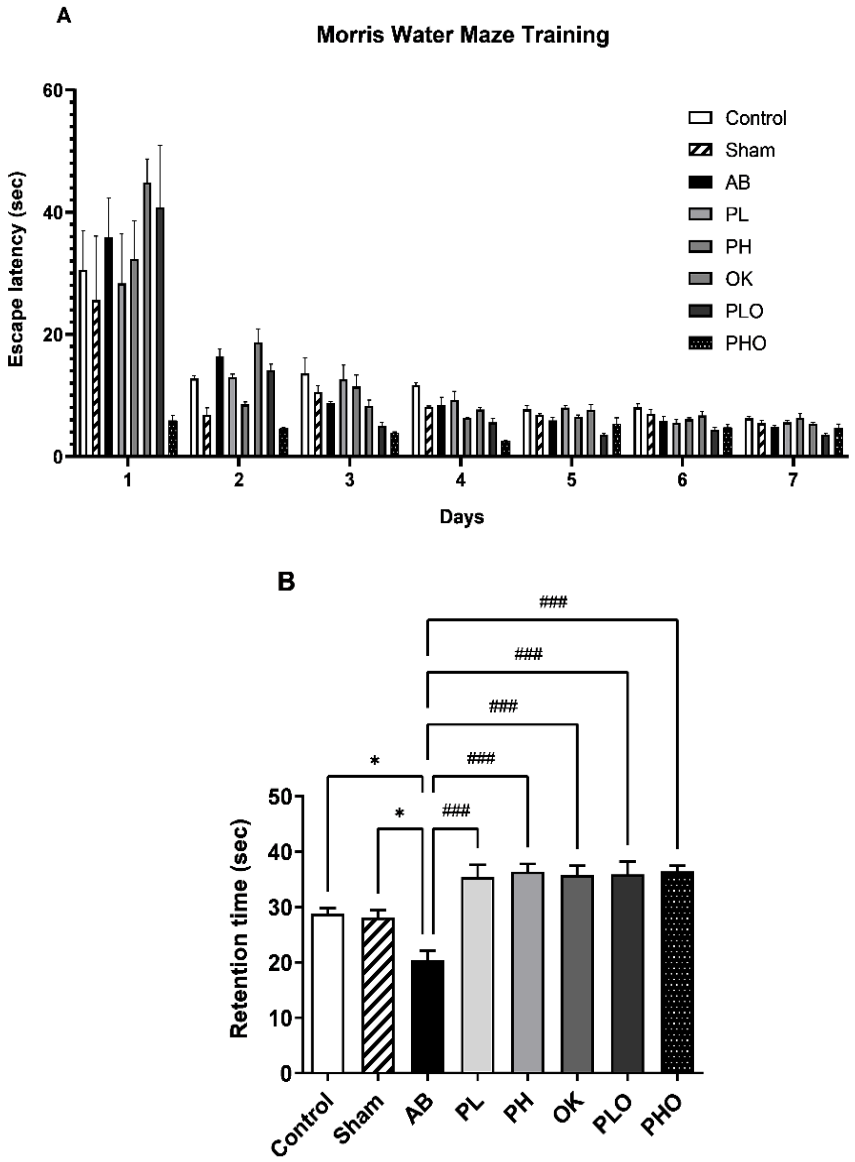

Figure 2 Effects of probiotics and okra on spatial memory: The escape latencies in a training session (A) and the retention time in a testing session (B). AB, amyloid beta; PL, low-concentration probiotics; PH, high-concentration probiotics; OK, okra; PLO, low-concentration probiotics with okra; and PHO, high-concentration probiotics with okra. Data were expressed as mean ± SEM (n = 7). $^{*}p < 0.05$ when compared to the control and sham groups and $^{###}p < 0.001$ when compared to the AB group.

Effects of probiotics and Okra on recognition memory ability

To further evaluate recognition performance, the Novel Object Recognition (NOR) test was conducted (**Figure 3**). Rats in the Aβ-treated (AB) group failed to distinguish between the novel and familiar objects, exhibiting significantly reduced exploration of the new object compared to the sham control group. This suggests a deficit in recognition memory, likely resulting from $A\beta_{25-35}$-induced cognitive impairment. However, the administration of probiotics alone (PL and PH), okra alone (OK), or their combinations (PLO and PHO) significantly increased exploration of the novel object (object C), as evidenced by a higher % recognition index compared to the AB group. These results indicated that both probiotics and okra could ameliorate the rats against recognition memory impairment which were induced by $A\beta_{25-35}$.

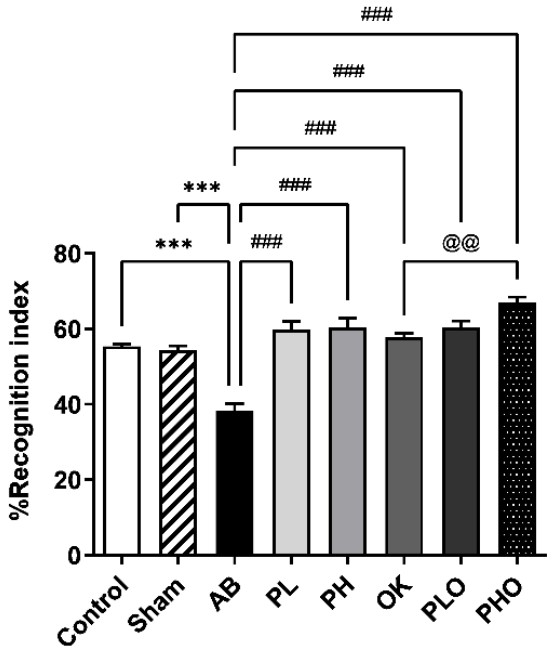

Figure 3 Effects of probiotics and okra on recognition memory. AB, amyloid beta; PL, low-concentration probiotics; PH, high-concentration probiotics; OK, okra; PLO, low-concentration probiotics with okra; and PHO, high-concentration probiotics with okra. Data were expressed as mean ± SEM (n = 7). $^{***}p < 0.001$ when compared to the control and sham groups, $^{###}p < 0.001$ when compared to the AB group, and $^{@@}p < 0.01$ when compared to the OK group.

Rats that received the intracerebroventricular $A\beta_{25-35}$ injection (AB group) exhibited significant impairments in both spatial and recognition memory, as demonstrated by decreased retention time in the MWM test and a reduced recognition index in the NOR test. These findings validate the successful establishment of an AD-like behavioral phenotype.

The probiotic strains *B. longum and L. rhamnosus* have been reported for their beneficial effects on brain health. Previous studies have shown that treatment of these strains, either alone or in combination, improved cognitive performance in mouse models of lipopolysaccharide (LPS)-induced systemic inflammation and cognitive deficits, as evidenced by Y-maze and NOR tasks [27]. Furthermore, oral administration of a probiotic mixture containing *B. longum and L. rhamnosus* has been shown to significantly improve cognitive function, memory, and depression in aging human populations [28]. Recent evidence has also highlighted the neuroprotective role of okra, which is rich in flavonoids and other antioxidant compounds. In AD mouse models, okra extract has been shown to improve learning and memory performance, as assessed by the Y-maze and MWM tests [29]. Consistent with these findings, our results demonstrated that administration of probiotics (PL and PH groups), okra (OK group), or their combination (PLO and PHO groups) significantly ameliorated $A\beta_{25-35}$-induced spatial and recognition memory deficits.

Effects of probiotics and Okra on lipid peroxidation and antioxidant activity

Lipid peroxidation was evaluated by measuring MDA levels in the prefrontal cortex (**Figure 4(A)**) and hippocampus (**Figure 4(B)**). The Aβ-treated (AB) group exhibited significantly elevated MDA levels in both brain regions compared to the control and sham groups. Treatment with probiotics (PL and PH), okra (OK), or their combinations (PLO and PHO) significantly reduced MDA levels compared to the AB group in both the prefrontal cortex and hippocampus. In the prefrontal cortex, the combination groups (PLO and PHO) demonstrated significantly lower MDA levels than the PL group alone. Notably, the PHO group exhibited the most reduction in MDA levels, with values significantly lower than those observed in the PH and OK groups.

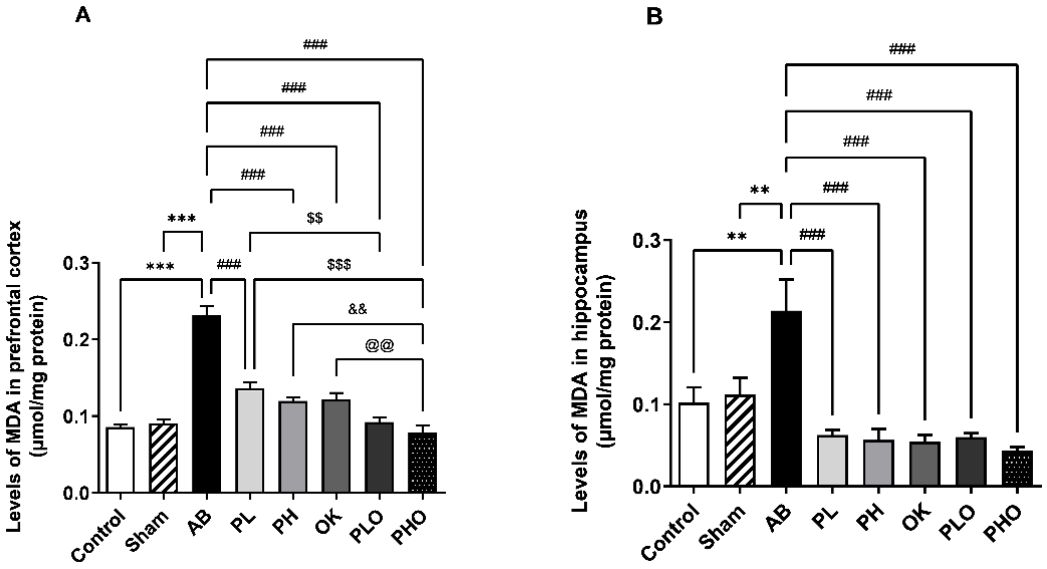

Figure 4 Effects of probiotics and okra on MDA levels in the prefrontal cortex (A) and the hippocampus (B). AB, amyloid beta; PL, low-concentration probiotics; PH, high-concentration probiotics; OK, okra; PLO, low-concentration probiotics with okra; and PHO, high-concentration probiotics with okra. Data were expressed as mean ± SEM (n = 7). $^{**}p < 0.01$ and $^{***}p < 0.001$ when compared to the control and sham groups, $^{###}p < 0.001$ when compared to the AB group, $^{\$\$}p < 0.01$ and $^{\$\$\$}p < 0.001$ when compared to the PL group, $^{\&\&}p < 0.01$ when compared to the PH group, and $^{@@}p < 0.01$ when compared to the OK group.

Superoxide dismutase (SOD) activity was significantly increased in the Aβ-treated (AB) group compared to the control and sham groups. However, treatment with probiotics, okra, or their combination led to a significant reduction in SOD activity compared to the AB group in both the prefrontal cortex (**Figure 5(A)**) and hippocampus (**Figure 5(B)**).

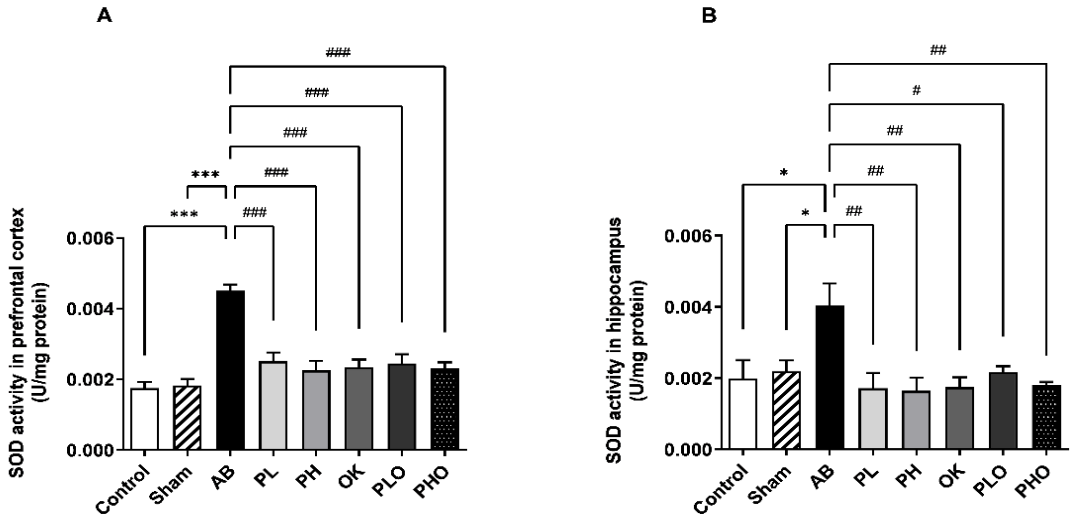

Figure 5 Effects of probiotics and okra on SOD activity in the prefrontal cortex (A) and the hippocampus (B). AB, amyloid beta; PL, low-concentration probiotics; PH, high-concentration probiotics; OK, okra; PLO, low-concentration probiotics with okra; and PHO, high-concentration probiotics with okra. Data were expressed as mean ± SEM (n = 7). $^{*}p < 0.05$ and $^{***}p < 0.001$ when compared to the control and sham groups, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$ when compared to the AB group.

Catalase (CAT) activity was significantly reduced in the Aβ-treated (AB) group compared to the control and sham groups in both the prefrontal cortex (**Figure 6(A)**) and hippocampus (**Figure 6(B)**). Conversely, the PL, PH, OK, PLO, and PHO groups exhibited a significant increase in CAT activity compared to the AB group. Notably, the PHO group demonstrated the most substantial enhancement in CAT activity, higher than those observed in the PL, PH, and OK groups.

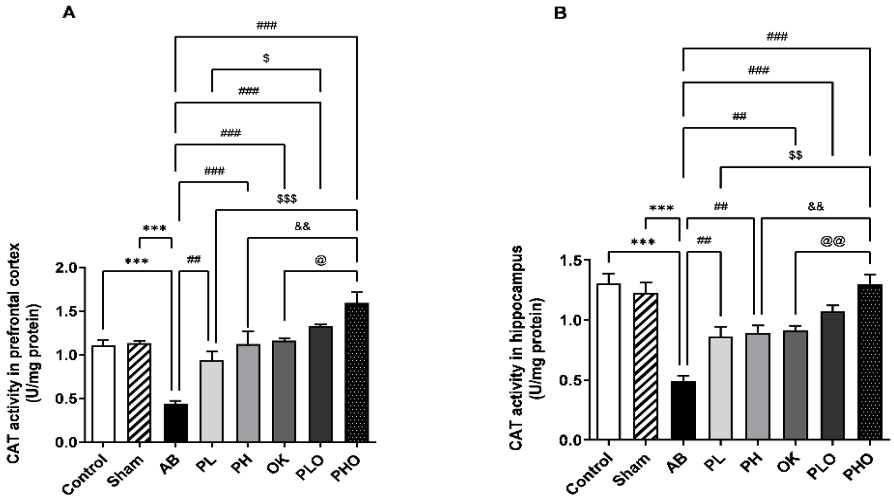

Figure 6 Effects of probiotics and okra on CAT activity in the prefrontal cortex (A) and the hippocampus (B). AB, amyloid beta; PL, low-concentration probiotics; PH, high-concentration probiotics; OK, okra; PLO, low-concentration probiotics with okra; and PHO, high-concentration probiotics with okra. Data were expressed as mean ± SEM (n = 7). $^{***}p < 0.001$ when compared to the control and sham groups, $^{\#\#}p < 0.01$ and $^{\#\#\#}p < 0.001$ when compared to the AB group, $^{\$}p < 0.05$, $^{\$\$}p < 0.01$ and $^{\$\$\$}p < 0.001$ when compared to the PL group, $^{\&\&}p < 0.01$ when compared to the PH group, $^{@}p < 0.05$ and $^{@@}p < 0.01$ when compared to the OK group.

In our study, $A\beta_{25-35}$-injected rats (AB) group exhibited significantly elevated levels of MDA and SOD activity, in association with reduced CAT activity in both the prefrontal cortex and hippocampus compared to the sham control group. These results are consistent with previous studies indicating that Aβ peptides induce oxidative damage by generating reactive oxygen species (ROS), leading to increased lipid peroxidation and disruption of the endogenous antioxidant defense system [30,31]. Elevated MDA reflects increased oxidative damage to lipids, while the observed dysregulation in SOD and CAT suggests an imbalance in redox homeostasis.

Importantly, treatment with probiotics (*B. longum and L. rhamnosus*) in both low (PL) and high (PH) doses, okra extract (OK), or their combinations (PLO and PHO), significantly ameliorated these oxidative abnormalities. All treatment groups showed decreased MDA level and SOD activity, and increased CAT activity, indicating restoration of the antioxidant defense system. Among the groups, the high-dose combination (PHO) produced the strongest effects, showing the greatest reduction in MDA levels and the highest increase in CAT activity. These findings suggest a synergistic interaction between probiotics and okra in strengthening antioxidant defenses.

The probiotic strains *B. longum and L. rhamnosus* have been reported for their beneficial effects on brain health. Previous studies have shown that treatment of these strains, either alone or in combination, improved cognitive performance in mouse models of lipopolysaccharide (LPS)-induced systemic inflammation and cognitive deficits, as evidenced by Y-maze and NOR tasks [27]. Furthermore, oral administration of a probiotic mixture containing *B. longum and L. rhamnosus* has been shown to significantly improve cognitive function, memory, and depression in aging human populations [28].

Consistent with previous reports, our results showed that treatment with probiotics, okra, or their

combination significantly attenuated $A\beta_{25-35}$-induced impairments in spatial and recognition memory. Importantly, this is the first evidence that combining probiotics with okra produces greater neuroprotective and antioxidant effects than either intervention alone. The synergistic benefit likely arises from okra's polyphenols and soluble fiber, which enhance probiotic viability and bioactivity, together with the ability of probiotics to modulate the gut-brain axis modulation making the combination more effective than each component independently. These neuroprotective effects are likely attributable to the complementary mechanisms of probiotics and okra: probiotics may modulate the gut-brain axis and suppress neuroinflammation, while the flavonoid-rich okra extract contributes through potent antioxidant actions, scavenging reactive oxygen species (ROS) and potentially interfering with Aβ oligomer formation [29]. Moreover, our recent *in vitro* study demonstrated that okra ethanolic extract, which contains Quercetin-3-O-glucoside (Q3G), effectively protected neuronal cells (SK-N-SH) from hydrogen peroxide-induced oxidative stress, reducing ROS accumulation and cellular senescence [19].

Conclusions

In conclusion, this study provides novel evidence that combining probiotics with okra yields synergistic neuroprotective and antioxidant benefits, surpassing the effects of either intervention alone. This multifaceted approach effectively counteracted $A\beta_{25-35}$-induced cognitive deficits, likely through attenuation of oxidative stress and restoration of antioxidant homeostasis. These results support further exploration of probiotics and plant-derived antioxidants as complementary therapeutic strategies in AD and related neurodegenerative disorders.

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Declaration of generative AI in scientific writing

During the preparation of this work the authors used Chat GPT to check grammar and spelling. After using this tool, the authors reviewed and edited the content as needed and took full responsibility for the content of the publication.

CRediT author statement

Nawapon Chaima: Methodology, Conceptualization, Formal analysis, Data curation, Writing-original draft. **Thanyaphon Photi:** Methodology. **Kotchakorn Klinprathap:** Methodology. **Narawadee Choompoo:** Methodology, Formal analysis, Data curation, Validation, Visualization, Writing-original draft. **Thanyarat Lekchaoum:** Methodology, Formal analysis, Data curation, Validation, Visualization, Resource, Writing-original draft. **Supita Tanasawet:** Methodology, Conceptualization, Data curation, Validation, Writing-original draft. **Suthkamol Suttikul:** Methodology, Conceptualization, Formal analysis, Data curation, Validation, Project administration, Resource, Writing-original draft. **Pennapa Chonpathompikunlert:** Methodology, Conceptualization, Formal analysis, Data curation, Supervision, Validation, Visualization, Investigation, Project administration, Resource, Writing-original draft. **Onrawee Khongsombat:** Methodology, Conceptualization, Formal analysis, Data curation, Supervision, Validation, Visualization, Investigation, Project administration, Resource, Writing-original draft.

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