Journal of Medicinal Materials, 2026, Vol. 31, No. 1+2 (pp. 123 – 130)

EVALUATION OF MEMORY-ENHANCING EFFECTS OF *MORINDA OFFICINALIS* HOW. ROOT EXTRACTS IN A TRANSGENIC *hAPP DROSOPHILA* MODEL OF ALZHEIMER'S DISEASE

Nguyen Van Hiep[1], Pham Thi Nguyet Hang[1,*], Trinh Dinh Luc[2], Leu Khanh Duy[1], Pham Anh Tung[1], Nguyen Thi Phuong[1], Nguyen Van Tai[1]

[1]*National Institute of Medicinal Materials (NIMM), Hanoi 11018, Vietnam;*

[2]*Hanoi University of Pharmacy, Vietnam*

*Corresponding author: nguyethangpt@nimm.org.vn

Received February 24th, 2026 Accepted March 12th, 2026

Summary

This study aimed to evaluate the memory-enhancing effects of the 50% ethanolic extract and four fractions (aqueous, *n*-butanol, ethyl acetate, and *n*-hexane) from *Morinda officinalis* How. (MO) roots using a transgenic hAPP *Drosophila* model of Alzheimer's disease. Screening of acetylcholinesterase (AchE) inhibitory activity showed that both the ethanolic and four fractions exhibited inhibitory effects, with the *n*-hexane fraction demonstrating the strongest activity. In the learning memory assay using *Drosophila* larvae, the ethanolic extract and three fractions (*n*-butanol, ethyl acetate, and *n*-hexane) improved odor memory more effectively than the aqueous fraction. Further assessments of locomotor activity and survival ability identified the ethanolic extract and *n*-butanol fraction as the most promising candidates. Moreover, the ethanolic extract showed antioxidant ability by significantly decreasing the concentration of MDA in the head homogenates of adult flies. These results suggest that MO extracts, especially the ethanolic extract and *n*-butanol fraction, have potential memory-enhancing and neuroprotective effects in a transgenic *Drosophila* model of Alzheimer's disease.

Keywords: Alzheimer's disease; *Transgenic hAPP Drosophila*; *Morinda officinalis* How.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by early memory loss and cognitive decline, eventually affecting behavior, speech, visuospatial orientation, and motor systems. The disease develops over 20-30 years, with symptoms becoming pronounced in individuals over 65 years old. AD is the most common form of dementia, accounting for approximately two-thirds of global dementia cases. Worldwide, it is estimated that around 41 million people with dementia remain undiagnosed, with only 25% of cases clinically identified [1],[2].

Current treatments for AD are symptomatic and primarily include acetylcholinesterase (AchE) inhibitors, which can reduce cognitive symptoms but often produce undesirable side effects. Non-pharmacological interventions such as physical exercise, memory training, music therapy, and art therapy aim to maintain or improve cognitive function and quality of life [3]. However, there remains a critical need for more effective therapies with fewer adverse effects.

In traditional medicine, the root of *Morinda officinalis* How. (MO) has been widely used as a tonic and for the treatment of various ailments, including kidney deficiency, inflammation, and cognitive disorders [4-6]. Oligosaccharides isolated from the roots of *M. officinalis* can ameliorate β-amyloid–induced dementia in rats by enhancing antioxidant capacity, activating brain energy metabolism, and improving cholinergic system dysfunction [7]. Previous studies have indicated that *M. officinalis* oligosaccharides can attenuate the progression of AD in animal models through modulation of gut microbiota diversity and metabolic profiles. However, the precise associations between gut microbial composition and metabolic alterations have not yet been fully clarified [8]. In addition, several classical prescriptions in traditional Chinese medicine, such as Tian-Si-Yin and Bajitianwan, include MO roots for the treatment of memory loss and osteoporosis [9],[10]. Despite its traditional use for improving memory, studies on the neuroprotective and memory-enhancing effects of MO in Alzheimer's disease are limited. Therefore, this study aims to investigate the memory-enhancing and neuroprotective potential of MO extracts using a transgenic *Drosophila* model of Alzheimer's disease.

2. Materials and Methods

2.1. Plant Material and Extract Preparation

Roots of *Morinda officinalis* How. (5 years old) were collected in November 2024 from Phu Tho Province, Vietnam, and authenticated by MSc. Phan Van Truong, the Center of Medicinal Plant Resources, National Institute of Medicinal

Materials, according to the Vietnamese Pharmacopoeia V. The roots were cleaned, debarked, sliced, and dried at 50°C until the moisture content was below 10%. Dried material (2 kg) was extracted with 50% ethanol (8 L × 3) at room temperature, followed by hot extraction at 60°C for 3 hours. The extract was concentrated under reduced pressure at 55°C to yield 1.25 kg of crude 50% ethanolic extract (MOE) with a moisture content of 1.65%. 500 g of MOE (equivalent to 800 g of dried roots) was suspended in 1 L of distilled water and successively partitioned with *n*-hexane, ethyl acetate, and *n*-butanol to yield corresponding fractions. Solvents were removed under reduced pressure. Extraction yields are shown in Table 1.

Table 1. Extraction yields obtained from dried, core-removed *M. officinalis* How. Roots

Extract/Fraction	Dry weight (g)	Yield (%)
50% Ethanolic extract (MOE)	725.00	62.500
Aqueous fraction (MOW)	414.29	51.780
n-Butanol fraction (MOB)	29.48	3.380
Ethyl acetate fraction (MOEt)	1.25	0.155
n-Hexane fraction (MOH)	0.195	0.024

2.2. Evaluation of Acetylcholinesterase Inhibitory Activity

Acetylcholinesterase (AchE) inhibitory activity was assessed using a colorimetric method using Ellman's reagent, originally described in 1961 [11]. The principle of the assay is as follows: the substrate acetylthiocholine iodide (AchI) is hydrolyzed by AchE to generate thiocholine. This product subsequently reacts with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) to form the yellow-colored compound 5-thio-2-nitrobenzoic acid, the intensity of which is proportional to AchE enzymatic activity. The absorbance of the reaction mixture is then measured at 412 nm to determine AchE activity.

MO extracts were dissolved in DMSO and diluted in distilled water to final concentrations of 31.25, 62.5, 125, 250, and 500 μg/mL. Tacrine (Tac, positive control) was dissolved in distilled water and diluted to concentrations of 3.125, 6.25, 12.5, 25, 50, and 100 ng/mL. The assay was conducted in 96-well ELISA plates with two groups: wells containing AchE enzyme (test wells) and wells without enzyme (blank wells). All experiments were carried out at room temperature (25°C), and each reaction was performed in triplicate.

Each well received 25 μL of distilled water (control group) or 25 μL of plant extract/Tac solution (test group), followed by 200 μL of reaction buffer containing 25 μL of 15 mM AchI, 125 μL of 3 mM DTNB, and 50 μL of 0.1 M phosphate buffer (pH 8.0). Then, 25 μL of AchE (0.025 U/mL) was added to the test wells, whereas 25 μL of 0.1 M phosphate buffer (pH 8.0) was added to blank wells. Plates were incubated for 15 minutes at room temperature in the dark, after which absorbance was recorded at 412 nm. The percentage inhibition of AchE activity (I%) was calculated using the following formula:

$$\%\,\mathrm{I} = \frac{(\mathrm{Odcontrol + enzyme - Odcontrol\ blank}) - (\mathrm{Odsample - Odsample\ blank})}{\mathrm{Odcontrol + enzyme - Odcontrol\ blank}}\ x\ 100$$

The half-maximal inhibitory concentration (IC_{50}) was calculated by nonlinear regression using a dose-response inhibition model with a variable slope (four-parameter logistic model) in GraphPad Prism 8 software.

2.3. Generation of an Alzheimer's Disease Drosophila Model

In this study, the expression of the human APP protein in the *Drosophila* brain was achieved using the GAL4/UAS binary gene expression system. This system uses two parental fly lines: a driver line carrying the genotype w; +; Elav-GAL4 and a responder line carrying w; UAS-hAPP.HA. When these two lines are crossed, the F1 progeny inherit both Elav-GAL4 and UAS-hAPP.HA transgenes. Under the control of the neuron-specific promoter Elav, the GAL4 transcription factor is expressed and binds to the upstream activation sequence (UAS) preceding the target hAPP gene, thereby activating its transcription. As a result, the human amyloid precursor protein (APP) is produced and expressed in the *Drosophila* brain. When APP is expressed and released from neurons, it can accumulate and form amyloid aggregates, hallmark pathological features of Alzheimer's

disease [12]. The aggregation of amyloid-β peptides and related toxic amyloid species can ultimately lead to neuronal death and progressive AD-like neurodegeneration. Following genetic crossing, the resulting transgenic flies were used for behavioral assays relevant to Alzheimer's disease. All experiments were performed in parallel among the control group (Elav-Gal4), the pathological group (Elav-hAPP), and the treated group.

Flies were maintained at 25±1°C under a 12-h light-dark cycle (light on: 07:00-19:00) on standard medium containing 1% agarose, 5% sucrose, 5% dry yeast, 3% milk powder, and preservatives (sodium benzoate, propionic acid). The food was replaced every 2-3 days to ensure optimal nutritional conditions for growth.

2.4. Odor-taste learning assays in Drosophila larvae

This assay was performed following the method described by Gerber et al. (2013) [13]. Short-term associative memory was assessed in F1 larvae derived from parental flies reared on diet supplemented with the 50% ethanolic extract of *Morinda officinalis* and its *n*-butanol, ethyl acetate, *n*-hexane, and aqueous fractions.

Third-instar larvae (day 3) were trained on two types of petri dishes: agar plates containing 1% agar and 2 M sucrose (dish X), and agar plates containing 1.5% agar without sucrose (dish Y). Two odorants were used: octanol (OCT; Sigma-Aldrich, St. Louis, MO, USA) and n-amyl acetate/paraffin (1:50, AM Merck Millipore, Burlington, MA, USA). A total of 24 larvae were collected, washed with 1× PBS to remove food residues, and sequentially trained on dishes X and Y containing different odorants for 5 minutes each. The training cycle was repeated three times.

For testing, larvae were placed for 3 minutes on an agar plate divided into two halves, one containing AM odor and the other containing OCT. After testing, the number of larvae on each side was counted. Learning performance was quantified using the Learning Index (LI):

$$LI = \frac{PREF_{AM} - PREF_{OCT}}{2}$$

where $PREF_{AM}$ and $PREF_{OCT}$ represent the preference indices for AM and OCT, respectively, and LI is the learning index. The preference indices were calculated as

$$PREF = \frac{\#AM - \#OCT}{\#Total}$$

(#: the number of larvae observed on the respective half of the test dish)

Each experimental batch consisted of approximately 144-168 larvae, with 24-28 larvae per group. Three replicates were conducted for AM-associated learning and three for OCT-associated learning.

2.5. Crawling Assay in Drosophila larvae

This experiment was conducted according to the method of Nichols et al. (2012) [14] to evaluate improvements in locomotor behavior in the Drosophila Alzheimer's disease model.

Approximately 30-32 male third-instar larvae were randomly selected and washed with PBS to remove residual food. Larvae were transferred onto 1.5% agar plates at a density of 3-4 larvae per dish. Their movements were recorded using a digital camera at 640×480 resolution, 30 frames per second, for 60 seconds. Recorded videos were processed and analyzed using ImageJ (NIH, USA) with the wrMTrck plugin (developed by Dr. Jesper Søndergaard Pedersen) to determine average crawling velocity (AV) and movement trajectories.

2.6. Viability Assay in Adult Drosophila

The survival assay was performed following Piper et al. (2016) [15] to evaluate whether MO extracts improved lifespan in adult *Drosophila* expressing Alzheimer's disease phenotypes.

Adult male and female flies were separated and placed into food vials according to their experimental groups. Each group consisted of 140-160 adult flies (1/2 male and ½ female). Vials were kept at 25±1°C, 50% humidity, under a 12 h light/12 h dark cycle. The food was replaced every 2-3 days; the number of dead flies was recorded at each transfer, and this process continued until no individuals survived.

2.7. Antioxidant Activity in Fly Head Homogenates (MDA Assay)

Malondialdehyde (MDA) is a lipid peroxidation product and serves as a biomarker of oxidative membrane damage. The assay relies on the reaction of MDA with thiobarbituric acid (TBA) under acidic and high-temperature conditions, forming a pink MDA-TBA adduct with a maximal absorbance at 532 nm. The measured absorbance is proportional to the MDA concentration in the sample, and elevated MDA levels indicate enhanced lipid peroxidation, a hallmark of oxidative stress. The assay was performed following the methods of Subedi et al. (2017) and Wu et al. (2025) [16],[17]. Approximately 240-280 three-day-old adult flies

from each experimental group were collected and stored at −80°C. Forty fly heads (20 males and 20 females) were dissected on ice and transferred to 2 mL eppendorf tubes. Samples were homogenized in 240 μL of 1× PBS using a tissue homogenizer at 30 strokes/s for 5 minutes, and this procedure was repeated twice. Then, 100 μL of the resulting homogenate was reacted with 450 μL of 0.37% TBA in acidified solution H_2SO_4, whereas 100 μL of the corresponding blank sample received 450 μL of 1× PBS instead. Tubes were incubated in a boiling water bath (100°C) for 15 minutes. After incubation, samples were cooled in ice water, and 5 μL of 1 N HCl was added to terminate the reaction. Tubes were centrifuged at 800 g for 10 minutes. A total of 150 μL of the supernatant was transferred to a 96-well plate, and absorbance was measured at 532 nm.

3. Results

3.1. Inhibitory Effect on Acetylcholinesterase Activity

The cholinergic system is severely impaired in AD due to the degeneration of acetylcholine (Ach) producing neurons, particularly those in the nucleus basalis of Meynert and other memory-related regions such as the hippocampus and cerebral cortex. The decline in Ach levels leads to deficits in learning, memory, and attention. Acetylcholinesterase is an enzyme involved in cholinergic neurotransmission, playing a key role in the hydrolysis of the neurotransmitter Ach into choline and acetate [18].

Through the assay evaluating the acetylcholinesterase inhibitory activity of various extracts from MO roots, using Tac as the positive control and the Ellman colorimetric assay, we obtained the half-maximal inhibitory concentration (IC_{50}) values presented in Table 2.

Table 2. IC_{50} values for AchE inhibition of MO root extracts and Tac.

Sample	IC_{50} (μg/mL)
MOW	437.7 (417.2 – 460.7)
MOE	420.4 (401.1 – 441.9)
MOEt	363.2 (352.1 – 375.0)
MOB	346.5 (326.6 – 368.5)
MOH	212.7 (203.3 – 222.7)
Tac	0.03978 (0.03629 – 0.0437)

All MO root extracts exhibited AchE inhibitory activity in a concentration-dependent manner. Among the tested samples, the *n*-hexane (MOH) and *n*-butanol (MOB) fractions showed the strongest inhibitory effects, followed by the ethyl acetate fraction (MOEt), 50% ethanolic extract (MOE), and aqueous fraction (MOW). Nevertheless, the inhibitory potency of all MO extracts was moderate and markedly lower than that of the reference inhibitor tacrine, indicating that MO possesses measurable but non-potent AchE inhibitory activity.

3.2. Short-Term Memory Improvement in Drosophila Larvae

AD is a progressive neurodegenerative disorder characterized by impairments in memory, cognition, and behavior; therefore, improving memory function is an essential parameter in evaluating potential therapeutic effects. We assessed short-term memory in F1 *Drosophila* larvae generated by crossing male flies carrying the hAPP transgene with virgin female flies of the Elav-Gal4 strain. The resulting larvae were reared on standard food supplemented with MOE and MOW at concentrations of 2, 4, and 6 mg/mL; MOB, MOEt, and MOH fractions at 0.5 and 1 mg/mL; and Tac as the positive control at 0.05 mg/mL. The results of the short-term memory assay are shown in Fig. 1.

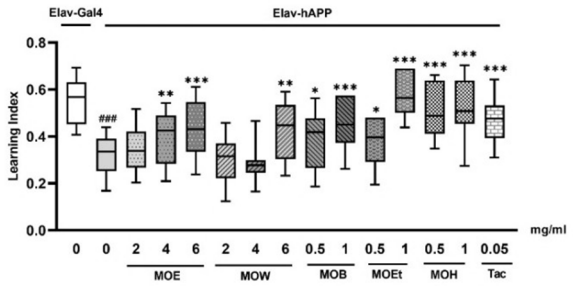

Fig. 1. Effects of MO extracts on short-term memory performance in *Drosophila* larvae.
(n = 24-30, one-way ANOVA); $^{###}p<0.001$ *compared to Elav-Gal4 group* $*p<0.05$, $**p<0.01$, $***p<0.001$ *compared to Elav-hAPP group.*

The data presented in Fig. 1 show that the Learning Index (LI) of larvae in the Elav-Gal4 group was significantly higher than that of the Elav-hAPP group ($p<0.001$). Treatment with MO extracts significantly improved learning index (LI) values in a concentration- and fraction-dependent manner. MOE significantly improved memory performance at 4 and 6 mg/mL ($p<0.01$ and $p<0.001$, respectively), whereas the aqueous fraction showed improvement only at the highest concentration

tested. Both MOB and MOEt significantly increased LI values at 0.5 and 1 mg/mL ($p<0.05$ and $p<0.001$, respectively). The MOH fraction produced robust improvements in memory at both tested concentrations ($p<0.001$). Positive control treatment with tacrine also significantly restored memory performance ($p<0.001$). These findings demonstrate that several MO, particularly MOE, MOB, and MOH, effectively ameliorate short-term memory deficits in the *Drosophila* Alzheimer's model.

3.3. Improvement of Locomotor Ability in Drosophila Larvae

Motor dysfunction is also a characteristic feature of AD progression. Restoration of motor function reflects the potential therapeutic benefits against AD. Third-instar *Drosophila* larvae expressing the AD phenotype were collected on day 3 and evaluated for locomotor performance based on their average crawling speed and trajectory patterns. The results are presented in Fig. 2.

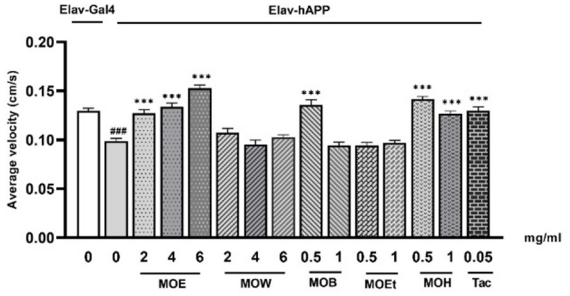

Fig. 2. Effects of MO extracts on locomotor ability in *Drosophila* larvae. (n = 28-32, one-way ANOVA); *###p<0.001 compared to Elav-Gal4 group;***p<0.001 compared to Elav-hAPP group.*

The results in Fig. 2 show that the average crawling speed (AV) of larvae in the Elav-Gal4 group was significantly higher than that of the Elav-hAPP group ($p<0.001$). Treatment with MOE significantly increased average crawling velocity at all tested concentrations ($p<0.001$). MOB improved locomotor ability at 0.5 mg/mL but not at 1 mg/mL, whereas MOEt did not produce significant effects. In contrast, MOH significantly enhanced crawling velocity at both concentrations tested ($p<0.001$). Tacrine treatment also significantly restored locomotor activity ($p<0.001$). These results indicate that MO, particularly MOE and MOH, improve motor dysfunction associated with APP expression.

3.4. Effects on Lifespan Extension in Adult Drosophila

The median survival time during the lifespan of *Drosophila* was assessed at the point when 50% of the individuals in each population remained alive. This assay was conducted to evaluate the ability of MO root extracts to extend the lifespan of adult flies. The results are presented in Fig. 3.

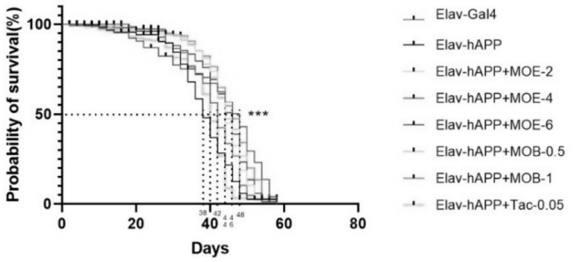

Fig. 3. Effects of MOE and MOB on the survival of adult *Drosophila* (n = 140-160, log-rank test, $p<0.001$).

The median survival time (50% survival) of the Elav-Gal4 group (48 days) was longer than that of the Elav-hAPP group (38 days) with a statistically significant difference ($p<0.001$). Adult flies expressing human APP showed a significant reduction in median lifespan compared with control flies. Dietary supplementation with MOE significantly extended median survival in a dose-dependent manner (42, 44, and 46 days, respectively). Similarly, MOB treatment at both tested concentrations markedly prolonged lifespan relative to the pathological control (44 and 46 days). In contrast, tacrine treatment did not significantly increase median lifespan (40 days). These results suggest that MOE and MOB exert protective effects on organismal survival in the *Drosophila* Alzheimer's model.

3.5. Evaluation of the antioxidant activity of MO in the flies' head homogenates

MDA is a byproduct of lipid peroxidation. In the brain, lipid-rich tissues are essential for maintaining structural integrity and supporting neuronal metabolic functions. Therefore, increased lipid peroxidation may lead to neuronal damage and contribute to various neurological disorders. Quantification of MDA is a widely used method to assess the antioxidant capacity of

MO root extracts in the hAPP transgenic *Drosophila* Alzheimer's model. A reduction in MDA levels compared with the pathological control indicates that the extract decreases lipid peroxidation. The results are shown in Fig. 4.

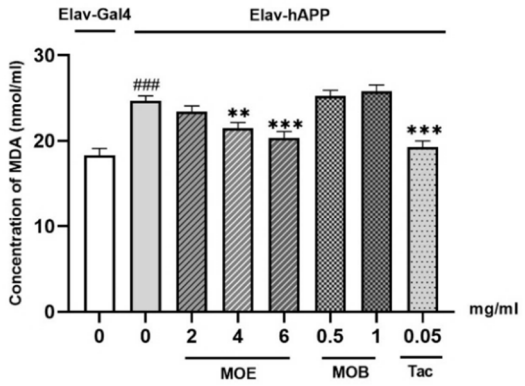

Fig. 4. Effects of MOE and MOB on MDA levels in *Drosophila* head homogenates. (n = 6-7, one-way ANOVA), $^{###}p<0.001$ *compared to Elav-Gal4 group;* $^{**}p<0.01$, $^{***}p<0.001$ *compared to Elav-hAPP group.*

APP-expressing flies exhibited significantly elevated malondialdehyde (MDA) levels, indicating increased lipid peroxidation and oxidative stress ($p<0.001$). Treatment with MOE significantly reduced MDA levels at 4 and 6 mg/mL ($p<0.01$ and $p<0.001$), whereas the lower dose showed no significant effect. MOB did not significantly alter MDA levels at the tested concentrations. Tacrine treatment significantly reduced MDA levels compared with the pathological control ($p<0.001$). These results indicate that MOE effectively attenuates oxidative damage in the fly brain, which may contribute to its neuroprotective effects.

4. Discussion

In the present study, we demonstrated that root-derived extracts of MO, particularly the 50% ethanolic extract (MOE) and the *n*-butanol fraction (MOB), exert pronounced memory-enhancing and neuroprotective activities in a transgenic *Drosophila melanogaster* model expressing human APP. These beneficial outcomes were reproducibly observed across multiple experimental endpoints, including moderate inhibition of AChE, restoration of short-term associative memory, improvement of larval locomotor activity, extension of adult lifespan, and mitigation of oxidative damage in *Drosophila* brains. Taken together, these findings indicate that MO mediates neuroprotection via a multi-target mode of action, consistent with reports on other neuroactive natural compounds.

Both MOE extract and fractions exhibited moderate AChE inhibitory effects, with MOH and MOB fractions displaying the greatest activity. This pattern aligns with phytochemical evidence indicating that MO roots are enriched in anthraquinones, iridoid glycosides, polysaccharides, coumarins, and triterpenoids, which preferentially distribute into relatively non-polar organic fractions [4, 19]. Anthraquinones and triterpenoids have been shown to interact with both the catalytically active site and the peripheral anionic site of AChE through hydrophobic contacts and π-π stacking interactions, leading to biologically meaningful yet moderate enzyme inhibition [20]. Although the AChE inhibitory efficacy of MO was lower than that of the reference compound tacrine, comparable degrees of inhibition have been documented for numerous multi-component herbal preparations, whose cognitive benefits arise from synergistic regulation of multiple pathological processes rather than strong suppression of a single molecular target [9],[10]. Accordingly, the observed AChE inhibition supports the view that the neuroprotective actions of MO reflect a multi-mechanistic pharmacological profile rather than potent cholinesterase inhibition alone.

MO extracts markedly alleviated short-term associative memory impairments in APP-expressing flies. This observation is consistent with a growing body of evidence demonstrating the cognition-enhancing potential of MO across diverse experimental paradigms. Previous studies in mammalian models have shown that polysaccharides and oligosaccharides derived from MO ameliorate learning and memory deficits induced by aging, scopolamine, or amyloid-β. These effects have been linked to activation of BDNF/CREB signaling pathways, attenuation of oxidative stress, and suppression of neuronal apoptosis [5],[21]. Furthermore, ethanolic extracts of MO have been reported to reduce amyloid burden and improve cognitive performance in APP/PS1 transgenic mice, supporting a direct

role of MO in modulating AD-related pathology [6].

The concordance between the present *Drosophila* findings and mammalian data suggests that MO influences evolutionarily conserved molecular pathways governing learning and memory. Rather than acting through a single molecular target, MO appears to exert neuroprotective effects via coordinated modulation of cholinergic neurotransmission, reinforcement of endogenous antioxidant defense systems, and activation of neurotrophic signaling cascades [20].

APP-expressing flies displayed pronounced locomotor deficits, reflecting neuromuscular dysfunction commonly observed in Alzheimer's disease models. Accumulation of APP/Aβ has been shown to induce synaptic degeneration and disruption of motor circuits in *Drosophila*, rendering locomotor activity, a relevant behavioral readout for neurodegenerative progression and therapeutic intervention [22],[23]. The most efficacious MO fractions are likely enriched in hydrophobic constituents with antioxidant and neuroprotective properties, and attenuation of oxidative stress and mitochondrial dysfunction may directly underlie the observed improvements in neuromuscular function [24].

In addition to behavioral abnormalities, APP-expressing flies exhibited a significant reduction in lifespan. Administration of MOE and MOB markedly prolonged median lifespan, consistent with previous findings indicating that suppression of oxidative stress and preservation of mitochondrial integrity can delay neurodegeneration and extend survival in *Drosophila* models of AD [24]. APP expression has been shown to increase the production of reactive oxygen species (ROS), particularly in the mitochondria of motor neurons. The accumulation of ROS can disrupt mitochondrial function, reduce ATP production, and impair the activity of neuromuscular junctions (NMJs), ultimately leading to locomotor dysfunction [25]. MDA is a major end-product of lipid peroxidation and is widely used as a biomarker reflecting oxidative damage to cellular and mitochondrial membranes caused by ROS. In the present study, treatment with MOE significantly reduced MDA levels, indicating an attenuation of oxidative stress and protection of cellular and mitochondrial membrane integrity. The reduction of oxidative damage at NMJs may help preserve mitochondrial function and neuromuscular transmission, thereby contributing to the improvement of locomotor performance observed in flies [26]. In contrast, tacrine treatment produced transient improvements in behavioral performance and oxidative stress markers but failed to extend lifespan upon prolonged exposure, consistent with its recognized limitations in long-term use and poor tolerability.

Despite these promising findings, several limitations should be addressed. The present study was conducted in a transgenic *Drosophila melanogaster* model, which does not fully reflect the complexity of AD in mammalian systems. In addition, the study primarily focused on behavioral outcomes and oxidative stress markers, while the precise molecular mechanisms underlying the neuroprotective effects of *M. officinalis* extracts remain unclear. Therefore, future studies should investigate the specific signaling pathways involved and identify the active compounds contributing to these effects, as well as further validate the findings in experimental mouse models.

5. Conclusions

This study demonstrates that *M. officinalis* How. root extracts, particularly the 50% ethanolic extract and the *n*-butanol fraction, significantly mitigate AD-like phenotypes in a transgenic *Drosophila* model expressing human APP. MO treatment improved associative memory and locomotor function, extended lifespan, reduced oxidative stress, and moderately inhibited acetylcholinesterase activity. Overall, these findings suggest that *M. officinalis* ameliorates memory impairment in experimental models of AD, indicating its potential for further investigation as a botanical therapeutic candidate.

Acknowledgements: *The findings of this study were generated from an institutional research project conducted at the National Institute of Medicinal Materials (2024–2025), entitled "Evaluation of the memory-enhancing and neuroprotective effects of Morinda officinalis How. roots in experimental models" supported under Contract No. 03/2024/HĐ-NVCV-DLSH.*

References

1. Schultz C., Del Tredici K., Braak H. (2004), *Neuropathology of Alzheimer's Disease. In Alzheimer's Disease Current Clinical Neurology*. Humana Press, 21-31.

2. Dubois B., von Arnim C. A., Burnie N., Bozeat S., Cummings J. (2023), Biomarkers in Alzheimer's disease: role in early and differential diagnosis and recognition of atypical variants. *Alzheimer's Research & Therapy*, 15(1), 175.

3. Khan A. H., Ijaz E., Ubaid B., Eddaki I., Edhi M., Shah M. N., Perry G. (2024), Analysis of Alzheimer's disease-related mortality rates among the elderly populations across the United States: an analysis of demographic and regional disparities from 1999 to 2020. *Current Alzheimer Research*, *21*(6), 384-394.

4. Do H. B., Dang Q. C., Bui X. C., Nguyen T. D., Do T. D., Pham V. H., Vu N. L., Pham D.M., Pham K.M., Doan T.N., Nguyen T., Tran T. (2004), *Medicinal Plants and Animals of Vietnam.* Hanoi Science and Technology Publishing House, Hanoi, 1, 101.

5. Zhang Y., Zhang M. (2022), Neuroprotective effects of *Morinda officinalis* How.: Anti-inflammatory and antioxidant roles in Alzheimer's disease. *Frontiers in Aging Neuroscience*, 14, 963041.

6. Xin Y., Diling C., Jian Y., Ting L., Guoyan H., Hualun L., Xiaocui T., Guoxiao L., Ou S., Chaoqun Z., Jun Z. (2018), Effects of oligosaccharides from *Morinda officinalis* on gut microbiota and metabolome of APP/PS1 transgenic mice. *Frontiers in Neurology*, 9, 412.

7. Chen, D. L., Zhang, P., Lin, L., Zhang, H. M., & Liu, S. H. (2013), Protective effect of oligosaccharides from *Morinda officinalis* on beta-amyloid-induced dementia rats. *China Journal of Chinese Materia Médica*, 38(9), 1306-1309.

8. Xin Y., Diling C., Tianlu C., Jun Z., Xiaocui T., Yinrui G., Guoyan H. (2019), Oligosaccharides from Morinda officinalis slow the Progress of aging mice by regulating the key microbiota - metabolite pairs. *Evidence-Based Complementary and Alternative Medicine*, 2019(1), 9306834.

9. Zhou L., Yang C., Liu Z., Chen L., Wang P., Zhou Y., Yuan M., Zhou L. T., Wang X., Zhu L. Q. (2024), Neuroprotective effect of the traditional decoction Tian-Si-Yin against Alzheimer's disease via suppression of neuroinflammation. *Journal of Ethnopharmacology*, 321, 117569.

10. Xu W., Liu X., He X., Jiang Y., Zhang J., Zhang Q., Wang N., Qin L., Xin H. (2020), Bajitianwan attenuates D-galactose-induced memory impairment and bone loss through suppression of oxidative stress in aging rat model. *Journal of Ethnopharmacology*, 261, 112992.

11. Ellman G. L., Courtney K. D., Andres Jr V., Featherstone R. M. (1961), A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, 7(2), 88-95.

12. Zhu Y., Lobato A. G., Zhai R. G., Pinto M. (2022), Human nmnat1 promotes autophagic clearance of amyloid plaques in a Drosophila model of Alzheimer's disease. *Frontiers in Aging Neuroscience*, 14, 852972.

13. Gerber B., Biernacki R., Thum J. (2013), Odor–taste learning assays in Drosophila larvae. *Cold Spring Harbor Protocols*, 3, pdb. prot071639.

14. Nichols C. D., Becnel J., Pandey U. B. (2012), Methods to assay Drosophila behavior. *Journal of Visualized Experiments*, 61, 3795.

15. Piper M. D., Partridge L. (2016), Protocols to study aging in Drosophila. *Drosophila: Methods and Protocols*, 291-302.

16. Subedi R. P., Vartak R. R., Kale P. G. (2017), Management of stress exerted by hydrogen peroxide in Drosophila melanogaster using Abhrak bhasma. *Journal of Applied Pharmaceutical Science*, 7(12), 065-071.

17. Wu Q., (2025), Guidelines for Studying the Oxidative Stress Damage Model on Drosophila melanogaster. *Future Postharvest and Food*, 2(2), 124-138.

18. Martyn J. A. J., Fagerlund M. J., Eriksson L. I. (2009), Basic principles of neuromuscular transmission. *Anaesthesia*, 64, 1-9.

19. Lee Y. K., Bang H. J., Oh J. B., Whang W. K. (2017), Bioassay-guided isolated compounds from *Morinda officinalis* inhibit Alzheimer's disease pathologies. *Molecules*, 22(10), 1638.

20. Howes M. J. R., Perry N. S., Houghton P. J. (2003), Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. *Phytotherapy Research*, 17(1), 1-18.

21. He M., Hu M., Wang T., Zuo Z., Li H., Zhao Z., Hao Y., Dai X., Wang J., Sun Y. (2025), *Morinda officinalis* oligosaccharides alleviate chronic unpredictable mild stress-induced depression through the BDNF/TrkB/CREB pathway and symptoms of sexual dysfunction in mice. *Frontiers in Neuroscience*, 18, 1509543.

22. Iijima K., Liu H. P., Chiang A. S., Hearn S. A., Konsolaki M., Zhong Y. (2004), Dissecting the pathological effects of human Aβ40 and Aβ42 in *Drosophila*: a potential model for Alzheimer's disease. *Proceedings of the National Academy of Sciences*, 101(17), 6623-6628.

23. Crowther D. C., Kinghorn K. J., Miranda E., Page R., Curry J. A., Duthie F. A. I., Gubb D. C., Lomas D.A. (2005), Intraneuronal Aβ, non-amyloid aggregates and neurodegeneration in a *Drosophila* model of Alzheimer's disease. *Neuroscience*, 132(1), 123-135.

24. Iijima-Ando K. and Iijima K. (2010), Transgenic Drosophila models of Alzheimer's disease and tauopathies. *Brain Structure and Function*, 214(2), 245-262.

25. Stavrovskaya I., Morin B. K., Madamba S., Alexander C., Romano A., Alam S., Peixoto P. M. (2025), Mitochondrial ROS modulate presynaptic plasticity in the *Drosophila* neuromuscular junction. *Redox Biology*, 79, 103474.

26. Chai S., Zhang N., Cui C., Bao Z., Wang Q., Lin W., Cheung W. H. (2026), Systematic review of mitochondrial dysfunction and oxidative stress in aging: A focus on neuromuscular junctions. *Neural Regeneration Research*, 21(5), 1947-1960.