ORIGINAL ARTICLE

Evaluating the Therapeutic Potential of Lavender Aroma on Memory and Biochemical Alterations in an Amyloid-beta 1-42 – Induced Dementia Model

Nachammai Vinaitheerthan, Santhepete Nanjundaiah Manjula

Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru, Karnataka, India

Abstract

Objective: Neurodegenerative disorders, such as Alzheimer's disease (AD), are progressive conditions that inflict irreparable harm on neuronal structure and function. The research aimed to evaluate the impact of lavender essential oil inhalation in an AD mouse model, and to examine the effects of donepezil in combination with essential oil inhalation on olfactory impairments and cognitive decline. **Methods:** Animals were treated with lavender oil (LO) aroma and donepezil, after which assessments of behavior, cognition, histology, and biochemical parameters were conducted. **Results:** Animals administered amyloid-beta had considerable olfactory deficits, cognitive impairment, neuronal degeneration, and neuroinflammation, with modified neurotransmitter concentrations. Inhalation of LO, particularly at higher concentrations, markedly enhanced behavioral performance, memory retention, neurotransmitter balance, and decreased neuroinflammatory markers. The combination of donepezil and LO produced synergistic effects, showing greater neuroprotection, improved cognition, and enhanced anti-inflammatory effects compared to individual treatments. **Conclusion:** This research effectively builds a solid foundation for the olfactory involvement in the development of AD. Inhalation of lavender essential oil, especially when combined with donepezil, is a potential supplementary approach for alleviating olfactory and cognitive impairments associated with AD. Additional research is required to investigate the long-term safety and effectiveness of essential oil inhalation in neurodegenerative disorders.

Key words: Alzheimer's disease, donepezil, lavender oil, neurodegenerative disease, neuroinflammatory markers

INTRODUCTION

Alzheimer's disease (AD), a type of neurodegenerative disorder, is characterized by progressive neuronal damage and a decline in cognitive functions.[1] Globally, AD affects nearly 50 million individuals, with cases expected to rise substantially by 2050.[2] In India, dementia prevalence among individuals over 60 years is estimated at 7.4%, representing a significant and growing public health burden.[3-5]

AD is pathologically defined by amyloid-beta (Aβ) plaque accumulation and neurofibrillary tangles composed of hyperphosphorylated tau protein, which trigger oxidative stress, neuroinflammation, synaptic dysfunction, and mitochondrial impairment, ultimately leading to progressive cognitive decline.[6,7] From a clinical standpoint, AD presents as a progressive decline in memory and cognitive abilities, hampering the ability to perform everyday activities and maintain personal autonomy.[8]

The etiology of AD is multifactorial, involving genetic, environmental, and lifestyle-related risk factors such as diabetes, hypertension, and cerebrovascular disorders.[9] The olfactory vector hypothesis proposes that early pathological changes may originate in the olfactory system, with

Address for correspondence:
Dr. Santhepete Nanjundaiah Manjula, Department of Pharmacology, JSS College of Pharmacy, JSS Academy of Higher Education and Research, Mysuru - 570 015, Karnataka, India.
E-mail: snmanjula@jssuni.edu.in

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degeneration of the olfactory epithelium and bulb serving as early indicators of disease progression and potential pathways for the spread of neurodegeneration.[10,11]

Current therapeutic strategies primarily focus on symptomatic management using pharmacological agents such as donepezil, along with emerging multitarget approaches.[12,13] However, these treatments provide limited efficacy in halting disease progression, highlighting the need for adjunct therapeutic strategies.

Essential oils and aromatic plant extracts have gained attention for their neuroprotective potential. Lavender (*Lavandula angustifolia*) has demonstrated antioxidant, anti-inflammatory, and cholinesterase-inhibitory properties, supporting its role in modulating neurodegenerative pathways.[14,15] Aromatherapy, as a non-invasive intervention, may influence both olfactory and central nervous system pathways, offering a complementary therapeutic approach.[16]

Experimental evidence supporting the olfactory route as a portal for neurotoxic agents further strengthens the relevance of targeting olfactory dysfunction in AD.[17] Therefore, combining essential oil-based interventions with conventional pharmacotherapy may provide enhanced therapeutic benefits.

The present study aims to evaluate the effects of lavender essential oil inhalation in an Aβ-induced AD model and to assess its combined impact with donepezil on olfactory dysfunction and cognitive decline.

METHODS

Essential oil, chemicals, reagents/kits

Lavender essential oil (*L. angustifolia*) was obtained as a gift sample from Y'aura, Bengaluru. All chemicals and reagents used in the study were of analytical grade and procured from standard commercial suppliers. Primary antibodies against β-amyloid (1–42), Glycogen synthase kinase-3 beta (GSK-3β), NOD-like receptor protein 3 (NLRP3), and olfactory marker protein (OMP), along with corresponding secondary antibodies, were used for protein analysis. Enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and IL-6, and reagents for neurotransmitter estimation were obtained from commercially available sources.

Animals

Female C57BL/6 mice (2–3 months old, 25–30 g) were procured from a Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)-approved breeders. Animals were housed under standard laboratory conditions with free access to food and water. All procedures were conducted in accordance with CPCSEA guidelines and approved by the Institutional Animal Ethics Committee (JSSAHER/IAEC/033/2020).

Treatment

Animals were acclimatized for 7 days and then randomly distributed into five groups (n = 6/group): Normal (exposed to water vapors), disease control (DC) (unilateral Aβ [1–42] intracerebroventricular [ICV]), vehicle control (1% Tween 80 [v/v] by inhalation for 30 min for 14 days after 7 days of ICV injection), LO 1% (LO 1% v/v by inhalation for 30 min for 14 days after 7 days of ICV injection), and LO 3% (LO 3% v/v by inhalation for 30 min for 14 days after 7 days of ICV injection).

AD was induced by ICV administration of Aβ (1–42). LO (1% and 3% v/v) was administered via inhalation for 30 min daily for 14 days following Aβ injection. Behavioral assessments were conducted during the light cycle. Lung toxicity was evaluated using Hematoxylin and Eosin staining.[18]

ICV administration of amyloid beta

Aβ (1–42) was prepared by dissolving in dimethyl sulfoxide and diluting in phosphate-buffered saline (PBS) to obtain oligomeric forms through incubation at 37°C for 4 days. Mice were anesthetized using ketamine (80 mg/kg) and xylazine (20 mg/kg) and positioned in a stereotaxic apparatus. Aβ (4 µL) was injected into the right lateral ventricle using standard coordinates (anteroposterior −0.9 mm, mediolateral 1.3 mm, dorsoventral −2.0 mm). Control animals received PBS injections. The procedure was performed as described previously.[19,20]

Preparation of oil sample and essential oil exposure

The study was conducted over 21 days using two models: Essential oil monotherapy and in combination therapy with a standard drug. The inhalation system was based on a modified breathing apparatus as previously described.[21,22] LO was diluted to 1% and 3% (v/v) using 1% Tween 80. Animals were exposed to aerosolized oil using a nebulizer (OMRON-NE C28) connected to an acrylic inhalation chamber. The nebulizer generated aerosol particles of approximately 5 µm at a 0.4 mL/min flow rate. Exposure was carried out for 30 min/day for 14 days. Animals were acclimatized to the exposure chamber before treatment. The chamber was cleaned after each exposure to avoid residue accumulation, and animals were allowed to rest briefly before being returned to their home cages.[23,24]

Behavioral assessment

Buried food pellet test

The buried food pellet test was used to assess olfactory function in mice. Animals were food-deprived for 6–8 h before testing with free access to water. The test was conducted in a clean cage containing 3 cm deep bedding. After acclimatization

to the testing environment (1 h) and test cage (15 min), a food pellet was kept 1 cm beneath the bedding at a random location. Each mouse was placed individually in the cage, and the time taken to locate and uncover the pellet was recorded, with a maximum observation period of 300 s.[24,25]

Habituation and dishabituation

Olfactory discrimination was assessed using the habituation/dishabituation paradigm. Mice were acclimatized to the testing room (1 h) and cage (5 min) before testing. Animals were sequentially exposed to odorants (water, vanilla, and pineapple; 1:200 dilution), each presented thrice for 2 min with 1 min inter-trial intervals using cotton applicators. Sniffing behavior, defined as nose proximity within 2 cm of the applicator, was recorded and duration measured. Longer sniffing time for novel versus familiar odors indicated intact olfactory discrimination.[26]

Memory assessment

Passive/inhibitory avoidance test

Memory retention was assessed using the step-down passive avoidance test. Mice were placed on an elevated platform above an electrified grid. During training, a mild foot shock (0.2–1.0 mA, 3 s) was given when the animal stepped onto the grid. In the test phase (no shock), step-down latency was recorded with a 180 s cutoff. Increased latency indicated improved memory retention.[27]

Left-right discrimination test by T-maze

Working memory was evaluated using a T-maze. Mice were allowed to explore the apparatus to determine arm preference, which was indicated as the reward arm (with a food pellet). Animals underwent acquisition training (~10 trials/day; max 100 s/trial) until reaching the reward arm. In the test phase, the reward arm was reversed, and the latency to reach the new reward arm was recorded. Reduced latency indicated improved spatial working memory.[27]

Animal euthanasia and tissue preparation

After the experimental period, animals were anaesthetised with ketamine (80 mg/kg) and xylazine (10 mg/kg). After confirming loss of reflexes, euthanasia was performed by cervical dislocation in accordance with CPCSEA and ARRIVE guidelines. The olfactory bulb and hippocampus were promptly dissected, homogenised in sodium phosphate buffer, and centrifuged at 10,000 rpm for 15 min at 4°c. The supernatants were collected for subsequent biochemical assays, and brain tissues were preserved in 10% formalin for histopathological examination.

Cresyl violet staining

Formalin-fixed brain tissues were sectioned (3–5 µm), deparaffinized, and rehydrated. Sections were stained with 0.1% cresyl violet for neuronal morphology and Nissl substance. Neuronal counts were performed at 40× magnification using image analysis software and expressed as neurons per 100 µm. Histopathological evaluation followed established protocols.[28]

Biochemical estimation

Animals were anesthetized using ketamine (80 mg/kg) in combination with xylazine (10 mg/kg) before sacrifice, and euthanasia was performed by cervical dislocation following confirmation of loss of reflexes. All procedures were carried out in accordance with CPCSEA and ARRIVE guidelines.

Western blot analysis

Tissue homogenates were prepared using a cell lysis buffer and centrifuged at 12,000 rpm to obtain supernatants for protein estimation by the Bradford method. Equal quantities of protein (30–40 µg) were resolved using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis gels and subsequently transferred to nitrocellulose membranes. Membranes were stained with Ponceau solution to confirm transfer efficiency, followed by blocking with 5% bovine serum albumin in Tris-Buffered Saline with Tween-20 (TBST) for 1 h at room temperature. Blots were then incubated overnight with primary antibodies against β-amyloid (1–42), NLRP3, GSK-3β, and OMP. After repeated washing with TBST (3–5 cycles, 5 min each), membranes were incubated with horseradish peroxidase -conjugated secondary antibodies for 1 h at room temperature. Protein bands were visualized using an electrochemiluminescence substrate and documented using a gel imaging system. All procedures were performed as described previously.[29]

Estimation of pro-inflammatory cytokines by ELISA

Supernatants from brain homogenates were used to quantify pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) using ELISA kits as per the manufacturer's protocols. Cytokine levels were determined from standard calibration curves and expressed as ng/mL protein. All measurements were performed in triplicate.[30]

Neurotransmitter estimation

Neurotransmitter concentrations (dopamine, serotonin, glutamate, gamma-aminobutyric acid [GABA], and acetylcholine [ACh]) were quantified using liquid chromatography-electrospray ionization-tandem mass spectrometry. Brain tissues were homogenized in an ice-cold buffer containing enzyme inhibitors and internal standards, followed by protein precipitation and centrifugation. Samples were analyzed using ultra-high-pressure liquid chromatography linked to a triple quadrupole mass spectrometer operated in positive electrospray ionization mode with multiple reaction monitoring. Where required, derivatization was performed to enhance the detection of

polar analytes. Calibration curves (0.1–1000 ng/mL) showed good linearity ($r^2 > 0.99$), and the method demonstrated acceptable accuracy, precision, and stability. Quantification was performed as described previously.[31,32]

Effects of donepezil and LO on Aβ (1–42)-induced mice

The potential combined action of donepezil and lavender essential oil was studied in an Aβ (1–42)-induced AD model. Based on earlier findings indicating greater efficacy of higher concentrations, LO (3% v/v) was selected for combination treatment. Animals were divided into five groups ($n = 6$/group): Normal (exposed to water vapors), DC (Aβ [1–42] ICV), vehicle control (1% Tween 80 inhalation), donepezil (3 mg/kg, p.o.), and donepezil + LO 3% v/v. Donepezil was administered orally for 14 days, post 7th day of Aβ injection.[33] LO inhalation was performed as described in the essential oil mono therapy model (30 min/day for 14 days). The total study duration was 21 days. All behavioral, biochemical, and histological assessments described in in the previous sections were performed to evaluate the combined effects on cognitive function, olfactory performance, and neurochemical parameters.

Statistical analysis

All data were expressed as mean ± standard error of the mean ($n = 6$ per group). Statistical analysis was performed using one-way or two-way analysis of variance followed by appropriate *post hoc* tests (Tukey's or Bonferroni's). $P \leq 0.05$ was considered statistically significant.

RESULTS

Behavioral assessment

Effect of LO and donepezil + LO on the olfactory buried pellet test

Aβ-injected animals showed a significant delay in locating and unburying the hidden food pellet, indicating impairment in olfactory-associated memory and cognitive function. LO inhalation improved performance in a dose-dependent manner. Animals treated with the lower concentration showed a significant reduction in latency ($P < 0.05$), while those treated with the higher concentration exhibited a more pronounced decrease ($P < 0.001$) in time required to uncover the pellet compared to the DC group.

On day 7 post-ICV injection, Aβ administration resulted in a marked elevation in latency relative to the normal group. Continued treatment with LO resulted in progressive improvement by day 21, with values significantly reduced compared to both the DC group and respective day 7 measurements. These findings indicate that LO effectively ameliorates Aβ-induced olfactory and cognitive deficits, with higher concentrations producing greater improvement [Figure 1].

Effect of LO and donepezil + LO on habituation and dishabituation

Aβ-injected animals showed a significant increase in sniffing time for both familiar and novel odors compared to the normal group ($P < 0.05$), indicating impaired olfactory discrimination. Treatment with LO (both low and high

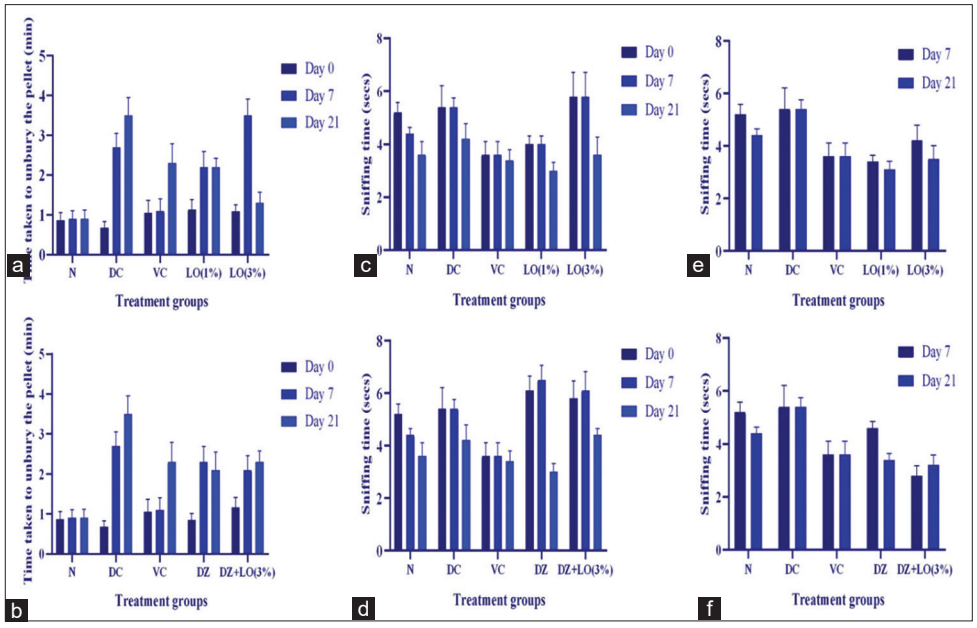


Figure 1: Effect of lavender oil inhalation and donepezil + lavender oil inhalation on the olfactory buried pellet test, habituation, and dishabituation test. (a) Lavender oil inhalation on olfactory buried pellet test, (b) donepezil + lavender oil inhalation on olfactory buried pellet test, (c) lavender oil inhalation on habituation test, (d) donepezil + lavender oil inhalation on habituation test, (e) lavender oil inhalation on dishabituation test, (f) donepezil + lavender oil inhalation on dishabituation test

concentrations) significantly reduced sniffing time compared to the DC group on the final day of assessment.

The donepezil-treated group alone did not show a significant improvement compared to the DC group. However, the combination of donepezil and LO resulted in a significant reduction in sniffing time for both familiar and novel odors compared to both DC and donepezil groups ($P < 0.05$), indicating enhanced olfactory discrimination with combination therapy [Figure 1].

Memory assessment

Effect of LO and donepezil + LO on the passive/inhibitory avoidance test (step-down latency)

Passive avoidance was used to assess memory retention on day 21. The normal group exhibited significantly higher step-down latency (180.66 ± 0.84 s), indicating intact memory, whereas Aβ-injected animals showed a marked reduction in latency (32.5 ± 4.99 s), reflecting impaired memory consolidation. LO inhalation significantly increased step-down latency in Aβ-treated animals in a dose-dependent manner, with the higher dilution showing greater improvement compared to the lower concentration.

A significant decrease in the latency was observed in the DC group when compared with the normal group ($P < 0.0001$). Donepezil treatment alone significantly increased latency in comparison to the DC group ($P < 0.0001$), indicating partial recovery. Notably, the combination of donepezil and LO (3%) resulted in a significantly greater increase in latency compared to both the DC and donepezil groups ($P < 0.0001$), suggesting enhanced memory retention. The vehicle control group showed no significant improvement. Overall, combination therapy produced the most pronounced effect in restoring memory performance [Figure 2].

Effect of LO and donepezil + LO on the left/right discrimination test by the T maze

Spatial working memory was assessed using the T-maze left–right discrimination task. $A\beta_{1-42}$-injected animals showed significant impairment, evidenced by increased time to reach the correct (reversed) arm and reduced correct choices compared to the normal group. LO inhalation (1% and 3%) significantly improved performance, as indicated by reduced latency and improved arm selection compared to the DC group. The vehicle control group showed no significant improvement. Donepezil treatment produced moderate improvement by day 21. In contrast, the combination of donepezil and LO (3%) resulted in significantly greater improvement compared to both DC and donepezil groups, indicating a synergistic enhancement of spatial memory [Figure 2].

Effect of LO and donepezil + LO on cresyl violet staining

Cresyl violet-stained sections of the hippocampus and olfactory bulb were examined to assess neuronal integrity following $A\beta_{1-42}$ administration. The DC group exhibited marked neurodegenerative changes, including neuronal cell degeneration, vacuolation, and mild hemorrhagic alterations in both regions. In contrast, the normal and vehicle control groups showed normal neuronal architecture without observable abnormalities.

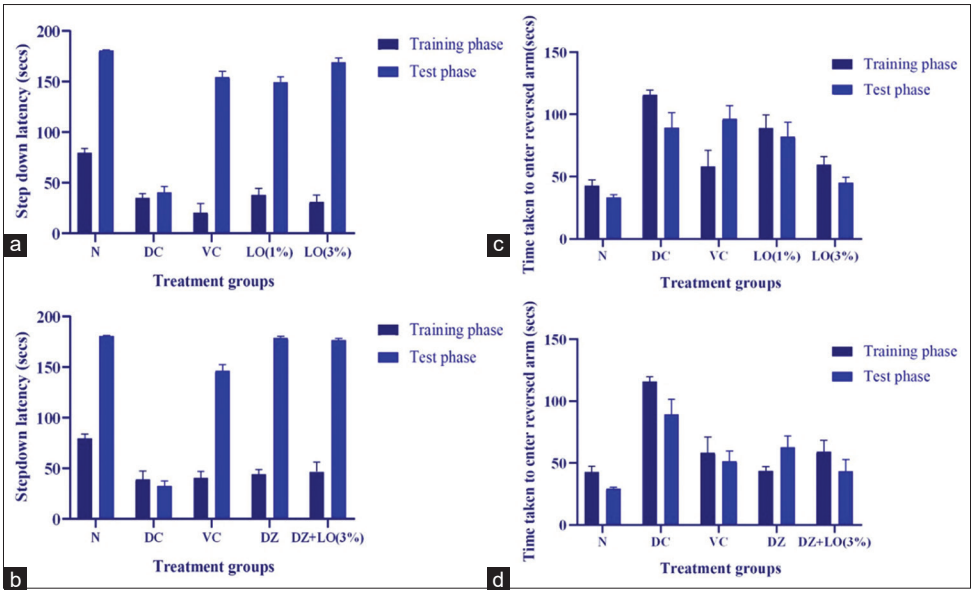


Figure 2: Effect of lavender oil and donepezil + lavender oil on the passive/inhibitory avoidance test (step-down latency) and left/right discrimination test by the T maze. (a) Lavender oil inhalation on passive/inhibitory avoidance test, (b) donepezil + lavender oil inhalation on passive/inhibitory avoidance test, (c) lavender oil inhalation on left/right discrimination test, (d) donepezil + lavender oil inhalation on left/right discrimination test

LO-treated groups (both low and high concentrations) showed mild vacuolation with partial preservation of neuronal structure compared to the DC group. Notably, the combination treatment (donepezil + LO 3%) demonstrated improved neuronal integrity with a marked reduction in degeneration and structural damage in both the hippocampus and olfactory bulb. These findings indicate that LO, particularly in combination with donepezil, exerts a protective effect against Aβ-induced neurodegeneration [Figures 3 and 4].

Biochemical estimation

Effect of LO and donepezil + LO on protein expression by western blot analysis

$A\beta_{1-42}$ injection significantly increased hippocampal β-amyloid expression compared to the normal group ($P < 0.001$), along with elevated levels of neuroinflammatory markers (GSK-3β and NLRP3) and a marked reduction in OMP expression. Bulbar OMP levels decreased significantly from 0.99 ± 0.06 in the normal group to 0.25 ± 0.11 in the DC group ($P < 0.01$), indicating olfactory dysfunction.

LO treatment showed a reduction in the expression of β-amyloid and inflammatory markers while increasing OMP levels. Donepezil treatment alone showed moderate improvement ($P < 0.05$), whereas the combination of donepezil and LO (3%) produced a more pronounced effect, significantly decreasing inflammatory markers and restoring OMP expression compared to both DC and donepezil groups ($P < 0.001$) [Figures 5 and 6].

Effect of LO and donepezil + LO on cytokine levels

The Aβ group showed a significant elevation in pro-inflammatory cytokine levels (TNF-α, IL-1β, and IL-6) compared to the normal control, indicating pronounced neuroinflammation. The vehicle control group showed no significant change.

LO inhalation (1% and 3%) significantly reduced cytokine levels, demonstrating an anti-inflammatory effect. Donepezil treatment alone resulted in a modest reduction, whereas the combination treatment (donepezil + LO 3%) produced a more substantial decrease in cytokine levels compared to both DC and donepezil groups [Figure 7].

Effect of LO inhalation and donepezil + LO inhalation on neurotransmitters

$A\beta_{1-42}$ injection caused a significant decrease in serotonin (5-HT), GABA, and ACh, along with an increase in glutamate levels, indicating neurotransmitter imbalance associated with cognitive decline. No significant change was observed in dopamine levels across groups. LO inhalation (1% and 3%) significantly normalized levels of 5-HT, GABA, ACh, and glutamate compared to the DC group. Donepezil treatment alone showed partial restoration, whereas the combination treatment (donepezil + LO 3%) resulted in greater normalization across most neurotransmitters [Figures 8 and 9].

DISCUSSION

This study demonstrates that lavender essential oil inhalation improves olfactory function, cognitive performance, and neurochemical alterations in an Aβ-induced AD model, with enhanced effects observed when combined with donepezil. These findings directly support the study objectives and highlight the olfactory pathway as a potential therapeutic target in AD.

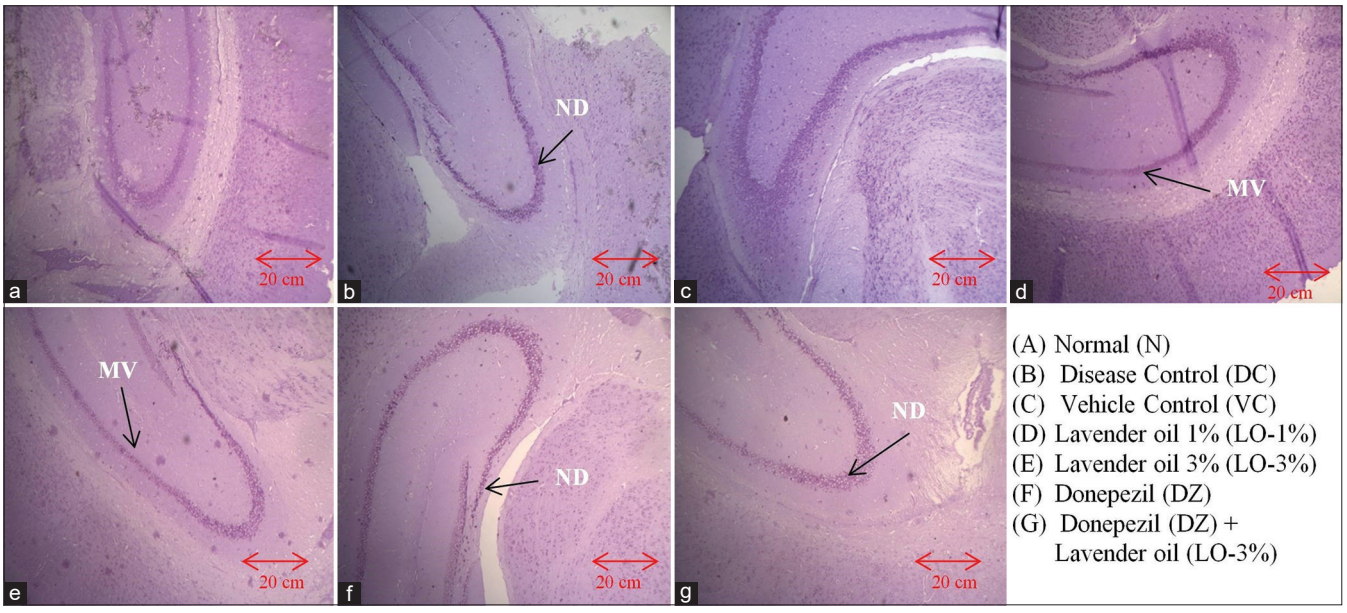


Figure 3: (a-g) Effect of lavender oil inhalation and donepezil + lavender oil inhalation on the hippocampus by cresyl violet staining. ND: Neuronal cell degeneration, MV: Mild vacuolation

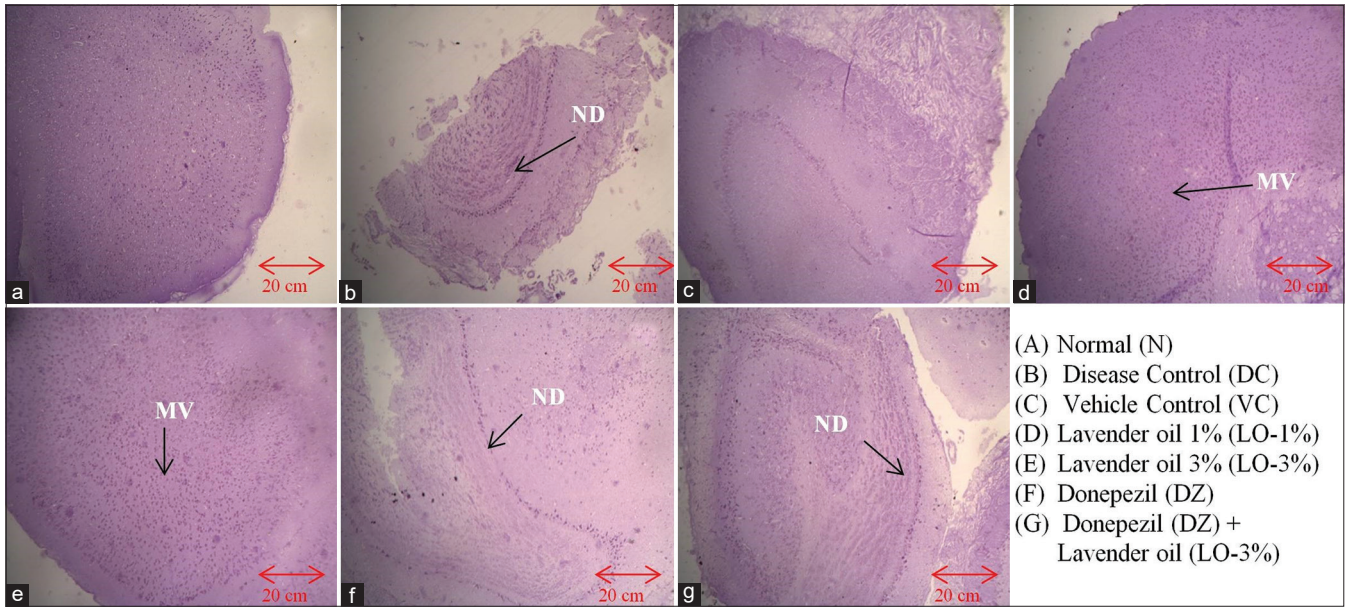


Figure 4: (a-g) Effect of lavender oil inhalation and donepezil + lavender oil inhalation on the olfactory bulb by cresyl violet staining. ND: Neuronal cell degeneration, MV: Mild vacuolation

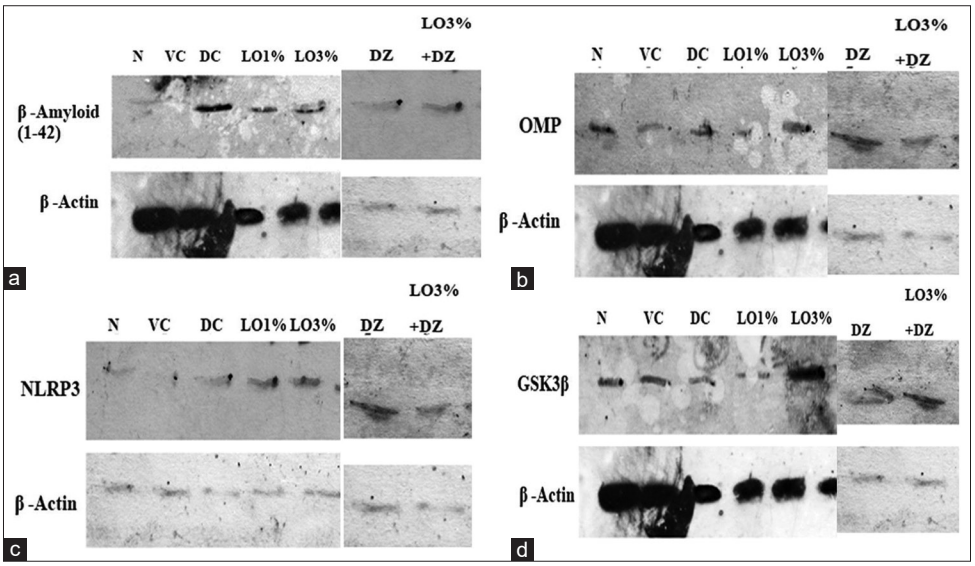


Figure 5: Effect of lavender oil and donepezil + lavender oil on protein expression by western blot analysis. (a) β-amyloid, (b) olfactory marker protein, (c) NOD-like receptor protein 3, (d) glycogen synthase kinase-3 beta

The primary outcomes showed that Aβ-induced animals exhibited olfactory deficits, memory impairment, neuroinflammation, and neurotransmitter imbalance. LO inhalation significantly improved behavioral performance and restored neurotransmitter levels, while reducing inflammatory markers. Notably, combination therapy (donepezil + LO) produced superior effects compared to monotherapy, indicating a synergistic interaction. Secondary outcomes, including histological analysis, further confirmed neuronal preservation in the hippocampus and olfactory bulb.

These findings are consistent with previous reports on olfactory dysfunction as an early feature of AD and its association with hippocampal memory circuits.[34] Prior research has shown that neuroinflammation, amyloid accumulation, and neurotransmitter imbalance contribute to cognitive decline, and the present results extend this by demonstrating that olfactory-based interventions can modulate these pathways simultaneously.[35] Unlike earlier studies focusing solely on pharmacological treatments, this work highlights a non-invasive aromatherapy-based adjunct strategy, thereby adding novelty.

Mechanistically, the observed improvements may be attributed to the antioxidant, anti-inflammatory, and cholinergic-modulating properties of LO. The reduction in Aβ, NLRP3, and GSK-3β expression, along with restoration

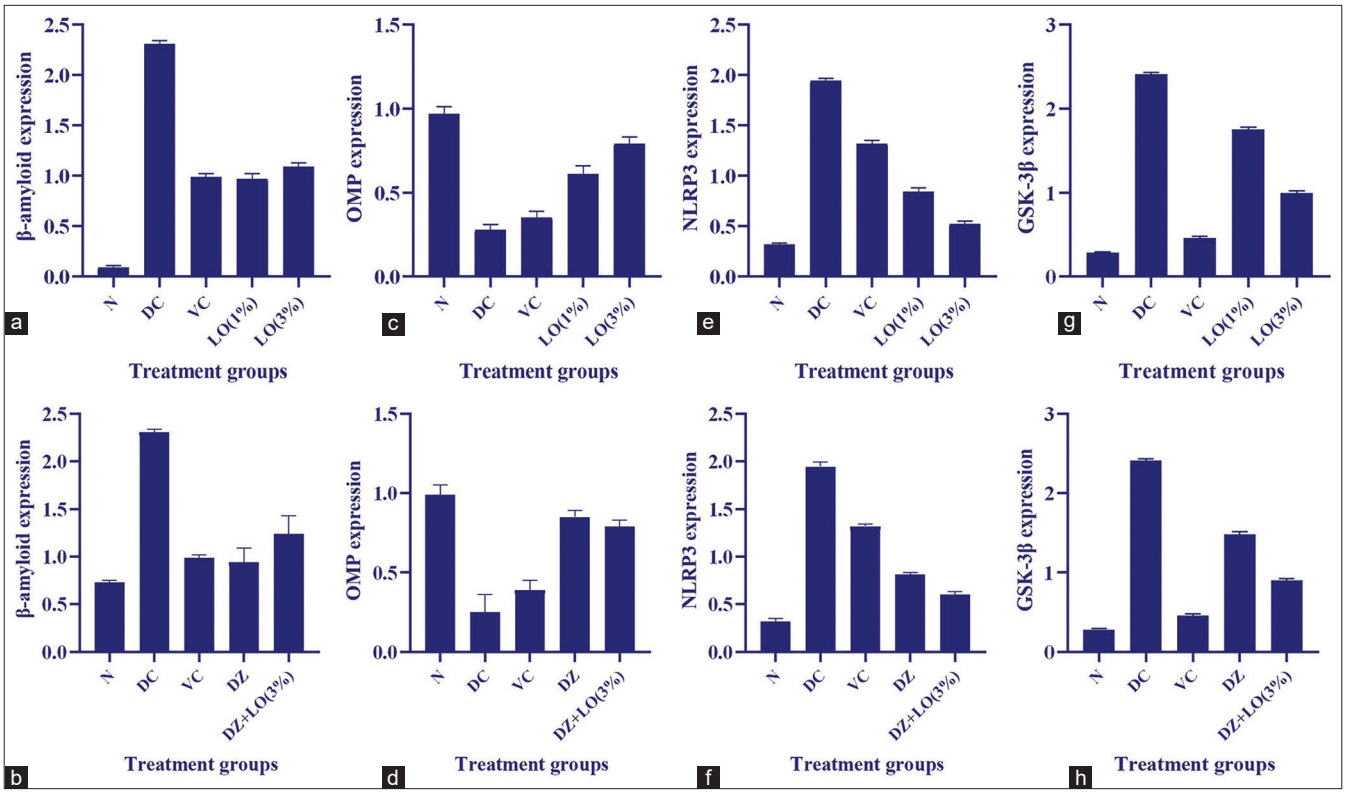


Figure 6: Effect of lavender oil and donepezil + lavender oil on β-amyloid, olfactory marker protein (OMP), NOD-like receptor protein 3 (NLRP3), and glycogen synthase kinase-3 beta (GSK-3) expression. (a) Lavender oil inhalation on β-amyloid expression, (b) donepezil + lavender oil inhalation on β-amyloid expression, (c) lavender oil inhalation on OMP expression, (d) donepezil + lavender oil inhalation on OMP expression, (e) lavender oil inhalation on NLRP3 expression, (f) donepezil + lavender oil inhalation on NLRP3 expression, (g) lavender oil inhalation on GSK-3β expression, (h) donepezil + lavender oil inhalation on GSK-3β expression

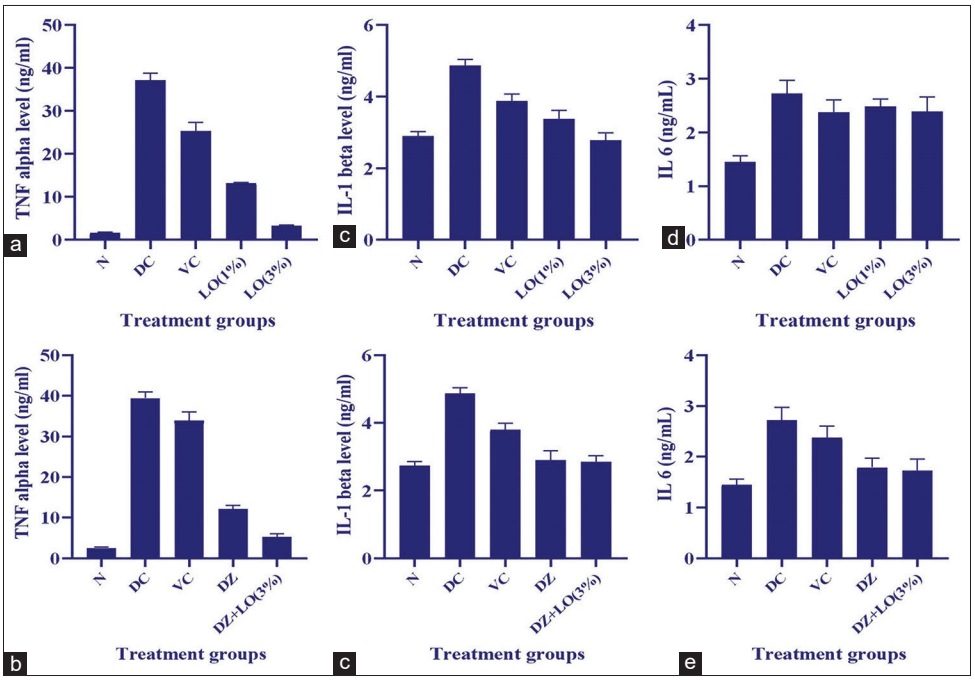


Figure 7: Effect of lavender oil and donepezil + lavender oil on the cytokine levels. (a) Lavender oil inhalation on tumor necrosis factor-alpha (TNF-α), (b) donepezil + lavender oil inhalation on TNF-α, (c) lavender oil inhalation on interleukin-1 beta (IL-1β), (d) donepezil + lavender oil inhalation on IL-1β, (e) lavender oil inhalation on IL-6, (f) donepezil + lavender oil inhalation on IL-6

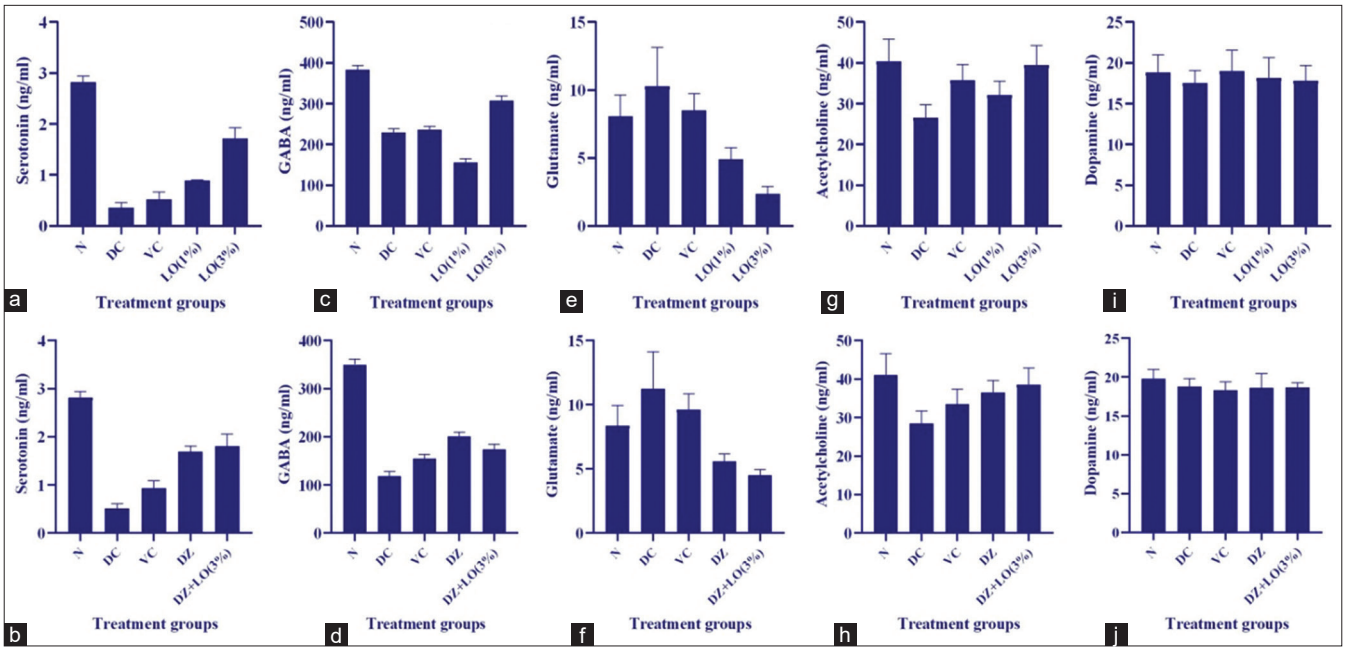


Figure 8: Effect of lavender oil and donepezil + lavender oil on neurotransmitters. (a) Lavender oil inhalation on serotonin, (b) donepezil + lavender oil inhalation on serotonin, (c) lavender oil inhalation on gamma-aminobutyric acid (GABA), (d) donepezil + lavender oil inhalation on GABA, (e) lavender oil inhalation on glutamate, (f) donepezil + lavender oil inhalation on glutamate, (g) lavender oil inhalation on acetylcholine, (h) donepezil + lavender oil inhalation on acetylcholine, (i) lavender oil inhalation on dopamine, (j) donepezil + lavender oil inhalation on dopamine

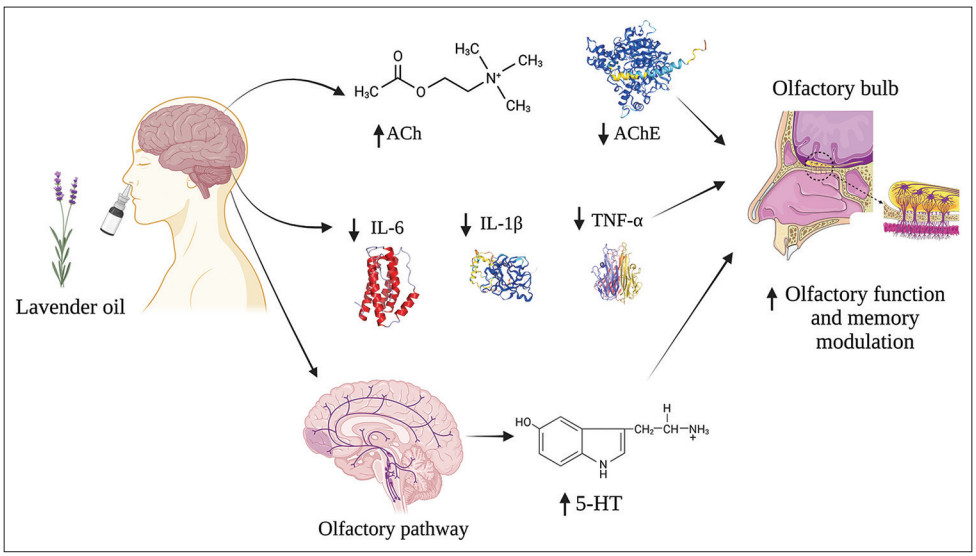


Figure 9: Molecular mechanism of lavender oil on the olfactory pathway

of OMP levels, suggests that lavender inhalation influences both olfactory and central neurodegenerative pathways. In addition, normalization of serotonin, GABA, and ACh levels indicates improved synaptic function, which aligns with previous reports linking neurotransmitter balance to cognitive recovery.

Strengths of the study

This study integrates behavioral, biochemical, and histopathological analyses, providing a multidimensional evaluation of treatment effects. The use of both monotherapy and combination therapy strengthens the interpretation of synergistic benefits. Furthermore, targeting the olfactory pathway represents a novel and underexplored therapeutic approach in AD research.

Limitations

The study is limited to an acute, non-transgenic mouse model, which may not fully replicate the complexity of human AD pathology. The sample size is relatively small, and the

long-term safety and efficacy of essential oil inhalation were not assessed. In addition, dose standardization and pharmacokinetic profiles of the inhaled compounds remain unclear, which may affect translational applicability.

Implications and contribution to existing evidence: This study adds to the growing evidence that non-invasive, multitarget approaches can enhance therapeutic outcomes in AD. The findings suggest that combining conventional drugs with complementary therapies like aromatherapy may improve both olfactory and cognitive deficits. From a translational perspective, this approach may have implications for early intervention strategies, particularly in patients presenting with olfactory dysfunction as a prodromal symptom.

Overall, the results support the concept that targeting olfactory pathways alongside conventional pharmacotherapy offers a more comprehensive strategy for managing AD progression, warranting further investigation in long-term and clinical studies.

CONCLUSION

This study successfully establishes a scientific basis for the olfactory contribution to AD progression. Lavender essential oil inhalation, particularly in combination with donepezil, offers a promising adjunct strategy for mitigating olfactory and cognitive deficits associated with AD. Further studies are necessary to explore the long-term safety and efficacy of essential oil inhalation in neurodegenerative conditions.

ETHICAL STATEMENT

All experimental procedures were conducted in accordance with the ARRIVE 2.0 guidelines and the CPCSEA (Government of India) standards. The study has been conducted as per the approval obtained from the Institutional Animal Ethics Committee (IAEC) of JSS College of Pharmacy, Mysuru, India, thoroughly examined, and granted approval for all experimental procedures (JSSAHER/IAEC/033/2020).

AUTHORS' CONTRIBUTIONS

Nachammai Vinaitheerthan: Research study, content, experimentation, and writing the article. Santhepete Nanjundaiah Manjula: Conceptualization, design, and supervision.

DATA AND CODE AVAILABILITY

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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