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Original Article

Neuroprotective Potential of *Marrubium vulgare* Linn. Extract against Scopolamine-Induced Alzheimer's Type Dementia in Mice

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ABSTRACT

Background: Alzheimer's Disease (AD) is a progressive neurodegenerative disorder characterized by memory impairment, cognitive decline and behavioural changes. It primarily affects individuals over the age of 65. It poses a substantial global health burden, with an increasing prevalence among the elderly population. Although the exact aetiology of AD is complex and multifactorial, emerging evidence suggests that dysfunction of glutamatergic neurotransmission, particularly involving the N-Methyl-D-Aspartate (NMDA) receptors, plays a crucial role in the pathophysiology of AD. **Materials and Methods:** Fresh plant material was collected, extracted and administered orally to experimental groups, the negative control group received scopolamine, while the control group received a vehicle. Behavioural assessments, biochemical assays, neurotransmitters and histo-pathological examinations were conducted. **Results:** Scopolamine administration significantly increased glutamatergic activity in the brain regions of mice, while *Marrubium vulgare* treatment mitigated this effect in a dose-dependent manner. Moreover, scopolamine-induced memory deficits were associated with elevated glutamate levels and oxidative stress, as indicated by decreased levels of glutathione and increased levels of malondialdehyde, superoxide dismutase and catalase. Treatment with *Marrubium vulgare* attenuated the scopolamine-induced oxidative stress by reducing glutamate and MDA levels and restoring GSH, catalase and SOD levels in the brain. **Conclusion:** These findings suggest that *Marrubium vulgare* exerts its memory-enhancing effects by modulating glutamatergic activity and reducing oxidative stress in this scopolamine-induced dementia model. Targeting these mechanisms may contribute to developing new AD therapies. Further research is needed to elucidate the molecular pathways and validate the potential clinical utility of *Marrubium vulgare* for AD management.

Keywords: Alzheimer's Disease, Glutamate, Neurotransmitter, Oxidative stress, *Marrubium vulgare*.

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INTRODUCTION

Alzheimer's Disease (AD) is a gradually developing neurodegenerative disorder of the brain, characterized by dementia, atypical behaviour, shift in personality and eventual mortality.[1] AD is the leading cause of dementia among older adults. It involves a slow, progressive decline in memory and thinking skills over some time. Examination of brain tissue in AD shows extensive shrinkage and build-up of abnormal protein deposits called tau tangles and beta-amyloid plaques.[2] Aging naturally leads to overall loss of brain volume, but different parts of the brain are affected at different rates. Other age-related brain changes include fewer synapses, less Gray and white matter and decreased blood flow.[3] Dealing with memory-related conditions like amnesia, attention issues and AD remains a tough task in the medical field.[4] The average age of onset for AD is around 75 years old, with an overall prevalence of about 1% in most developed countries. Looking at different age groups, the rate of AD increases sharply from 1% in those aged 60-64 years to over 25% in people over age 85.[5] The National Institutes of Health (NIH) has forecasted that based on current trajectories, Alzheimer's disease cases will surpass 8.5 million in the United States by the year 2030.[6] Drugs like the nootropics Piracetam and Aniracetam, along with cholinesterase inhibitors such as Donepezil, are now used to improve memory, mood and behaviour in AD patients.[7] Both in humans and animals, the cholinergic signalling pathway holds considerable importance in memory and learning processes.[8] A widely recognized centrally acting cholinergic probe is the nonselective muscarinic cholinergic antagonist, namely

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scopolamine, which induces learning and memory impairment in rodents and humans.[9] The drug scopolamine has been used as a model to screen potential anti-amnesia medications. According to estimation, approximately 75% of herbal remedies used worldwide were first discovered from leads in traditional local medicine. The World Health Organization reports that around 25% of modern pharmaceutical drugs originate from medicinal plants first utilized in traditional practices. Many other drugs are synthetic analogues constructed based on compound models isolated from plants. In India, almost 70% of modern medicines are derived from natural products. The basis of the variety of *Marrubium vulgare* as a nootropic plant is that it contains some constituents that strengthen the nervous systems according to Ayurveda and some standard books.[10] The amyloid beta protein initiates an NMDA-mediated influx of Ca^{2+}, which in turn triggers excitotoxicity and pathways related to stress in neurons. This process results in heightened oxidative stress, compromised energy metabolism and disrupted calcium ion homeostasis as shown in Figure 1.[11]

The Central Nervous System's (CNS) NMDARs, which are cationic channels regulated by the neurotransmitter glutamate, are crucial for excitatory transmission, synaptic integration, learning and memory. Elevated glutamate levels are associated with a robust but temporary influx of Calcium ions (Ca^{2+}), which hampers the functionality of mitochondria by triggering the activation of permeability transition pores situated within the mitochondrial inner membrane. This process results in the release of cytochrome c, a reduction in ATP levels and the concurrent generation of ROS, culminating in neurodegeneration.[12]

MATERIALS AND METHODS

Collection of *Marrubium vulgare* plant

Marrubium vulgare plant was collected from Kanchipuram, Tamil Nādu. The taxonomic identity of the plant was confirmed by Dr. S.S Yadav in the Dept. of Botany, Maharshi Dayanand University, Rohtak (Haryana).

Preparation of *Marrubium vulgare* extract

The entire plant sample that was gathered was carefully cleaned in running water before being air-dried in the shade. By using the cold maceration process, the dried herb was pulverized, sieved (60-80) and 50% hydroalcoholic solvent extracted. For 10 days, the powdered sample (100 g) was stored at room temperature with the solvent (1:15) and sometimes stirred. The resulting extract was concentrated, lyophilized and kept at low temperatures for later research in an airtight container.[13,14]

Chemicals and reagents

The following substances were obtained from Sigma-Aldrich (St. Louis, MO, USA): sodium hydroxide, Copper sulphate, Sodium chloride, Triton X-100, 5, 5′-dithiobis (2-nitro-benzoic acid, Bovine Serum Albumin, Folin-Ciocalteu's reagent, trichloroacetic acid, HCl and Scopolamine.

Experimental Animals

Mice of Swiss albino strain weighed 25-30 g were utilized in this study. This strain was obtained from the animal housing facility at Maharshi Dayanand University, Rohtak, Haryana, India (CPCSEA Registration No. 426/2). Mice were kept under a 12 hr light/dark cycle in polypropylene cages (L: 29 cm, W: 22 cm, H: 14 cm). Standard rodent feed pellets and tap water were provided ad libitum via stainless steel nozzles suspended within each cage. Cages underwent manual cleaning and sanitation daily. All experimental procedures strictly adhered to ethical guidelines set forth by the Institute Animal Ethical Committee under the purview of the CPCSEA.

Experimental Grouping

The study divided the experimental animals into four groups as follows:

Group I: Normal control (treated with Saline).

Group II: Vehicle+Scopolamine (1 mg/kg).

Group III: *Marrubium vulgare* (700 mg/kg)+Scopolamine (1 mg/kg).

Group IV: *Marrubium vulgare* (1400 mg/kg)+Scopolamine (1 mg/kg).

Drug administration

In the current study, scopolamine was used. To assess behavioural and biochemical markers, the mice received intraperitoneal injections of scopolamine at a dosage of 1 mg/kg of body weight over seven consecutive days.[15] This was done 30 min before the test medication was given to the mice as shown in Figure 2.

Evaluation of behavioural parameters:

Morris Water Maze (MWM) test

The study employed the MWM, a circular pool measuring 60 cm in diameter and 26 cm in height. The water, kept at 26±1°C, was 20 cm deep and made opaque by adding white colouring. The pool was divided into four imaginary quadrants and a submerged platform served as an escape. Four different starting points around the pool's edge were used for variety. Over five days of training, each of the four starting points was used once, following a specific order. The maze was set up in a room adorned with visual cues to aid navigation. In a trial, a mouse was positioned in the water, facing the pool's wall from a starting point. In cases where the mouse failed to locate the platform within a 60 sec timeframe, it was assisted to find it and permitted to remain on it for 20 sec. The experimental protocol consisted of four trial sessions per day, carried out consecutively over a span of five days. The time taken

by the mouse to reach the concealed platform termed the Escape Latency Time (ELT), was recorded to gauge learning progress.[16-18]

Open Field Test (OFT)

The assessment involved observing ambulatory behaviour, which refers to movement from one area to another, within the OFT framework. The setup consisted of a box with transparent acrylic walls and a black floor, spanning dimensions of 30 cm×30 cm×15 cm in height. The floor was neatly divided into nine equal squares. To initiate the session, a mouse was gently placed at the centre of a square and allowed to freely explore the surroundings. During this 5 min exploration period, the count of squares traversed using all four paws and the count of instances where the mouse stood on its rear legs (rearing behaviour) were meticulously tallied and documented.[19]

Elevated plus Maze Test

To evaluate the learning and memory of the mice in this study, an elevated plus maze apparatus was employed. This maze consisted of two open arms (each measuring 16 cmX5 cm) and two enclosed arms (each measuring 16 cmX5 cmX12 cm). These arms extended from a central platform measuring 5 cmX5 cm and the entire maze was elevated 25 cm above the ground. At the start of the testing session, every mouse was initially placed at the remote end of an open arm, with its back turned to the central platform. The Transfer Latency (TL) was measured by recording the duration it took for the mouse to shift all four legs into one of the enclosed arms. On the initial day, TL was documented. If a mouse did not enter an enclosed arm within a 90 sec period, it was gently directed into one and assigned a Time Limit (TL) of 90 sec. After a 10 sec exploration of the maze, the mouse was returned to its enclosure. To assess memory, a second trial was conducted 24 hr later on the following day.[20]

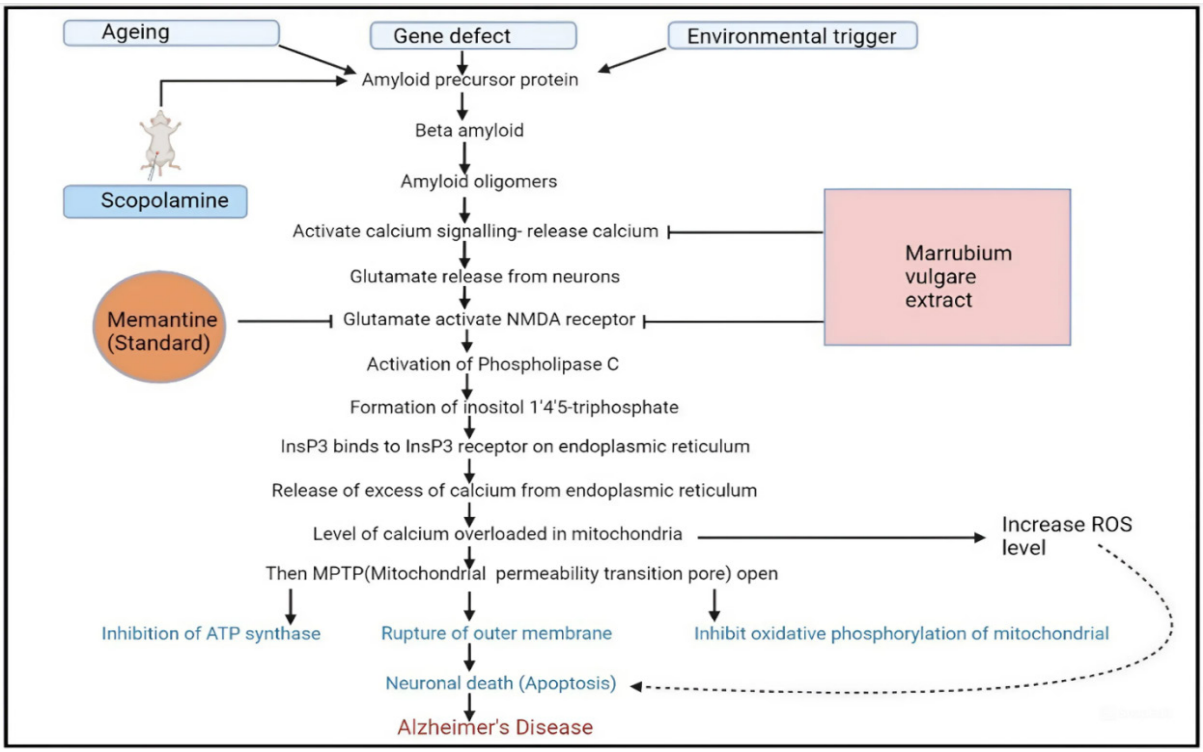

Figure 1: Mechanistic approach of *Marrubium vulgare* extract for treating Alzheimer's disease.

Biochemical estimation

Preparation of Brain homogenate

Once the behavioural tests were concluded, the animals were humanely euthanized within a CO_2 chamber. Following this, their brains were swiftly extracted and cleansed using a 0.9% ice-cold saline solution. The brain tissues were subsequently weighed and then homogenized in a 0.1 M phosphate buffer (pH 7.4) using a mortar and pestle. To isolate the supernatant, the homogenate was subjected to centrifugation at 2500 rpm for 15 min at 4°C. The resulting supernatant was employed for the measurement of various parameters, including TPC, NO, CAT, SOD, GSH, MDA, GABA, Glutamate and others.[21]

Determination of Total Protein Concentration

The protein levels in the various samples were assessed utilizing the method introduced by Lowry and colleagues in 1951.[22] Initially, 20 µL of each sample, a BSA standard solution covering a range from 10-200 µg and an empty control tube were prepared. The volume in each tube was then adjusted to 0.5 mL by adding 0.1 N NaOH solution. Subsequently, a unique mixture known as "reagent C" was prepared by combining 48 mL of 2% Na_2CO_3, 1 mL of 1% copper sulfate and 1 mL of 2% sodium potassium tartrate. A total of 0.5 mL of reagent C was gently blended into each tube. After thorough mixing, the tubes were left to stand at room temperature for 10 min. Following this, approximately 0.5 mL of 1N Folin Ciocalteau's reagent was added and mixed promptly. The tubes were left undisturbed for 30 min at room temperature. To determine the protein content, the absorbance of the solutions was recorded at 660 nm and this was compared to the absorbance of an empty reagent tube. A standard curve was constructed, covering a range from 10 to 200 µg, allowing for the calculation of the protein concentration.

Measurement of SOD Enzyme activity

The assessment of SOD activity followed the original protocol outlined by Kono in 1978. The reaction commenced with the addition of 0.1 mM hydroxylamine hydrochloride to a reaction mixture composed of 24 µM Nitroblue Tetrazolium, 50 mM $NaCO_3$ buffer, 0.1 mM Ethylenediamine tetraacetic acid and 0.03% Triton X. After a 2 min incubation period, the enzyme extract was introduced and the reduction in NBT was determined by measuring the absorbance of the reaction mixture at 560 nm over a 2 min interval. The unit of SOD activity is determined as the quantity of enzyme necessary to induce a 50% decrease in NBT reduction per minute per gram of fresh weight.[23]

Under these conditions, at a pH of 10.2 and in the presence of EDTA, hydroxylamine underwent auto-oxidation, leading to the

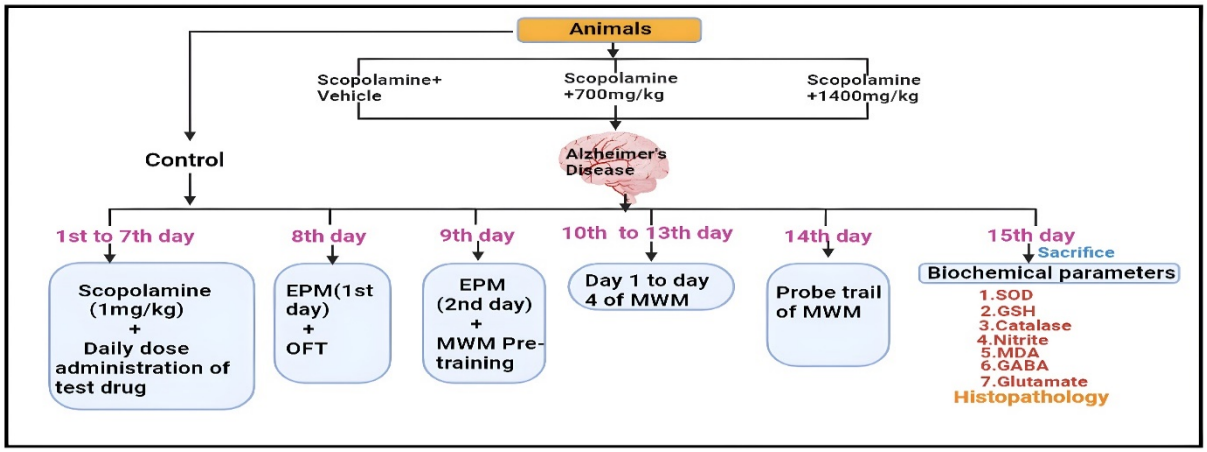

Figure 2: The schedule of the experimental and treatment design of mice.

generation of nitrite. Simultaneously, NBT was reduced, resulting in the creation and accumulation of a blue formazon product, leading to an increase in absorbance at 560 nm. The introduction of the SOD enzyme effectively inhibited the reduction of NBT and the formation of the blue formazon.[24]

Estimation of Catalase Activity

To assess Catalase (CAT) activity, 100 µL of tissue homogenate was blended with 1000 µL of freshly prepared 2 mmol/L H_2O_2 in a cuvette. This mixture was gently mixed and left to incubate at 37°C for 3 min. Subsequently, 1000 µL of dichromatic acetic acid was introduced. The tubes were then exposed to a 10 min incubation at 100°C. After cooling and centrifugation at 2500 g for 5 min to remove precipitated proteins, the changes in absorbance were measured at 570 nm against the reagent blank.[25]

Determination of Nitrite

The concentration of nitrite was determined spectrophotometrically by employing the Griess reagent, which consisted of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 0.1% sulphanilamide and 2.5% phosphoric acid. After centrifugation, the supernatant obtained was mixed with an equal volume of the Griess reagent and incubated for 10 min at room temperature. The absorbance of the reaction mixture was then assessed at 540 nm and compared to a blank solution created using 100 µL of distilled water. To quantify the produced Nitric Oxide (NO), a standard curve was established using sodium nitrite as a reference, encompassing concentrations ranging from 5-30 µmol/mL. The nitrite concentration was subsequently expressed as micro moles of NO per milligram of protein.[26]

Determination of GSH

Reduced Glutathione (GSH) was determined using the Ellman method. To begin, 0.5 mL of plasma was extracted and subsequently precipitated with 2.0 mL of 5% trichloroacetic acid. Following centrifugation at 3000-3500 rpm for 10 min, 1 mL of the supernatant was collected. To this, 0.5 mL of 5,5-Dithio-bis-2-Nitrobenzoic acid (DTNB) reagent and 3.0 mL of phosphate buffer (0.2 M, pH 8.0) were added. The resulting color was measured at 412 nm. A set of standards underwent a similar process, including a blank sample consisting of 3.5 mL of buffer. Instead of using tissue samples, established GSH concentrations were employed to construct a standard curve.[27]

Estimation of Lipid Peroxidation

It was resolute by TBARs as described by Okhawa *et al.* (1979). 1.5 mL of 20% acetic acid, SDS (0.2 mL) and TBA (1.5 mL) were added to tissue homogenate (0.5 mL). Using distilled water, the mixture was prepared up to 4.0 mL and then it was heated at 95 C (60 min) with a glass ball as a condenser. After cooling, the butanol-pyridine mixture (4.0 mL) was added and mixed well. After centrifugation (10 min) at 4,000 rpm, absorbance was taken at 532 nm using the organic layer. Blank and standards were treated in a parallel way. Malondialdehyde (MDA) generated per gram of tissue was used to measure the amount of lipid peroxidation.[28]

Estimation of Glutamate

For the estimation of glutamate, a modified version of Wiggins' (1986) method was applied. In screw-capped tubes, 0.25 mL of each sample was placed. To both the standard solutions and test samples, the following components were added: 0.01 mL of ADP solution, water, 0.1 mL of NAD solution and 1 mL of Tris-EDTA-Hydrazine buffer, resulting in a total reaction volume of 2 mL. To establish the background reading, the absorbance (C1) at 340 nm was measured using a spectrophotometer (Shimadzu mini). Following a 40 min incubation period, 0.02 mL of L-glutamic dehydrogenase was introduced into each tube and the absorbance (C2) was once again measured at 340 nm.[29] The net absorbance was determined by subtracting C1 from C2. The standard curve was utilized as a reference to calculate the glutamate levels in the samples.

GABA Estimation

Mix 0.5 M ninhydrin with a carbonate-bicarbonate buffer that has a pH of 9.5 to create a ninhydrin solution. Add 200 mL of the ninhydrin solution to 100 mL of the supernatant fluid. To reach room temperature, let the mixture cool. When the liquid has cooled, add 5 mL of copper tartrate reagent. To ensure thorough blending, vortex the solution. At a steady temperature of 25°C, incubate the mixture for 15 min. At two distinct wavelengths, A at 377 nm and B at 451 nm, measure the absorbance of the solution. We'll use these numbers in our analyses moving forward. The measurement most likely has to do with how much light at those wavelengths is absorbed or transmitted by the solution.[30]

Histopathology

Dissection of brain tissue was done after sacrifice in the CO_2 chamber. Tissues were embedded in buffered formalin (10%), dehydrated with alcohol, cleared with xylene, impregnated with paraffin, fixed in paraffin wax and sectioned with a microtome to get 3-5 µm thick paraffin sections.[31] The sections that were dewaxed were stained using H&E stain and observed under a microscope for scopolamine-induced changes and neuroprotective efficiency of *Marrubium vulgare*.

Statistical Analysis

The analysis involved using the ANOVA (Analysis of Variance) to assess potential statistically significant differences in means across the groups. Subsequently, Tukey's *post hoc* test was utilized for conducting multiple comparisons of the means. Statistical significance was considered when *p*-values were less than 0.05. The data analysis was conducted using GraphPad Prism 9 software.

RESULTS

Behavioural Parameters

Effect of Marrubium vulgare on Open Field

The initial step in the analysis involved conducting a One-way ANOVA, which was followed by a subsequent Tukey's *post hoc* examination. The results revealed a substantial decrease ($p<0.001$) in the number of squared crossings within the group administered the vehicle (53±1.807) when compared to the control group (124±2.82). Conversely, a significant raise ($p<0.001$) in the count of squared crossings was observed in the groups receiving M.V at doses of 700 mg/kg (75±2.74) and 1400 mg/kg (104±3.105) in comparison to the group that received the vehicle treatment as shown in Figure 3(A).

Elevated plus maze

The initial step in the analysis involved conducting a One-way ANOVA, which was followed by a subsequent Tukey's *post hoc* examination, which indicated a notable increase ($p<0.001$) in transfer latency in the vehicle-treated groups (34±1.23) when compared to control group (16.6±0.80). A statistically significant reduction ($p<0.05$) in transfer latency was observed in the M.V. 700 mg/kg group (26.6±1.38). A highly significant reduction ($p<0.001$) in transfer latency was noted in the M.V 1400 mg/kg group (19.66±0.84) in comparison to the group that received the vehicle treatment as shown in Figure 3(B).

Morris water maze

Effect of Marrubium vulgare on the time spent in the target quadrant

The analysis began with univariate analysis of variance (One-way ANOVA), followed by a subsequent Tukey's *post hoc* examination, which indicated that there was statistically significant reduction ($p<0.001$) in the time spent in the target quadrant when compared to control group in the vehicle-treated groups. Conversely, a statistically significant rise ($p<0.05$) in the time spent in the target quadrant was observed in the M.V. 700 mg/kg group. A highly significant increase ($p<0.001$) was noted in the M.V 1400 mg/kg group in comparison to the group that received the vehicle treatment as shown in Figure 3(C).

Effect of *Marrubium vulgare* on No. of target platform crossings

The analysis began with univariate analysis of variance (One-way ANOVA), followed by a subsequent Tukey's *post hoc* examination, which indicated a substantial reduction ($p<0.001$) in the number of crossings when comparing the vehicle-treated groups to the control group. A statistically notable rise ($p<0.05$) in the number of crossings was noted in the M.V. 700 mg/kg group. A significant increase ($p<0.001$) in the number of crossings was observed in the M.V 1400 mg/kg group in comparison to the group that received the vehicle treatment as shown in Figure 3(D).

Effect of *Marrubium vulgare* on escape latency

The analysis began with univariate analysis of variance (One-way ANOVA), followed by a subsequent Tukey's *post hoc* examination, which indicated that there was statistically significant increase

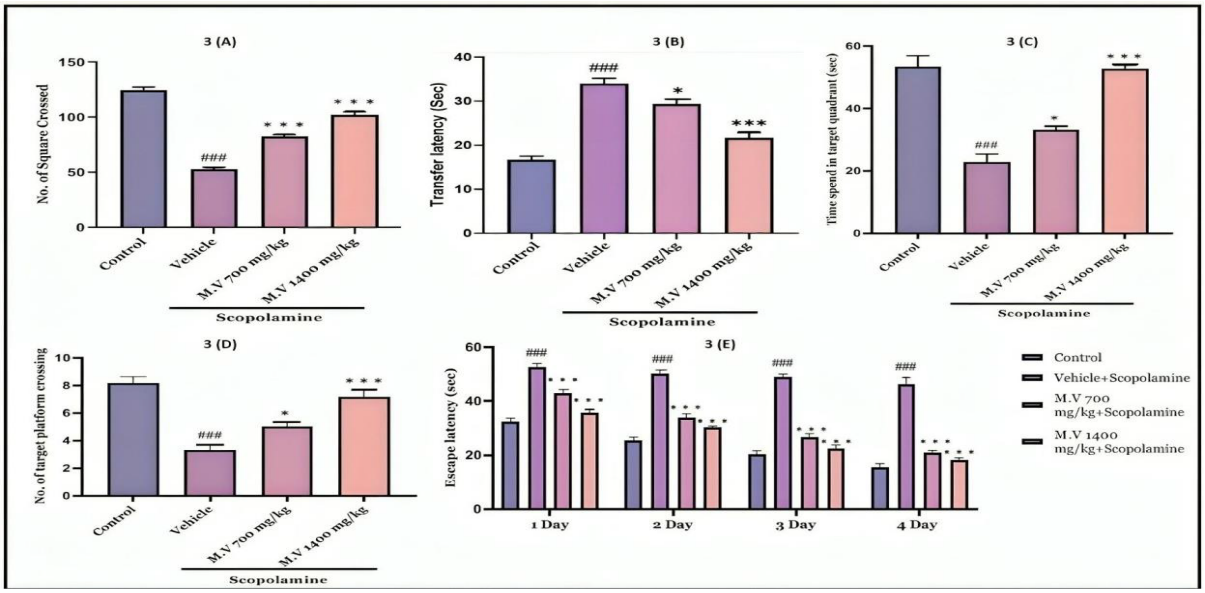

Figure 3: Effect of *Marrubium vulgare* on Neurobehavioral Parameters: 3(A) Open field tests; 3(B) Elevated plus-maze; 3(C), 3(D) and 3(E) Morris water maze. Each bar represents the mean±SEM ($n=6$). The results showed significant differences: ###$p<0.001$ when compared to the Control group and ***$p<0.001$ when compared to the Scopolamine+Vehicle group.

(p>0.05) in escape latency observed in the vehicle-treated groups from day 1 to day 4 when compared to control group. However, a significant decrease (p<0.001) in escape latency was noted in the M.V. 700 mg/kg and M.V. 1400 mg/kg groups in comparison to the group that received the vehicle treatment as shown in Figure 3(E).

Evaluation of biochemical parameters

Effect of Marrubium vulgare on total protein content level in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination indicated a significant reduction (p<0.001) in protein levels in the vehicle-treated groups (0.309±0.02) when compared to control group (0.82±0.019). Furthermore, there was a statistically significant rise (p<0.001) in total protein content in both the M.V 700 mg/kg group (0.4±0.0048) and the M.V 1400 mg/kg group (0.62±0.049) in comparison to the group that received the vehicle treatment as shown in Figure 4(A).

Effect of *Marrubium vulgare* on Malondialdehyde level (MDA) in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination revealed a substantial rise (p<0.001) in MDA levels within the vehicle-treated cohorts (12.45±0.11) when compared to control group (2.85±0.20). Notably, a statistically significant reduction (p<0.001) in MDA levels was evident in both the M.V 700 mg/kg group (6.30±0.29) and the M.V 1400 mg/kg group (5.56±0.68) in comparison to the group that received the vehicle treatment as shown in Figure 4(B).

Effect of *Marrubium vulgare* on Glutathione level (GSH) in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination indicated a significant reduction (p<0.001) in GSH levels in the vehicle-treated groups (12.2±0.91) when compared to control group (34.5±1.74). There was a statistically significant elevation (P<0.001) in GSH levels in both the M.V 700 mg/kg group (24.12±1.43) and the M.O 1400 mg/kg group (29.9±0.32) in comparison to the group that received the vehicle treatment as shown in Figure 4(C).

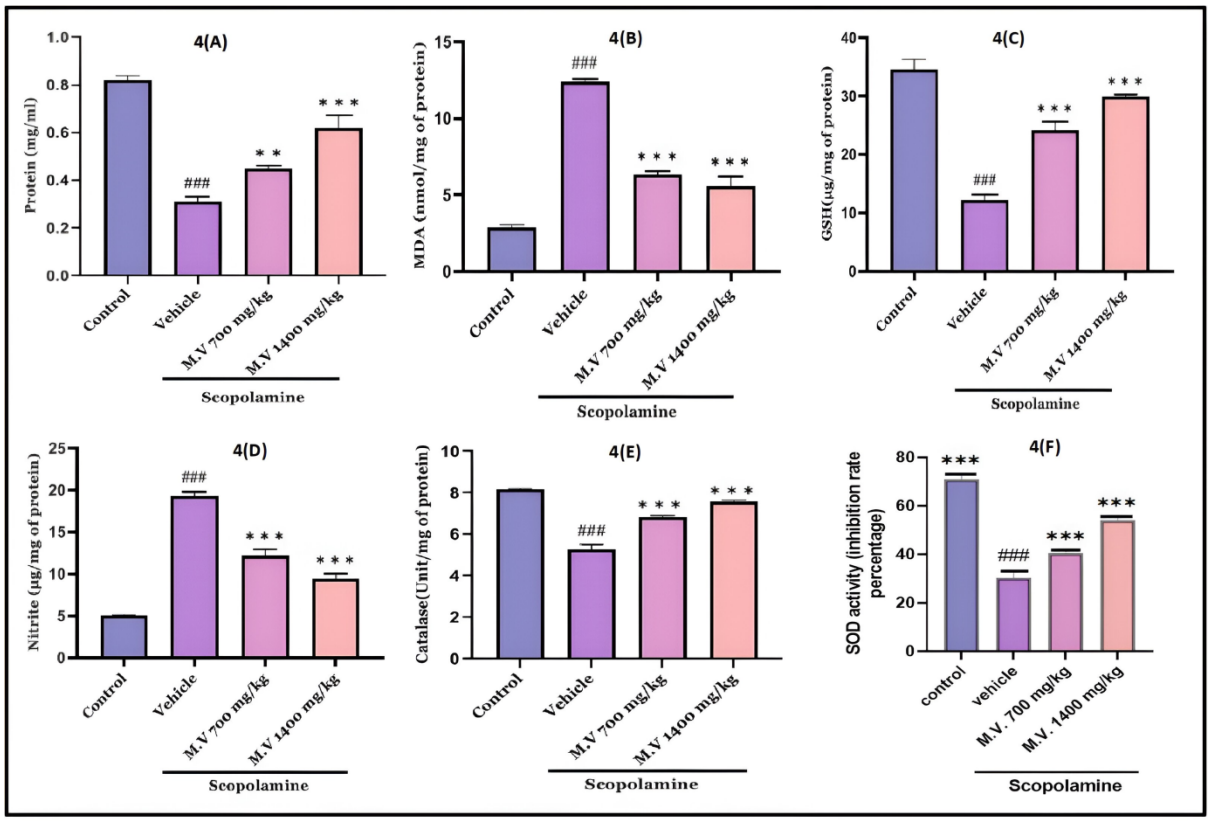

Figure 4: Effect of *Marrubium vulgare* on biochemical parameters: 4(A) Total protein content; 4(B) malondialdehyde level; 4(C) Glutathione level; 4(D) Nitrite; 4(E) Catalase; 4(F) Superoxide dismutase. Each bar represents the mean±SEM (n=6). The results showed significant differences: ###p<0.001 vs Control, ***p<0.001 vs Scopolamine+Vehicle, **p<0.01 vs Scopolamine+Vehicle.

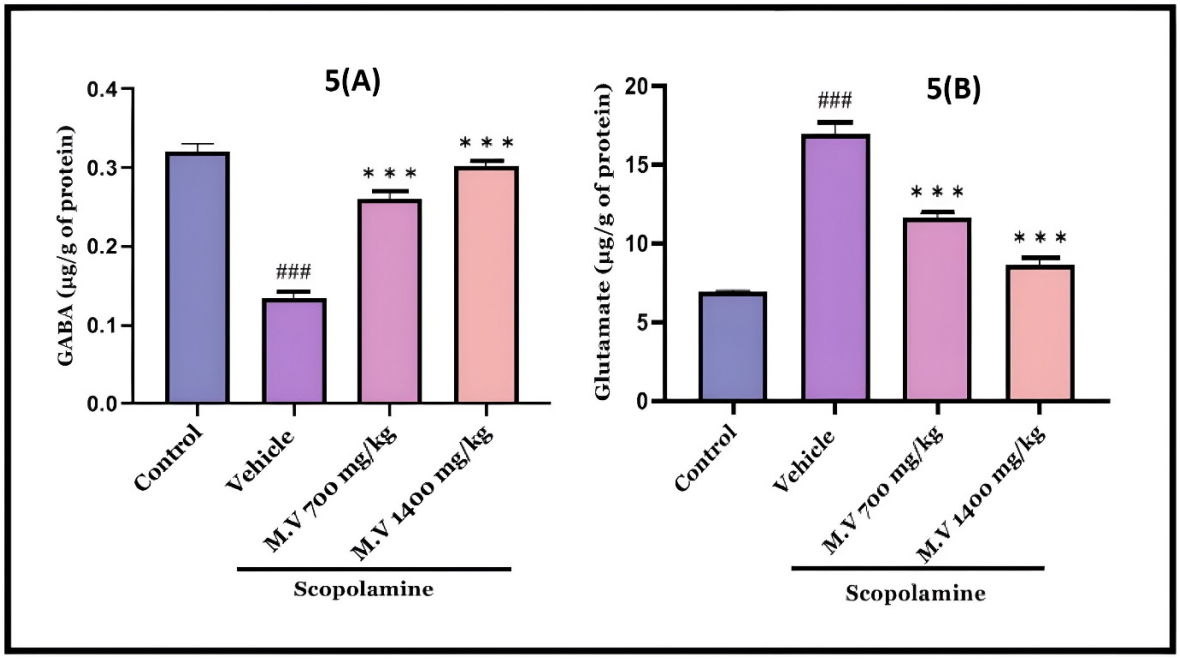

Figure 5: Effect of *Marrubium vulgare* on Neurotransmitters levels. 5(A) GABA; 5(B) Glutamate. Each bar represents the mean±SEM (*n*=6). The results showed significant differences: ###*p*<0.001 vs control, ***p<0.001 vs vehicle+Scopolamine.

Effect of *Marrubium vulgare* on Nitrite Level (NO) in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination revealed a statistically notable rise (p<0.001) in NO levels in the vehicle-treated cohorts (19.3±0.43) when compared to control group (5.03±0.14). Conversely, a significant reduction (p<0.001) in NO concentrations was evident in both the M.V 700 mg/kg group (12.15±0.77) and the M.V 1400 mg/kg group (9.44±0.66) in comparison to the group that received the vehicle treatment as shown in Figure 4(D).

Effect of *Marrubium vulgare* on Catalase (CAT) level in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination revealed a noteworthy decrease (p<0.001) in catalase levels in the vehicle-treated groups (5.1±0.63) when compared to the control group (8.1±0.09). Additionally, a statistically significant rise (p<0.001) in catalase concentrations was observed in the M.V 700 mg/kg group (7.01±0.26) and the M.V 1400 mg/kg group (7.64±0.53) in comparison to the group that received the vehicle treatment as shown in Figure 4(E).

Effect of *Marrubium vulgare* on Superoxide dismutase (SOD) in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination indicated a significant reduction (p<0.001) in SOD activity in the vehicle-treated groups (30.16±0.048) when compared to control group (70.83±0.037). Conversely, there was a statistically significant increase (p<0.001) in SOD levels observed in both the M.V 700 mg/kg group (40.3±0.026) and the M.V 1400 mg/kg group (54±0.081) in comparison to the group that received the vehicle treatment as shown in Figure 4(F).

Neurotransmitters

Effect of Marrubium vulgare on GABA level in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination revealed a statistically notable decrease (p<0.001) in GABA levels in the vehicle-treated cohorts (0.23±0.043) when compared to control group (0.32±0.014). Conversely, a significant rise (p<0.001) in GABA concentrations was evident in both the M.V 700 mg/kg group (0.26±0.071) and the M.V 1400 mg/kg group (0.28±0.065) in comparison to the group that received the vehicle treatment as shown in Figure 5(A).

Effect of *Marrubium vulgare* on Glutamate level in mice

The initial step in the analysis involved conducting univariate analysis of variance (One-way ANOVA), which was followed by a subsequent Tukey's *post hoc* examination indicated a substantial increase (p<0.001) in the glutamate level in the vehicle-treated groups (16.9±0.63) when compared to control group (6.9±0.09). Additionally, a statistically significant reduction (p<0.001) in

glutamate levels was observed in both the M.V 700 mg/kg group (11.67±0.26) and the M.V 1400 mg/kg group (8.64±0.53) in comparison to the group that received the vehicle treatment as shown in Figure 5(B).

Histopathology

Histopathological examination of hippocampal lides from mice brains stained with Haematoxylin and Eosin was conducted. Figure 6(A) displays the control group with normal histoarchitecture. A marked degeneration of neurons was observed in the scopolamine-treated group, as shown in Figure 6(B). Mice treated with scopolamine and M. vulgare at 700 mg/kg demonstrated reduced neuronal degeneration, as depicted in Figure 6(C). A further reduction in degeneration was evident in the group treated with scopolamine and M. vulgare at 1400 mg/kg, as illustrated in Figure 6(D).

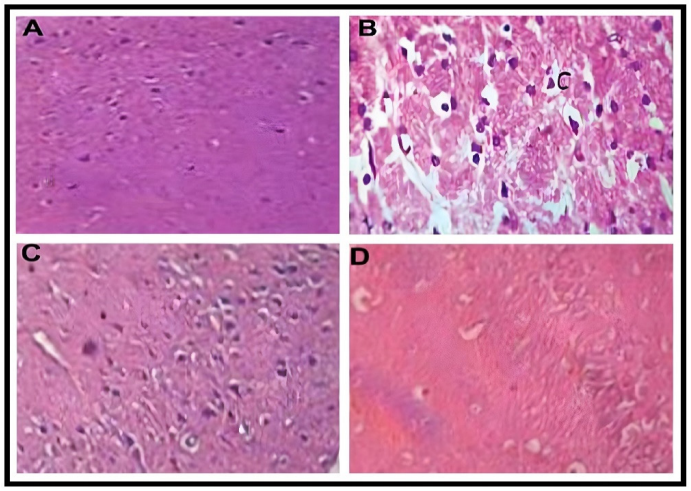

Figure 6: Histopathological slides of hippocampus in brain of mice stained with Haematoxylin and Eosin (a) Control group showing normal histoarchitecture (b) Mice treated with Scopolamine with prominent degeneration of neuron (c) Mice treated with Scopolamine and *M. vulgare* 700 mg/kg (d) Mice treated with Scopolamine and *M. vulgare* 1400 mg/kg.

DISCUSSION

Dementia manifests as a significant decline in cognitive abilities beyond typical age-related changes, affecting memory, attention, language and problem-solving skills. Alzheimer's Disease (AD) stands as the primary cause of dementia among the elderly, with onset typically occurring after the age of 65. The neuropathological hallmarks of AD include the accumulation of senile plaques and neurofibrillary tangles, leading to neuronal degeneration. Recent research indicates that cholinergic deficits in AD patients are closely correlated with the severity of cognitive decline and memory impairment. Additionally, oxidative imbalance is increasingly recognized as a significant contributor to the development and progression of AD. Studies have highlighted the involvement of calcium signalling and glutamatergic activity in the regulation of cognitive function, underscoring their importance in AD pathophysiology. Alzheimer's Disease (AD) is characterized by the excessive activation of glutamatergic activity, leading to elevated glutamate levels and subsequent neurodegeneration. Currently, one of the most effective treatments for AD involves using the NMDA blocker memantine to reduce endogenous glutamate levels. Inhibition of glutamatergic activity has been shown to improve learning and memory in rodents, nonhuman primates and humans. Scopolamine, a muscarinic receptor antagonist, disrupts memory formation and learning processes, particularly affecting short-term memory in both humans and animals. To investigate the potential of *Marrubium vulgare* in ameliorating memory impairment through its glutamatergic activity-blocking action, scopolamine was employed in this study. The Morris Water Maze (MWM), Elevated plus Maze (EPM) and Open Field Test (OFT) were utilized to assess memory function in mice. The MWM is commonly used to evaluate spatial learning and memory in behavioural neuroscience. In this paradigm, mice are placed in a water maze with a hidden platform and visual cues for navigation. The animals' ability to locate the platform improves over trials, indicating learning and memory of its location relative to visual cues. The elevated plus maze was used as an external behavioural model to assess the memory and learning abilities of the mice. No discernible decrease in transfer latency time in mice after scopolamine administration suggested memory impairment in mice. Further, in MWM mice treated with scopolamine had significantly higher latency to reach the hidden platform. In OFT memory impairment in mice is indicated by slow locomotion activity. These findings agree with previous reports showing memory impairment following scopolamine injection in rodents. The effects of *Marrubium vulgare* were examined in a mouse model of scopolamine-induced memory impairment. Administration of *Marrubium vulgare* displayed a dose-dependent influence on the dementia caused by scopolamine in the mice. In the MWM test, there was a significant reduction in latency to reach the hidden platform suggesting amelioration of dementia caused by scopolamine. A significant decrease in retention latencies was observed in EPM, following *Marrubium vulgare* treatment in mice. In OFT, there was a significant increase in locomotion activity. The collective findings demonstrated that *Marrubium vulgare* ameliorates memory impairment induced by scopolamine administration in mice. Prior research has shown that the memory deficits caused by scopolamine are associated with the overactivity of NMDA receptors and oxidative stress occurring in the brain. The objective of this investigation was to explore the potential anti-Alzheimer effect of *Marrubium vulgare* by evaluating its influence on oxidative stress and glutamatergic activity. The levels of glutamate in various regions of mice brains were measured to assess the involvement of NMDA receptors. The results of this study demonstrated a significant augmentation in glutamatergic activity within the brain regions of mice following the administration of scopolamine. However, this effect was

alleviated in a dose-dependent manner by *Marrubium vulgare*. *Marrubium vulgare's* inhibition of glutamatergic activity could potentially restore glutamate balance in the brain, contributing to its anti-amnesic effects in the scopolamine-induced model. This suggests glutamatergic activity inhibition might address diminished glutamate levels in AD. Additionally, this study examined whether scopolamine-induced memory deficits correlated with alterations in oxidative stress markers. Oxidative imbalance results from the disparity between free radical production and their neutralization by antioxidants. Previous research has unveiled a significant link between the memory impairments observed in scopolamine-induced amnesia models and the oxidative damage found in individuals with mild cognitive impairment. Additionally, extensive clinical investigations have verified that oxidative stress performs a critical function in driving the advancement of AD. In this study, we assessed the levels of endogenous antioxidants, including GSH, catalase and SOD, along with the marker for lipid peroxidation, MDA. Elevated MDA levels signify neuronal degeneration, while reduced levels of GSH, catalase and SOD indicate an upsurge in the production of free radicals. Following the completion of behavioural assessments in the scopolamine-induced dementia model, we measured the levels of MDA, GSH, catalase and SOD to evaluate oxidative imbalance. Mice treated with a control substance exhibited a noteworthy increase in MDA and a decrease in GSH, catalase and SOD levels in the brain compared to baseline values, signifying heightened oxidative stress. *Marrubium vulgare* exhibited a significant reduction in the scopolamine-induced increase in MDA and a decrease in GSH, catalase and SOD levels within the mouse brain. These results indicate that the memory-enhancing effects of *Marrubium vulgare* in the scopolamine model may be linked to improved cholinergic function and reduced oxidative stress.

CONCLUSION

In this study, we aimed to explore how *Marrubium vulgare* extract could help with Alzheimer's type dementia induced by scopolamine in mice. Our findings shed light on the potential benefits of using *Marrubium vulgare* as a treatment for Alzheimer's disease. Our findings demonstrate that treatment with *Marrubium vulgare* extract effectively attenuated the cognitive impairments induced by scopolamine. The mice treated with the extract exhibited significant improvements in behavioural outcomes and cognitive performance when compared to the scopolamine-treated group. These results suggest that *Marrubium vulgare* extract has the potential to mitigate the memory deficits associated with Alzheimer-type dementia. Furthermore, our study revealed the presence of potential neuroprotective effects of *Marrubium vulgare* extract. The extract appeared to protect against neuronal damage caused by scopolamine, leading to the preservation of synaptic integrity and neuronal function. These neuroprotective properties of *Marrubium vulgare* extract could be attributed to its antioxidant and anti-inflammatory activities, as evidenced by the reduction in oxidative stress markers and inflammatory cytokine levels observed in the treated mice. In this study, the administration of scopolamine significantly increased glutamatergic activity in the brain regions of mice. Treatment with *Marrubium vulgare* extract ameliorated this scopolamine-induced increase in glutamatergic activity in a dose-dependent manner. The ability of *Marrubium vulgare* to inhibit glutamatergic activity may help restore normal glutamate levels in the brain. This glutamatergic activity inhibition is likely responsible for the antiamnesic effects of *Marrubium vulgare* observed in the scopolamine-induced amnesia mouse model. The observed effects of *Marrubium vulgare* extract in our study provide support for its potential as a natural alternative for the treatment of AD. The extract's ability to modulate cognitive function and protect against neurodegeneration suggests that it may act through multiple mechanisms, including enhancement of neuronal plasticity, regulation of neurotransmitter systems and inhibition of neuroinflammation. However, further investigations are required to elucidate the underlying mechanisms of action of *Marrubium vulgare* extract in AD. Additionally, studies evaluating the long-term safety, optimal dosage and potential drug interactions are necessary to ensure its potential clinical relevance. In conclusion, our findings demonstrate the promising pharmacological potential of *Marrubium vulgare* extract in mitigating scopolamine-induced Alzheimer-type dementia in mice. This study provides a basis for future research and supports the exploration of *Marrubium vulgare* extract as a potential therapeutic agent for AD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ETHICAL APPROVALS

The experimental protocols (IAEC/MDU/2022/27) were approved by the Institutional Animal Ethics Committee (IAEC) of the Maharshi Dayanand University Rohtak, Haryana, India.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

ABBREVIATIONS

AD: Alzheimer's disease; **ROS:** Reactive oxygen species; **Ach:** Acetylcholine; **TNF:** Tumour necrosis factor; **OFT:** Open field test; **MWM:** Morris's water maze; **EPM:** Elevated plus maze;

AChE: Acetylcholinesterase; **CAT:** Catalase; **GSH:** Reduced glutathione; **SOD:** Superoxide dismutase.

SUMMARY

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder affecting older adults and causing significant global health challenges. Glutamate dysfunction, particularly involving NMDA receptors, contributes to AD's complexity. This study investigated *Marrubium vulgare's* effects on a mouse model of dementia induced by scopolamine. Results showed significant memory improvement, attributed to *Marrubium vulgare's* ability to reduce glutamate activity, oxidative stress and restore neurotransmitter levels. These findings suggest potential therapeutic benefits in managing AD by targeting glutamate modulation and oxidative stress reduction. Further research is required to confirm *Marrubium vulgare's* clinical efficacy.

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