ORIGINAL ARTICLE

Oxymatrine-mediated prevention of amyloid β-peptide-induced apoptosis on Alzheimer's model PC12 cells: in vitro cell culture studies and in vivo cognitive assessment in rats

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Abstract

Alzheimer's disease (AD) is a major neurological disease affecting elderly individuals worldwide. Existing drugs only reduce the symptoms of the disease without addressing the underlying causes. Commonly, Aβ25–35 peptide aggregation is the main reason for AD development. Recently, the discovery of multiple protein-targeting molecules has provided a new strategy for treating AD. This study demonstrates the neuroprotective potential of oxymatrine against multiple mechanisms, such as acetylcholinesterase, mitochondrial damage, and β-amyloid-induced cell toxicity. The in vitro cell culture studies showed that oxymatrine possesses significant potential to inhibit acetylcholine esterase and promotes antioxidant, antiapoptotic effects while preventing Aβ25–35 peptide aggregation in PC12 cells. Furthermore, oxymatrine protects PC12 cells against Aβ25–35-induced cytotoxicity and down-regulates the reactive oxygen species generation. The in vivo acute toxicological studies confirm the safety of oxymatrine without causing organ damage or death in animals. Overall, this study provided evidence that oxymatrine is an efficient neuroprotective agent, with a potential to be a multifunctional drug for Alzheimer's disease treatment. These findings present a reliable and synergistic approach for treating AD.

Keywords Alzheimer's disease (AD) · Anti-aggregation · Biocompatibility · Cytotoxicity · Neurodegenerative · Oxymatrine

Introduction

Alzheimer's disease (AD) is the most common neurological disorder worldwide, causing cognitive impairment. According to the World Health Organization (WHO), more than one billion people worldwide are affected by neurodegenerative disorders; 35 million people suffer from Alzheimer's disease and 50 million people suffer from epilepsy and other neurodegenerative diseases. The expected number of AD cases and incidence is 115 million by 2050 (Long and Holtzman 2019; Yiannopoulou and Papageorgiou 2020; Alexander and Karlawish 2021; AI-Atroshi et al. 2022; Folch et al. 2016;

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Jeyakumar et al. 2019). The amyloid peptides targeted drug discovery is a promising method for preventing β-amyloid aggregation in AD treatment. Amyloid-peptide (Aβ25–35) is a bioactive component of the Aβ peptide that leads to oxidative stress, memory loss, and apoptotic effects in brain cells. Neurotoxic short Aβ25–35 peptide fragments affect the tissues of the neurones (Jeyakumar et al. 2022; Wang et al. 2014; Zhang et al. 2018; Caruso et al. 2022; Michaels et al. 2020; Hu et al. 2021; Sun et al. 2021; Matuszyk et al. 2022). Currently, most of the scientific studies demonstrate that the aggregation of Aβ peptide causes oxidative stress-mediated necrosis in neuronal cells and develops AD. To date, several natural and synthetic curative agents have been discovered for AD. Among commercial drugs, only a few provide satisfactory cholinesterase inhibition against AD. Most medicines are used only for short-term relaxation of cognitive deficit relaxation (Nirale et al. 2020; Thoe et al. 2021; Wong et al. 2019; Abeysinghe et al. 2020). Intense biomedical research is mainly interested in the prevention of Aβ peptide deposition in the brain. In contrast, medicinal plants exhibit progressive effects and symptoms in various

diseases, including AD. Many studies have evaluated neuro-protective effects and isolated active compounds from plant extracts (Peng et al. 2021; Thakur et al. 2019; Ovais et al. 2018; Gul et al. 2021). Many plant compounds, such as res-veratrol, melatonin, galantamine, and catechin are known to have excellent neuroprotective effects against AD (Yan et al. 2020; Arbo et al. 2020; Roy et al. 2021; El-Ganainy et al. 2021; Okello and Mather 2020). Plant-derived alkaloids act as acetylcholinesterase (AChE) inhibitors and are considered model AD drugs (e.g., donepezil, galantamine, and rivastig-mine) (Nguyen et al. 2021; Piemontese et al. 2018). Health technology assessments have undermined the adverse side effects of neuroprotective drugs in patients with AD (Terao et al. 2022). Therefore, it is imperative to discover AChE-inhibiting compounds with a biocompatible central nervous system (Tan and Ismail 2020; Franchini et al. 2020; Sinha et al. 2020).

In this study, we demonstrated the multifactorial neuro-protective efficacy of the plant compound oxymatrine in a PC12 cell model of AD. The antioxidant, anticholinesterase, and Aβ peptide aggregation inhibition were evaluated by in vitro cell culture techniques. Cognitive memory improve-ment in oxymatrine-administered rats was examined using an in vivo step-down passive avoidance test. We believe that this investigation will be important for the future develop-ment of AD drugs. In the future, oxymatrine could be an efficient drug for the long-term prevention of AD.

Materials and methods

Chemicals and reagents

Oxymatrine (MW: 264,36 g/mol), MTT, Dulbeccos modi-fied eagle's medium (DMEM), Aβ25–35 (MW: 1.060,27 g/mol), Dimethyl sulfoxide (DMSO), Annexin V-FITC, Rho-damine 123, Donepezil, Galantamine, Acetylthiocholine iodide (ATCI) were purchased from Aldrich. Aβ25–35 was pre-treated with Hexafluoroisopropanol (HFIP) with N_2 evaporation and maintained at $-20\ °C$ for further use. All the strains and reagents were used without further purifi-cation. DCFH-DA was obtained from Nanjing Jiancheng Bioengineering Institute, China. PC12 cells were purchased from Peking Union Medical College, Chinese Academy of Medical Sciences, School of Basic Medicine, Cell Centre.

Assessment of cell viability using MTT assay

The cytotoxic effect of oxymatrine on neuronal cells was examined using the MTT assay (Prabhu et al. 2019). PC12 cells were seeded in a 96-well plate (1×10^5 cells/well) and incubated at 37 °C for 24 h. The cytotoxicity of only oxymatrine was examined at different concentrations

(25–200 μM/L) against PC12 cells. The IC_{50} of oxymatrine was quantified from triplicate MTT cell viability assays. The oxymatrine-mediated prevention of cytotoxicity of Aβ25–35 peptide-induced PC12 cells was examined at various con-centrations (1, 10, and 100 μM/L) of oxymatrine pretreat-ment under optimum conditions for 48 h. Subsequently, 50 μM/L of Aβ25–35 peptide was added to the culture plates, and cells were incubated for 48 h. After that, the cells were stained with 50 μl of MTT solution, and incuba-tion was continued for another 4 h at 37 °C. Excess MTT was removed with dimethyl sulfoxide. The absorbance of the cell suspension was measured at 570 nm using an ELISA plate reader. In a comparative study, cells were treated with oxymatrine (1,10, and 100 μM/L) loaded into a well plate before Aβ25–35 treatment:

$$Cell\ Viability\ \% = \frac{Absc - Abst}{Absc} \times 100$$

where *Absc*—absorbance of control cells, *Abst*—absorbance of treated cells.

Downregulation of reactive oxygen species (ROS) levels induced by Aβ25–35 protein

The antioxidant potential of oxymatrine was examined using a DCFHDA assay (Jeyakumar et al. 2022). The fluorescent probe DCFH-DA was added to PC12 cells and incubated at 37 °C for 1 h. The treated cells were washed with phosphate buffer solution (PBS). The fluorescence intensity of DCF-HDA was recorded at 485 nm excitation and 530 nm emis-sion ranges. Inhibition of superoxide dismutase (SOD), cata-lase (CAT), and glutathione peroxidase (GPx) was measured according to manufacturer's instructions of a commercial kit (Nanjing Jiancheng Bioengineering Institute, China). SOD, CAT, and GPx inhibition levels were quantified at 405, 412, and 550 nm, respectively.

Assessment of apoptosis from Caspase-3 activation assay

To assess the anti-apoptotic properties of oxymatrine in PC12 cells, a Caspase-3 activation assay was performed (Pei et al. 2022). Caspases-3 protease is a primary indicator of apoptosis. Caspase 3 activity in oxymatrine-treated PC12 cells was measured using the acetyl-Asp–Glu–Val–Asp p-nitroanilide (Ac-DEVDpNA) enzyme to hydrolyse the substrate p-nitroaniline (PNA). The reaction mixture con-tained 200 μM Ac-DEVD–pNA, and 50 μg of Aβ25–35 pro-teins. The reaction mixtures were loaded into 24 well plates and incubated at room temperature for 1 h. Donepezil was used as a positive control. The percentage inhibition of cas-pase-3 was measured at 405 nm and p-nitroaniline was used

as the reference. The quantification results are expressed as μ mol of pNA/min/mg of protein. The percentage of Caspases 3 activity was calculated using the following equation:

$$Specific\ activity\,(\%)$$
$$= \frac{(\mu MpNA \times dilution\ factor)}{Time \times volume\ of\ sample \times protein\ concentration} \times 100$$

Free radical scavenging assay

The antioxidant potential of oxymatrine was examined using the DPPH free-radical scavenging assay. The hydrogen peroxide (H_2O_2) scavenging ability of oxymatrine was evaluated according to the method described by Jeyakumar et al. (Jeyakumar et al. 2019). A H_2O_2 solution of 50 mM at pH 7.4 was added with different doses (10–50 μg/ml) of oxymatrine. The reaction mixture was incubated at room temperature for 15 min. The inhibition was quantified from the maximum absorbance of H_2O_2 at 550 nm. PBS solution was used as a reference. L-ascorbic acid was used as a positive control. The total percentage of H_2O_2 scavenging was calculated using the following equation:

$$\% \, of \, inhibition = \frac{Absc - Abst}{Absc} \times 100$$

where Absc—absorbance of control, and Abst—absorbance of treated group.

Determination of mitochondrial membrane potential

Mitochondrial membrane potential was assessed by spectrofluorimetric and fluorescence microscopic analyses using JC-1 staining (Raju et al. 2022). PC12 cells were treated for 24 h in the IC_{50} concentration 82.43 ± 0.02 μM/L of oxymatrine. After 24 h of exposure, 5 μL of JC-1 stain was added and the cells were incubated for 20 min. Stained PC12 cells were washed with PBS to remove the free-floating dye. The fluorescence intensity of the cell culture medium was recorded at excitation and emission wavelengths of 480 and 530 nm, respectively. Cellular changes were monitored, and images were captured using a fluorescence microscope at $20 \times$ magnification.

Apoptotic and live cell staining

The Acridine orange and ethidium bromide (AO/EtBr) dual-staining method was used to evaluate apoptotic and live/dead cells (Raju et al. 2022). PC12 cells were pretreated with different concentrations of oxymatrine (1, 10, and 20 μM/L) and Aβ25–35 peptide (50 μM/L) based on the MTT assay.

Treated cells were incubated at 37 °C under a carbon dioxide incubator. Treated cells were washed with PBS and transferred onto microscopic glass slides. Next, AO/EtBr dual stains (10 μl) were added to treated cells, and incubation was continued for another 10 min. Excess stain was washed with PBS. Images were captured using a fluorescence microscope (Nikon ECLIPSE Ti-E, Japan).

Assessment of anti-cholinesterase activity

The AChE inhibitory potential of oxymatrine was examined as previously reported (Jeyakumar et al. 2019). Briefly, two different concentrations (5 and 10 μg/ml) of oxymatrine were incubated with Aβ25–35 peptide (20 βL) at room temperature. After completion of the incubation period, 1 mM of DTNB (100 μl) was added with Tris–HCl buffer solution at pH 8.0 and made up the total volume of 1 mL. Furthermore, Acetylthiocholine iodide (50 μl) was added to the reaction mixture. Finally, 1 mM 5-thio-2-nitrobenzoate anion was added for yellow colour formation. The absorbance of the reaction solution was recorded at 405 nm using UV–Vis spectroscopy. The experiment was performed in triplicate. Donepezil was used as a positive control. Percentage of acetylcholine inhibition was calculated using the following formula:

$$\% \, of \, inhibition \, = \frac{Sc - St}{Sc} \times 100$$

where Sc is the specific activity of the control and St is the specific activity of the treated group.

Aβ25–35 peptide anti-aggregation assay

The Aβ25–35 peptide anti-aggregative potential of oxymatrine was quantified using oligomer and fibril anti-aggregation methods (Jeyakumar et al. 2022). Next, 0.5 mg of Aβ25–35 powder was dissolved in 1 ml of hexafluoro-2-propanol and left at room temperature for 24 h to form monomers.

Phase I

Inhibition of oligomer aggregation: in this experiment, Aβ25–35 monomers (100 μM) were dissolved in 100 mM Tris–HCL buffer and incubated for 24 h. After the incubation oxymatrine (5 and 10 μg/ml) was treated with oligomer and incubated for another 24 and 48 h. Galantamine (100 μg/ml) was considered a positive control. Finally, the treated samples were subjected to Thioflavin-T assay.

Phase II

Inhibition of fibrils aggregation: in this experiment, Aβ25–35 powder (100 μM) was dissolved in 50 mM of Tris–HCL buffer solution and incubated at 37 °C for 96 h to allow fibril maturation. The aggregated fibrils were treated with different concentrations of oxymatrine (5 and 10 μg/ml) and Galantamine (100 μg/ml) and incubated for another 96 h and 9 days, respectively. The total mature fibrils formation was quantified using a T assay.

Assessment of apoptosis in Aβ25–35-induced PC12 cell by flow cytometry

The anti-apoptotic effect of oxymatrine in PC12 cells was examined using Annexin V-FITC/PI staining (Pei et al. 2022). The IC_{50} concentration of oxymatrine treated cells was 82.43 ± 0.02 μM/L and 2.5 μl of annexin V-FITC/PI was used to stain the cells. The total percentage of apoptotic cells was quantified using the CytoFLEX flow cytometer. Early/late apoptotic cells were quantified using the FlowJo software.

Animal maintenance and groupings

The healthy 12-week-old Sprague–Dawley (SD) rats (180–250 g weight) were obtained from the Medicine Centre of Jinzhou Medical University (Jinzhou, China). Rats were maintained at 25 °C in 12 h dark/light cycle conditions with $60 - 70\%$ humidity. The animals experiments were approved by the Experimental Animal Ethics Committee of Jinzhou Medical University, China. Experimental animals were divided into four groups. Toxicity tests were performed according to the Organization for Economic Cooperation and Development test guidelines (OECD Guideline 423) for acute oral toxicity tests. Before starting the experiment, the body weights of the animals were individually recorded to calculate the appropriate treatment dosage. The volume was adjusted depending on the body weight of the rat using 10 ml/kg to be used in the rat as mentioned. SD rats were administered standard rat pellets and reverse osmosis (RO) water ad libitum. They were acclimatised to laboratory conditions for 7 days before the experiments and were housed in groups of three for acute oral toxicity. The rats were maintained at a room temperature of 25 °C, with a 12 h light/dark cycle.

Evaluation of acute toxicity

The healthy 12-week-old SD rats (180–250 g weight) were obtained from the Comparative Medicine Center of Yangzhou University (Yangzhou, China). Rats were maintained at 25 °C in 12 h dark/light cycle conditions with $60 - 70\%$ humidity. The animals were handled according to the guidelines of the Animal Ethics Committee of China Pharmaceutical University. The animals were then divided into corresponding groups (Al-Afifi et al. 2018). Oxymatrine was orally administered to the test animals 5 days before euthanization. Testing compound oxymatrine was dissolved in 0.5% of carboxymethylcellulose sodium salt solution (500, 1000, and 1500 mg/kg) and fed via oral administration. After oxymatrine administration, behavioural changes and mortality were continuously monitored for the first 24 h. One set of animals was occasionally observed for another 14 days to assess delayed acute toxicity. The PBS-treated rat group was used as the control group. All oxymatrine-treated rats were sacrificed on the 15th day. Organ damage in the heart, liver, and kidneys was evaluated macroscopically. In vivo acute toxic effects of oxymatrine in rats were examined using eosin–haematoxylin staining. The slides were observed and images were captured using an Olympus fluorescence microscope (SZX7 Zoom Stereo Microscope).

In vivo step-down passive avoidance test

A modified step-down passive avoidance test was performed to examine the learning ability and cognitive improvement in rats (Karimi et al. 2022). A total of 30 rats were used in the experiment (six rats/group. The experimental apparatus consisted of a plastic box divided into five equal compartment, separated by spaced steel bars measuring 1 cm. Rats were subjected to separate training and recall trials. Before the training trials, the animals were allowed to habituate for 5 min to the test environment. An electric current (24 V, 0.5 mA) was delivered to the apparatus platform, and the testing animals were gently placed on the platform. When the rats stepped down onto the platform, electric shock intermittently allowed them to return. Different doses of oxymatrine (20, 40, and 80 mg/kg) or a standard drug donepezil (5.0 mg/kg) were orally administrated prior to training trial. Donepezil was used as a positive control. After 30 min, scopolamine (3 mg/kg)-induced cognitive impairment. The learning and memory performance of the animals was evaluated from the latency step-down of first-time errors within 300 s of the shock.

Statistical analysis

Statistical analysis of all experimental data was performed using GraphPad Prism 8 software. The results were expressed as mean $\pm$ standard deviation (SD) and the comparisons were performed using one-way analysis of variance (ANOVA). P values of < 0.05 were considered to be statistically significant.

Results

Cytotoxicity of oxymatrine and Aβ25–35 peptide against PC12 cells

Prevention of cell death is essential for AD control. Enhancement of neuronal cell viability can protect against neurological disorders (Calvo-Rodriguez and Bacskai 2021). In this study, the cytotoxicity of oxymatrine in PC12 cells was examined using the MTT assay. The IC_{50} of oxymatrine alone was calculated to be 82.43 ± 0.02 μM/L. Aβ25–35 peptide treatment significantly ($29.05 \pm 1.2\%$) reduced the viability and density of PC12 cells (Fig. 1A) at the concentration of 50 μM/L. In this context, we investigated oxymatrine-treated PC12 cells, which showed non-toxic effect in PC12 cells. Furthermore, cells were pre-treated with oxymatrine at 1, 10, and 100 μM/L concentrations for 3 h. Aβ25–35 peptide-treated cells were cultured for another 24 h. The result revealed that treatment of oxymatrine prevented the cytotoxic effects of Aβ25–35 peptide, as shown in Fig. 1B, and mediation of oxymatrine rehabilitated the Aβ25–35 peptide-induced cell death. When the cells were treated at 100 μM/L, oxymatrine showed $86.50 \pm 0.5\%$ of viable cells, which was much higher than in the Aβ25–35 peptide. Morphological changes of Aβ25–35 peptide and oxymatrine were observed, and images were captured under an inverted phase contrast microscope. The control and oxymatrine-treated cells showed a clear cell structure and high cell density. In Aβ25–35, peptide-treated cells showed irregular morphology and a reduction in cell density.

The incorporation of Aβ25–35 peptide damaged the cell wall region and lead to cell shrinkage post-exposure. Oxymatrine-loaded cells were similar to the control cells. When the concentration of oxymatrine was increased, cell density and viability were also enhanced (Fig. 2). The cell viability assay confirmed that the incorporation of oxymatrine prevented the cytotoxic effect of Aβ25–35 in a dose-dependent manner. Therefore, oxymatrine is suitable for neuroprotective applications in cellular studies.

Reduction of ROS production against Aβ25–35-induced PC12 cells

Oxidative damage is closely associated with neurodegenerative diseases, such as AD, dementia, and Parkinson's disease, contributing to cellular inflammation and nitrated-protein dysfunctions (Good et al. 1996). The total production of ROS and cell morphology changes of Aβ25–35 and oxymatrine-treated PC12 cells was evaluated using DCFH-DA assay. The exposure of Aβ25–35 peptide at the dose of 50 μg/ml significantly induces ROS production in PC12 cells. The results of the DCFH-DA assay revealed maximum levels of SOD, GPx, and CAT enzyme activities, with some decreases compared to the control group (Fig. 3). The oxymatrine-pretreated cells showed low level of ROS production and enhanced SOD, GPx, and CAT activities. Presence of oxymatrine prevented oxidative stress in Aβ25–35 treated groups. The group treated with 10 g/mL oxymatrine exhibited similar levels of protective activity to that of commercial drug donepezil. The oxymatrine-treated group exhibited increased enzymatic activity in all three phases. CAT activity did not differ from that of the control. Whereas,
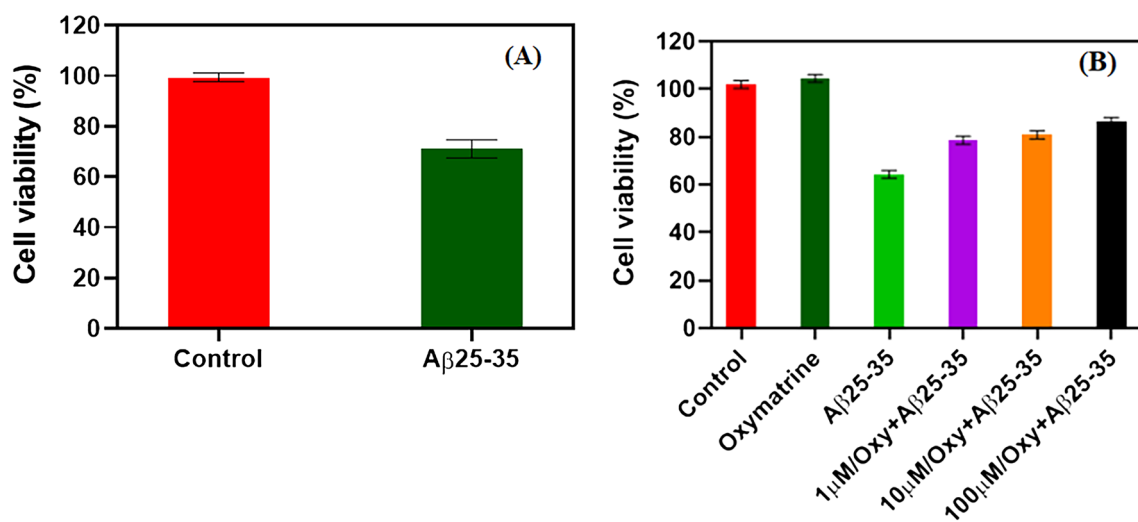

Fig. 1 Oxymatrine improved cell growth after Aβ25–35-induced PC12 cells. **A** Cell viability of Aβ25–35 (25 μM/L) treated PC12 cells by MTT. **B** Cell viability of oxymatrine (1 μM/L, 10 μM/L, 100 μM/L) doses pre-treated PC12 cells with Aβ25–35 (25 μM/L) cultured for another 48 h. The data represent Mean ± SD of triplicates with significance ($P < 0.05$) compared to the control
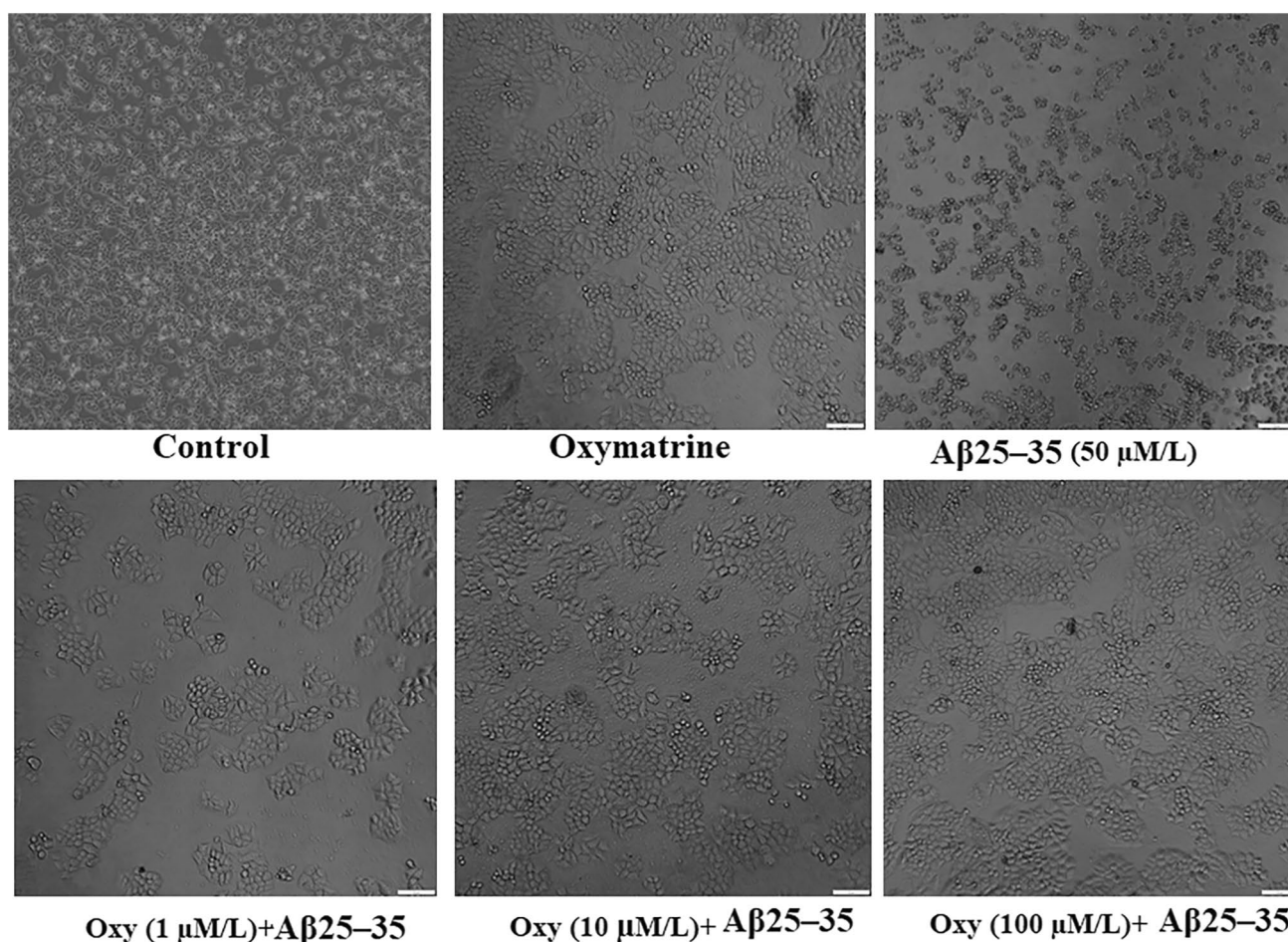
Fig. 2 Microscopic observation of oxymatrine and Aβ25–35-induced PC12 cells. Phase contrast microscopic images represent the cell morphological changes of oxymatrine and Aβ25–35-treated PC12 cells after 48 h post-exposure (20×)

the enzyme activities of SOD and GPx threefold increase compared to Aβ25–35 treated cells (Fig. 4A–C). After oxymatrine interruption, the intracellular ROS level was lower than the Aβ25–35 treated cells, indicating that oxymatrine protected the cells from ROS-mediated cell damage.

The anti-apoptotic potential of oxymatrine against Aβ25–35 peptide

Caspase-3 activity mediates cellular apoptosis The cleavage of cellular proteins in the Aβ25–35 treated PC12 cells and oxymatrine-treated cells were examined by caspase-3 activation assay. The results of PC12 cells with Aβ25–35 (50 μg/ml) treatment exposed 0.824 ± 0.007 μM of pNA/min/mg of protein increase in caspase-3. Control cells exhibited only 0.384 ± 0.040 μM of pNA/min/mg of protein increase in caspase-3 activity. Compared with a control group, Aβ25–35 treated cells showed a significant amount of caspase-3 activity-mediated apoptosis. In this context, cells were pretreated with oxymatrine and the commercial drug donepezil

(Fig. 4D) showed a significant decrease in caspase-3 values such as 0.559 ± 0.016, 0.5789 ± 0.006, and 0.533 ± 0.015 μM of pNA/min/mg of protein. The results of the caspase-3 activation assay suggested that the oxymatrine prevented the Aβ25–35-induced apoptotic cell death in PC12 cells.

Reduction of oxidative stress by oxymatrine pre-treatment

Free radical scavengers prevent the ROS forming and eliminate them from cell damage. Plant-derived compounds are well-known antioxidants. Antioxidants neutralize the free radical scavenging of cells by donating their electrons (Raju and Natarajan 2021; Raju et al. 2020). They act as natural switches that control free radicals. The antioxidant potential of oxymatrine was evaluated using the DPPH assay. The results showed excellent H_2O_2 scavenging activity under ambient conditions. Oxymatrine exhibited significant free radical reducing potential at the concentration of 50 μg/ml. Positive control L-ascorbic acid showed $67.70 \pm 1.32\%$
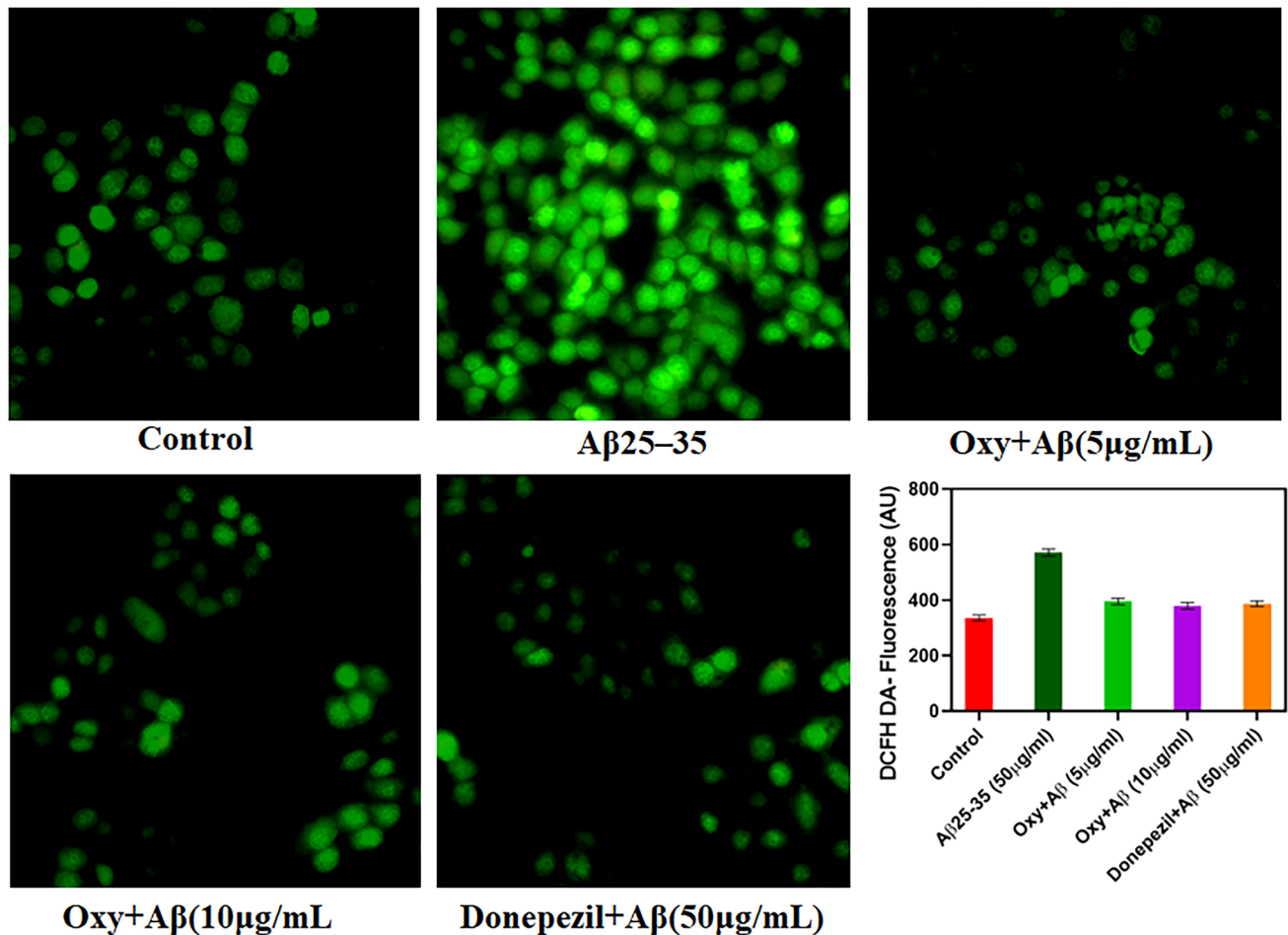
Fig. 3 Neuroprotective effect of oxymatrine (5 and 10 µg/ml), Aβ25–35 and commercial drug donepezil treated PC12 cells from ROS formation by DCFDA assay. Fluorescence microscopic images representing the inhibition of ROS production in oxymatrine pre-treated cells (20x). The data represent mean ± SD of triplicates with significance ($P < 0.05$) compared to the control

and oxymatrine was $68.01 \pm 1.21\%$, respectively (Fig. 5A). Compared to L-ascorbic acid, oxymatrine exhibited an equal hydrogen peroxide inhibition potential.

Enhancement of mitochondrial membrane potential by oxymatrine

The shape of the mitochondria is controlled by a balance of fusion–fission events. Damage to these mechanisms is closely associated with cell state. Loss of extracellular glucose supply leads to mitochondrial membrane potential (MMP) damage. Damage to the mitochondrial membrane potential harnesses cell protein production and energy flow (Wang et al. 2019; Misrani et al. 2021). In this experiment, fluorometric and microscopic changes of Aβ25–35 and oxymatrine-treated PC12 cells were assessed by Rh 123 staining technique. Aβ25–35 (50 µg/ml) treated PC12 cells showed a significant decline in MMP, as evidenced by a $59.92 \pm 2.64\%$ of cellular fluorescence decrease of Rhodamine 123. On the contrary, the cells pre-treated with oxymatrine exhibit an increased fluorescence intensity, such as $95.92 \pm 2.53\%$ and $96.91 \pm 3.65\%$, respectively. Oxymatrine treatment maintains mitochondrial integrity and protects cells from MMP damage. However, the effect of oxymatrine pre-treatment on cellular mitochondrial membrane integrity was similar to that in the control group (Fig. 5B). The experimental outputs confirmed that oxymatrine protected the mitochondria from Aβ25–35-induced MMP damage-mediated neurotoxicity.
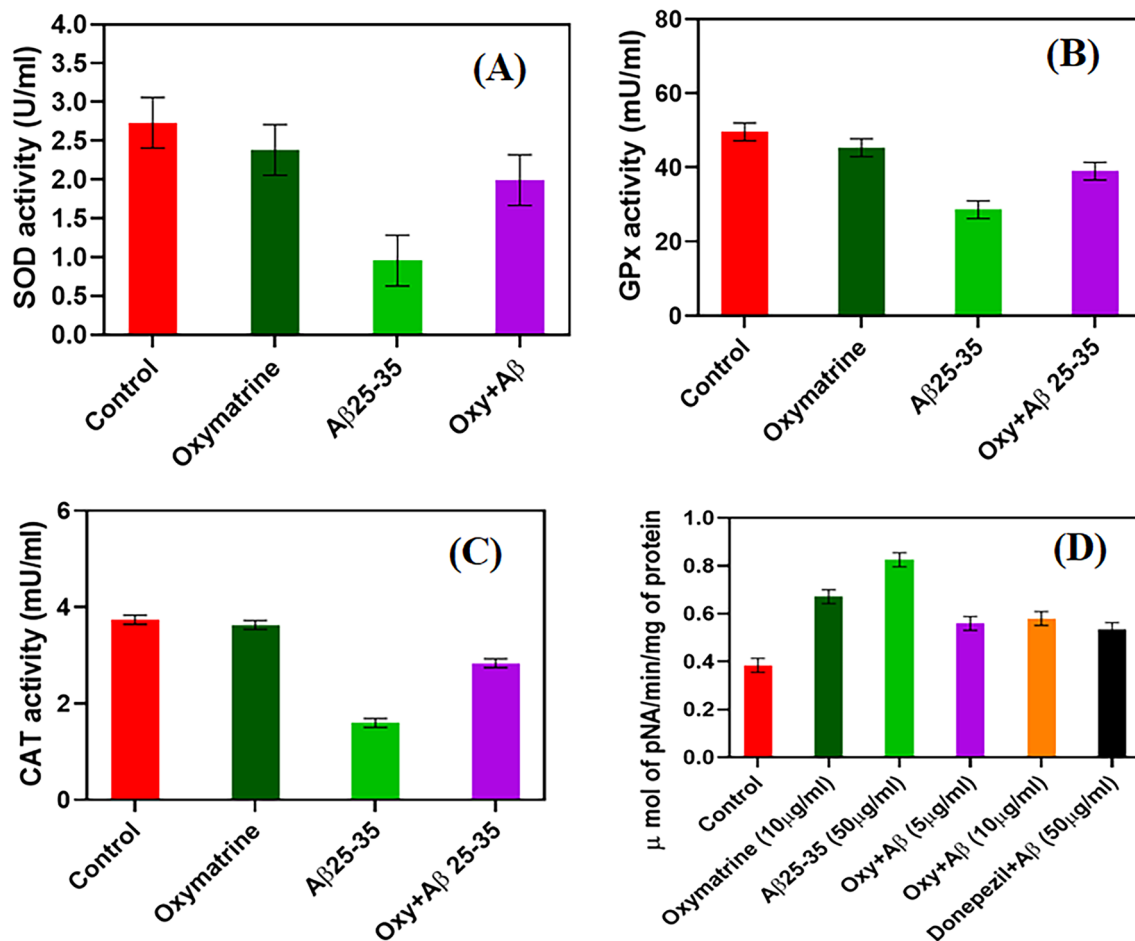
Fig. 4 Oxymatrine upregulated SOD, GPx, and CAT activity in PC12 cells **A–C** after treatment of Aβ25–35 for 48 h. **D** Quantification of Caspase-3 activity by pre-treatment of oxymatrine, and commercial drug donepezil against PC12 cells. The data represent Mean ± SD of triplicates with significance ($P < 0.05$) compared to the control
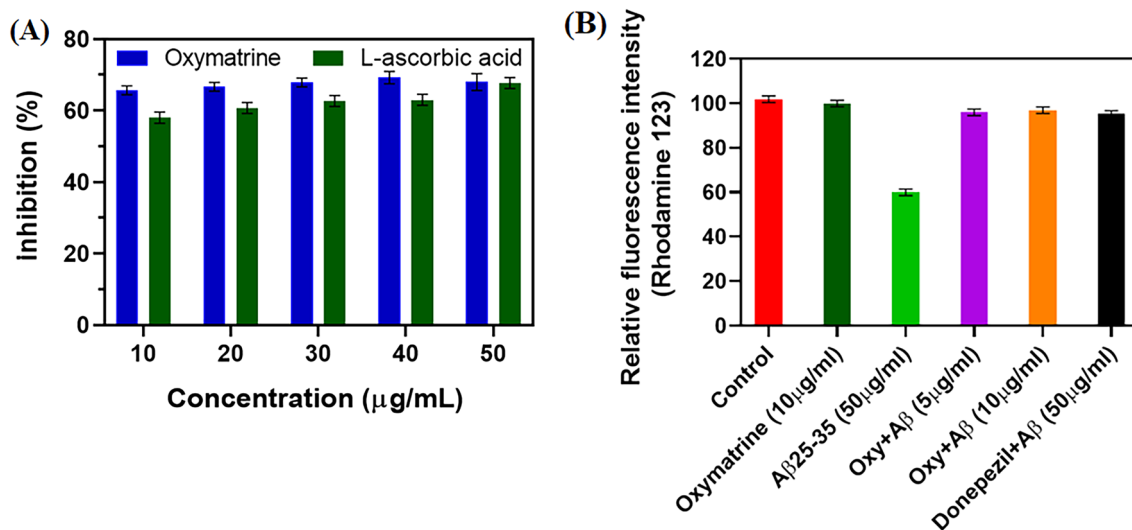

Fig. 5 DPPH free radical scavenging activity of oxymatrine. **A** Hydrogen peroxide (H_2O_2) inhibitory activity of oxymatrine and L-ascorbic acid at different concentrations. **B** Protective effect of oxy-matrine on MMP function in PC12 cells was assessed by Rhodamine 123 dye. The data represent Mean ± SD of triplicates with signifi-cance ($P < 0.05$) compared to the control

Anti-apoptotic activity of oxymatrine by AO/EtBr dual staining

Apoptosis is a highly sophisticated energy-dependent molecular event. Apoptosis triggers cellular injury and the disposal of damaged cells in an orderly fashion (Yu et al. 2020; Chu et al. 2020). In this AO/EtBr dual staining method, here Aβ25–35 treated cells exposed $40.90 \pm 2.56\%$ of dead cells with apoptotic effects. Control cells showed only $5.45 \pm 2.07\%$ of dead cells. In contrast, cells pre-treated with oxymatrine showed a significant decrease in apoptotic activity ($10.66 \pm 1.47\%$) compared with the Aβ25–35 treated group, i.e., threefold lesser (Fig. 6). Morphological changes and nuclear damage were evaluated using fluorescence microscopy. The microscopic images of AO/EtBr dual staining, Aβ25–35-induced cells showed a higher level of red fluorescence which represented the apoptotic cells. Cells pretreated with oxymatrine can also prevent apoptotic cell death and exhibit a regular cell structure with bright fluorescence. These fluorometric and microscopic evaluations suggested that oxymatrine have an excellent potential to prevent apoptotic cell death in brains affected by AD.

Anticholinesterase activity of oxymatrine in PC12 cells

Acetylcholinesterase (AChE) plays a vital role in the regulation of cholinergic mechanisms and neurotransmission. Decreased cholinergic activity is a common cause of neuronal cell death. According to cholinergic kinetics, a declining level of acetylcholine in the brain is the leading cause of memory destruction in AD (Lao et al. 2019; Stanciu et al. 2019; Majdi et al. 2020). The results revealed that oxymatrine enhanced the cholinergic activity and prevented the aggregation of Aβ25–35 peptide by AChE inhibition. Oxymatrine higher dose (50 μg/ml) showed $98.56 \pm 0.21\%$ of AChE inhibition. Standard drug donepezil showed $99.65 \pm 0.837\%$ of inhibition (Fig. 7A). Recent reports
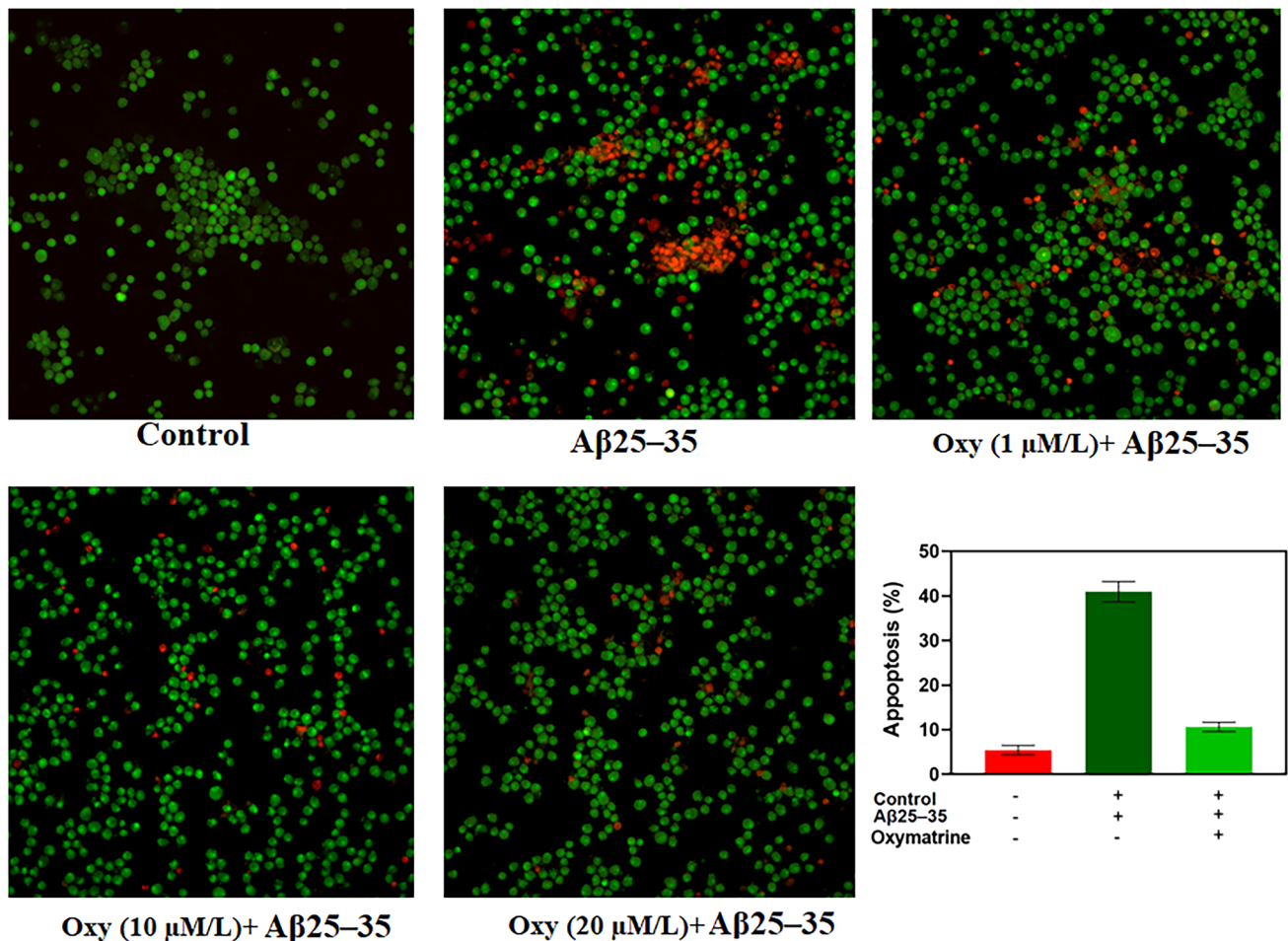

Fig. 6 Evaluation of the anti-apoptotic effect of oxymatrine against Aβ25–35 treated PC12 cells. Fluorescence microscopic images of AO/EtBr double stained PC12 cells after treatment (20×). The bar graph represents the apoptotic percentage of Aβ25–35 and oxymatrine-treated cells. The data represent Mean ± SD of triplicates with significance ($P < 0.05$) compared to the control
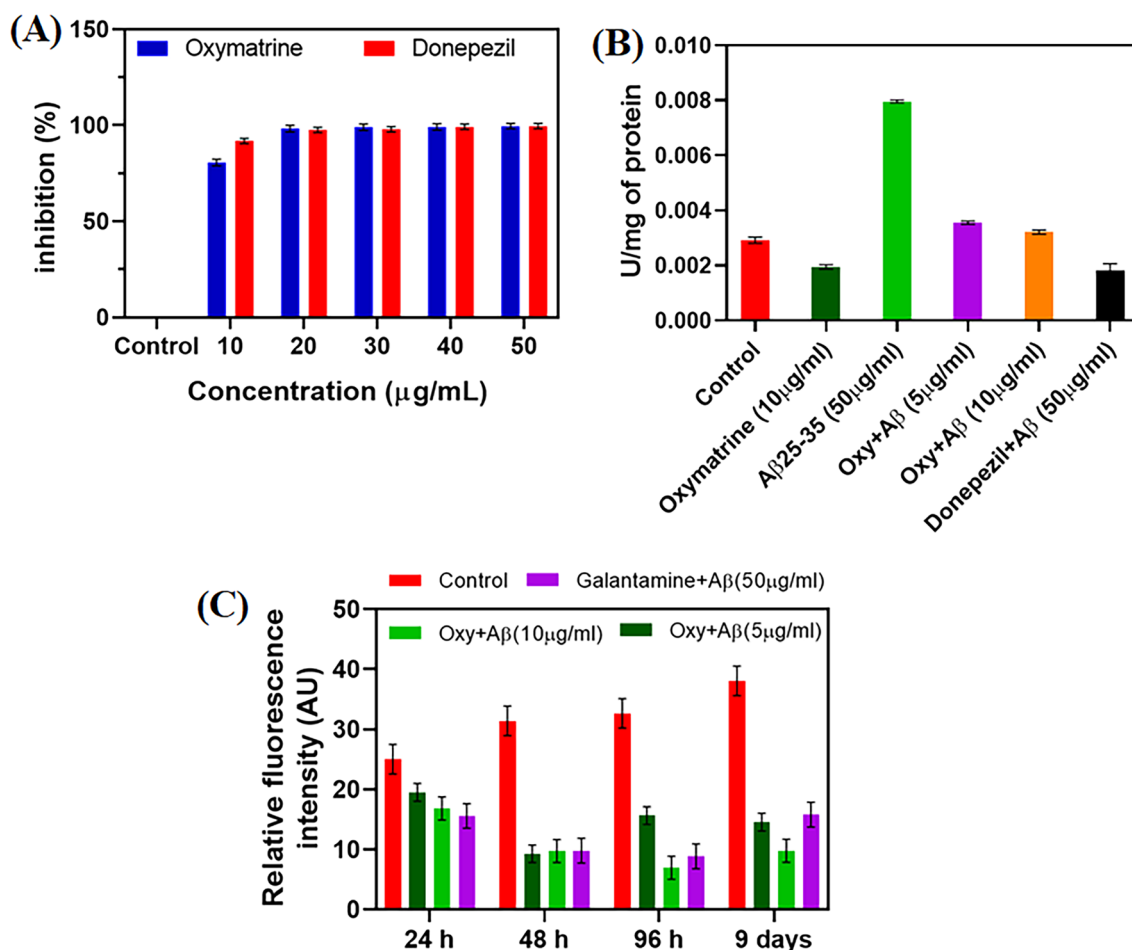
Fig. 7 Assessment of anti-acetylcholinesterase activity **A** Oxymatrine with a comparison of commercial drug donepezil. **B** Acetylcholinesterase inhibitory activity of oxymatrine against Aβ25–35-induced PC12 cells. **C** Anti-aggregation and disaggregation effects of Aβ pep-tide in different time intervals (24, 48, 96 h, and 9 days) in presence of oxymatrine (and Galantamine). The data represent Mean ± SD of triplicates with significance ($P < 0.05$) compared to the control

demonstrate phytocompounds as the new bio-functional scaffold for cholinesterase and β amyloid aggregation targeting. The Aβ25–35 treated cells exhibited a notable expansion in AChE level with enzyme activities (0.0078 ± 0.0001 U/mg of protein segregation. In this case, oxymatrine co-treated cells exhibited a significant level of AChE reduction with specific activities of (0.0035 ± 0.0002 and 0.0037 ± 0.0005 U/mg) of protein increase. The oxymatrine co-treatment effectively enhanced the cholinergic activity and prevented the Aβ25–35 peptide aggregations (Fig. 7B). The results of the anticholinesterase activity experiment confirmed that oxymatrine was a promising anti-cholinergic agent for the treatment of AD.

Anti-aggregation activity of oxymatrine against Aβ25–35 in PC12 cells

Extracellular aggregation of amyloid-β peptides is a leading cause of brain damage in AD. The additional formation of Aβ oligomers and β-amyloid deposition leads to brain plaques in AD. With the urgent need for alternative therapies, natural compounds serve as vital resources for novel drug developments (Zhao et al. 2022; Gomes et al. 2020; Zhang et al. 2021). The anti-aggregation ability of oxymatrine was assessed using an in vitro Thioflavin-T assay. The experiment was performed at different time intervals (24–96 h and 9 days) and in different phases: phase I (oligomer aggregation) and phase II (fibril formation). In the phase I study, the fluorescence intensity of Thioflavin-T was

found to increase in Aβ with oxymatrine 5 µg/ml treated group from 24 h (19.49 ± 2.31 AU) to 48 h (9.26 ± 1.54) indicating the reduction of Aβ aggregation. However, the treatment of oxymatrine at a dose of 10 µg/ml showed excellent Thioflavin-T fluorescence reduction at 24 h (16.70 ± 0.76 AU and 48 h (6.79 ± 0.75 AU), respectively. The standard drug galantamine showed (50 µg/ml) (15.51 ± 2.54 AU at 24 h and 9.70 ± 1.75 AU at 48 h), respectively. The results of the phase II fibril formation study showed that a reduced (ThT) fluorescence intensity in oxymatrine-treated cells and standard drug galantamine in 96 h (15.5 ± 1.03 AU, 6.83 ± 1.2 AU) and (8.82 ± 1.02 AU) and 9 days (13.79 ± 1.87AU, 9.73 ± 1.79 AU) and (15.74 ± 1.14 AU), respectively (Fig. 7C). The results showed that the pretreatment of PC12 cells with oxymatrine potentially disaggregated mature fibrils. These results highlighted the inhibitory potential of

oxymatrine against Aβ25–35 aggregation and phase I and II disaggregation. These experimental results provided a novel idea for the development of natural drugs for the treatment of AD.

Assessment apoptosis by flow cytometry

The total number of Aβ25–35- and oxymatrine-induced cell death and apoptotic stages was quantified by flow cytometry. The annexin-V/FITC and PI dual stains were utilised to evaluate the Aβ25–35-induced apoptosis and oxymatrine-mediated protective effects. Results showed that the control group exhibited (0.3%) apoptotic cells. However, when compared to PC12 cells treated with the dose of (50 µg/ml) Aβ25–35 showed 8.99% of early apoptosis and 16.7% of late apoptosis. Interestingly, oxymatrine (10 g/mL) pretreatment
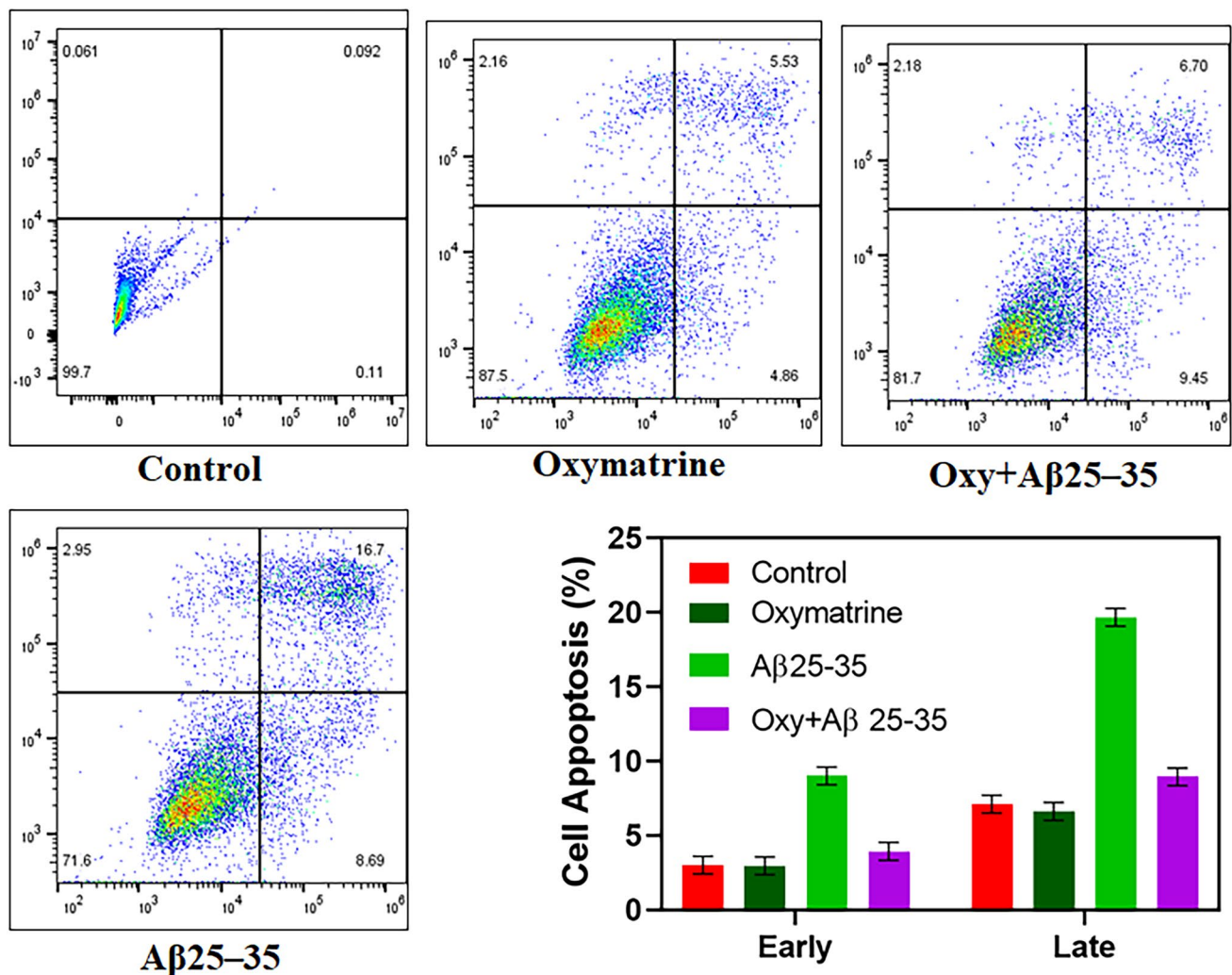

Fig. 8 Anti-apoptotic activity of Oxymatrine against Aβ25–35-induced PC12 cells. **A** Flow cytometric analysis of apoptosis in PC12 cells. **B** The percentage of early and late apoptotic PC12 cells in the Oxymatrine and Aβ25–35 treatments. The data represent Mean ± SD of triplicates with significance ($P < 0.05$) compared to the control

showed excellent protective effects in PC12 cells Aβ25–35 leading to apoptosis (Fig. 8). Overall, the flow cytometry study suggested that oxymatrine was an effective drug for Aβ25–35-induced neuronal cell death.

Evaluation of oxymatrine-induced acute toxicity in rats

Toxicity testing is essential for drug discovery before human use. The advantages of toxicological studies include clearly defining the genetic composition of test animals and their convenience of exposure. The toxicity of all tissues and the psychomotor activities of test animals can be evaluated using acute toxicity studies (Al-Afifi et al. 2018; Zhang et al. 2021; Tripathi et al. 2022). Acute toxicological studies also provide guidance for model drugs. In this study, oxymatrine-induced acute toxic effects in rat organelles such as the liver, heart, lungs, kidney, and spleen were investigated by histopathology (Fig. 9). Histopathological results showed no significant differences between the oxymatrine-administered animal groups and were similar to those in the PBS-treated control group. No significant tissue changes were observed in any of the groups (500–1500 mg/kg) in either of the acute oral toxicity tests. This result revealed that oxymatrine did not cause any inflammation or adverse effects on metabolic processes in the test animals. The cell morphology of the oxymatrine-treated groups was not different from that of the control group which was administered PBS. The present acute toxicological experiment demonstrated the safety of oxymatrine, suggesting that it is a promising neuroprotective drug for the treatment of AD.

Enhancement of cognitive activities by oxymatrine in the rat model

Cognitive decline is a common cause of memory impairment in patients with Alzheimer's disease. Neuropathological damage to the cerebral cortex and limbic system leads to impaired learning, memory, and spatial skills (Karimi et al. 2022). The present study explained the improvement in cognitive ability following oxymatrine administration in rat models using a step-down passive avoidance test. The
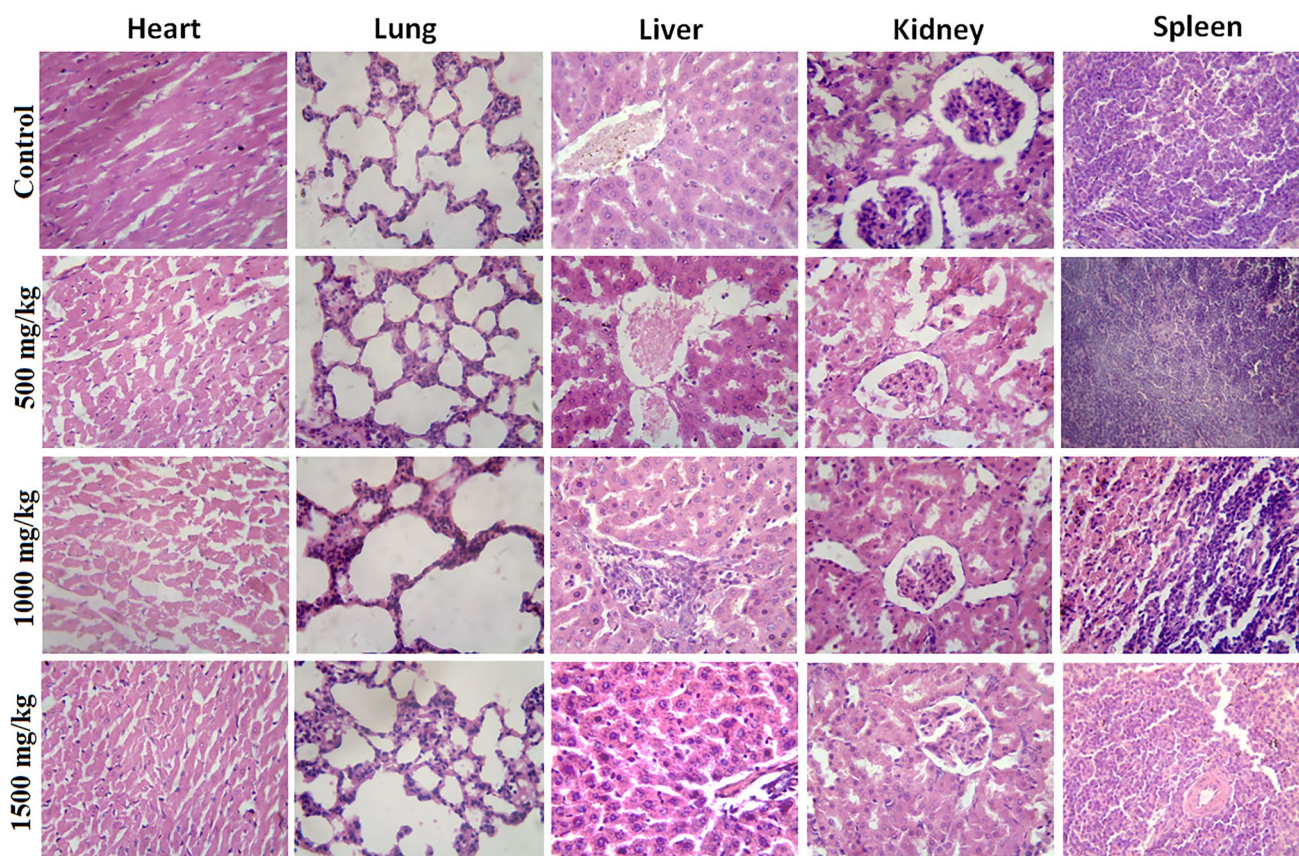

Fig. 9 Histopathological images of oxymatrine-treated rats in oral administration. Microscopic images of essential organs of experimental animal (rat) from the control and oxymatrine (500–1500 mg/kg) oral administrated groups (20×). There is no inflammation and irregular cell morphology appeared in oxymatrine-treated groups. The data represent Mean ± SD of triplicates with significance (*P* < 0.05) compared to the control
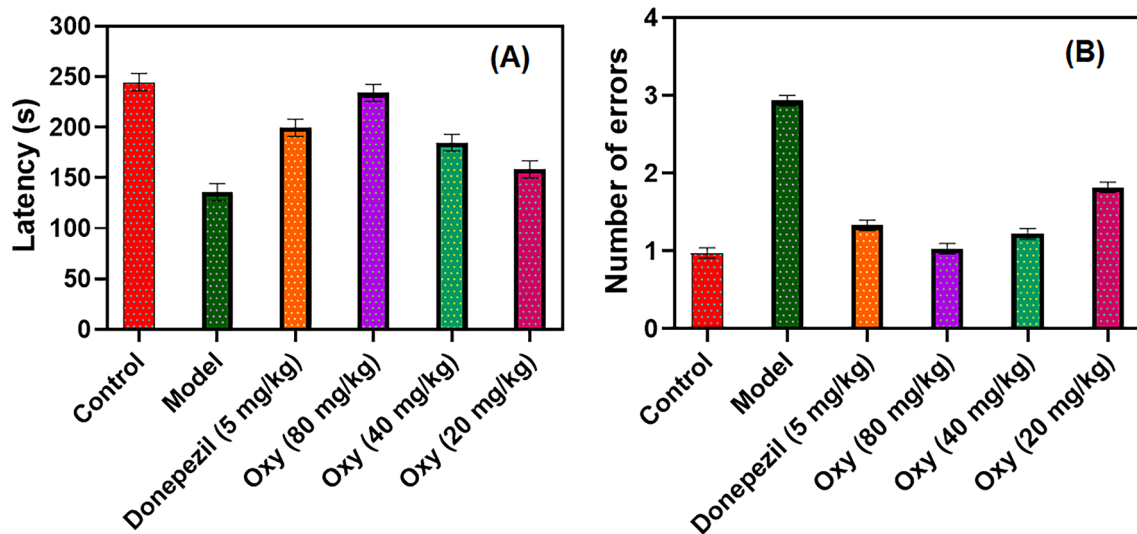
Fig. 10 Effects of oxymatrine on step-down test **A** latency and **B** number of errors in cognitive impairment. The significance value (mean ± SD) are representative of five independent experiments ($n = 5$)

commercial anticholinergic drug scopolamine was used to induce cognitive deficits in the animal model, and the commercial Alzheimer's drug donepezil was used as a positive control. The experimental results of the scopolamine-treated groups (Fig. 10) showed a significant reduction in latency and errors. When the animals were treated with oxymatrine, latency impairment and errors were reversed. The cognitive potential of the oxymatrine-treated groups showed a dose-dependent increase in learning and visual skills. The high-dose oxymatrine group (80 mg/kg) showed highest latency (233.83 s) and very few errors (1). This was better than that of the commercial drug donepezil group (5 mg/kg, 199.33 s, 1.2). In this context, the medium dose (40 mg/kg) of oxymatrine treated group exhibited a similar effect (184.79 s; 1.2) to donepezil. In the low-dose group (20 mg/kg), oxymatrine did not cause any notable differences in latency or errors (158.33 s; 1.9). The in vivo cognitive study results indicated that oxymatrine prevented cognitive deficits through anticholinergic activity-mediated brain damage.

Discussion

AD, a common form of dementia in the elderly, greatly impacts human life quality. Unfortunately, there is currently no successful treatment for AD. Recent studies have implicated inflammation and immunity in the disease. Researchers have also linked neurotoxicity mediated by ROS to β-amyloid. Oxymatrine, an alkaloid compound derived from the Chinese herb *Sophora flavescens*, has been investigated for its effects on oxidative stress, apoptotic mechanisms, and the progression of hypoxic–ischemic brain damage. Some

studies have explored its potential in the pathogenesis of AD. In this study, we aimed to investigate the neuroprotective efficacy of oxymatrine using PC12 cell lines as an in vitro model for AD. Our findings demonstrated that oxymatrine exhibited a protective role in preventing AD. We engineered an in vitro Alzheimer's model by inducing PC12 cell injury with the Aβ25–35 peptide. Pre-treatment with oxymatrine effectively protected PC12 cells from Aβ25–35 peptide-induced damage and apoptosis. While oxymatrine showed mild toxicity at a concentration of 200 μM/L, lower concentrations were found to be safe. Notably, oxymatrine pre-treatment significantly prevented cell death in Aβ25–35 peptide-induced PC12 cells. The DCFHDA assay revealed that oxymatrine inhibited ROS production in Aβ25–35 peptide-treated PC12 cells in a dose-dependent manner. We evaluated the effects of oxymatrine on caspase-3 expression in Aβ25–35-treated PC12 cells using a caspase-3 activation assay. The results showed that Aβ25–35 peptide treatment significantly increased caspase-3 expression, which was prevented by oxymatrine pre-treatment. Oxymatrine-treated cells alone exhibited minimal caspase-3 expression due to its mild toxic effect. The Amyloid-β anti-aggregation assay demonstrated that oxymatrine co-treatment reduced AChE activity in PC12 cells. Furthermore, the anti-inflammatory properties of oxymatrine protected Aβ25–35 peptide-induced PC12 cells from MMP damage and apoptosis. In in vivo assays, our acute toxicity study on rats revealed no toxic effects on major organs (liver, kidney, lungs, heart, and spleen). Rats pre-treated with oxymatrine exhibited improved cognitive development, showing significant facial memory improvement after 5 days of administration. These rats also displayed normal behaviour and reduced latency

errors. Oxymatrine pre-treatment effectively prevented scopolamine-induced cognitive deficits in rats. Overall, our investigation highlighted oxymatrine's ability to protect PC12 cells from Aβ25–35 peptide-induced MMP damage, apoptosis, caspase-3 gene expression, and ROS overproduction. The cellular protection observed with oxymatrine pre-treatment supports its potential neuroprotective role in AD. Future preclinical trials may establish its efficacy as an effective neuroprotective drug for AD.

Conclusions

This study highlights the significant protective effect of oxymatrine against neuronal cell death induced by β-amyloid. In vitro cell culture experiments demonstrate that oxymatrine pre-treated PC12 cells inhibit the mitochondrial damage and ROS generation caused by Aβ25–35 peptide. Oxymatrine's excellent antioxidant and anti-acetylcholinesterase properties effectively prevent amyloid aggregation. The antiapoptotic potential of oxymatrine in neuronal cells is confirmed through the caspase-3 activity assay. However, it is important to note that oxymatrine does exert cytotoxic effects on PC12 cells. Furthermore, in vivo acute toxicological experiments establish the safety of oxymatrine as a neuroprotective drug. These findings collectively support the development of effective neuroprotective agents for AD treatment, with oxymatrine emerging as a promising multifunctional drug in this regard.

Declarations

Conflict of interest The authors declare no competing interests.

References

Abeysinghe AADT, Deshapriya RDUS, Udawatte C (2020) Alzheimer's disease; a review of the pathophysiological basis and therapeutic interventions. Life Sci 256:117996

Al-Afifi NA, Alabsi AM, Bakri MM, Ramanathan A (2018) Acute and sub-acute oral toxicity of Dracaena cinnabari resin methanol extract in rats. BMC Complement Altern Med 18(1):1–14

AI-Atroshi C, Rene Beulah J, Singamaneni KK, Pretty Diana Cyril C, Neelakandan S, Velmurugan S (2022) Automated speech-based evaluation of mild cognitive impairment and Alzheimer's disease

detection using with deep belief network model. Int J Healthc Manag. https://doi.org/10.1080/20479700.2022.2097764

Alexander GC, Karlawish J (2021) The problem of aducanumab for the treatment of Alzheimer disease. Ann Intern Med 174(9):1303–1304

Arbo BD, Andre-Miral C, Nasre-Nasser RG, Schimith LE, Santos MG, Costa-Silva D, Hort MA (2020) Resveratrol derivatives as potential treatments for Alzheimer's and Parkinson's disease. Front Aging Neurosci 12:103

Calvo-Rodriguez M, Bacskai BJ (2021) Mitochondria and calcium in Alzheimer's disease: from cell signaling to neuronal cell death. Trends Neurosci 44(2):136–151

Caruso G, Godos J, Privitera A, Lanza G, Castellano S, Chillemi A, Grosso G (2022) Phenolic acids and prevention of cognitive decline: polyphenols with a neuroprotective role in cognitive disorders and Alzheimer's disease. Nutrients 14(4):819

Chu Q, Zhu Y, Cao T, Zhang Y, Chang Z, Liu Y, Zhang Y (2020) Studies on the neuroprotection of osthole on glutamate-induced apoptotic cells and an Alzheimer's disease mouse model via modulation oxidative stress. Appl Biochem Biotechnol 190(2):634–644

El-Ganainy SO, Gowayed MA, Agami M, Mohamed P, Belal M, Farid RM, Hanafy AS (2021) Galantamine nanoparticles outperform oral galantamine in an Alzheimer's rat model: pharmacokinetics and pharmacodynamics. Nanomedicine 16(15):1281–1296

Folch J, Petrov D, Ettcheto M, Abad S, Sánchez-Lopez E, Garcia ML, Camins A (2016) Current research therapeutic strategies for Alzheimer's disease treatment. Neural Plast 2016:1–15

Franchini S, Linciano P, Puja G, Tait A, Borsari C, Denora N, Sorbi C (2020) Novel dithiolane-based ligands combining sigma and NMDA receptor interactions as potential neuroprotective agents. ACS Med Chem Lett 11(5):1028–1034

Gomes LM, Bataglioli JC, Storr T (2020) Metal complexes that bind to the amyloid-β peptide of relevance to Alzheimer's disease. Coord Chem Rev 412:213255

Good PF, Werner P, Hsu A, Olanow CW, Perl DP (1996) Evidence of neuronal oxidative damage in Alzheimer's disease. Am J Pathol 149(1):21

Gul R, Jan H, Lalay G, Andleeb A, Usman H, Zainab R, Abbasi BH (2021) Medicinal plants and biogenic metal oxide nanoparticles: a paradigm shift to treat Alzheimer's disease. Coatings 11(6):717

Hu KW, Fan HF, Lin HC, Huang JW, Chen YC, Shen CL, Tu LH (2021) Exploring the impact of glyoxal glycation on β-amyloid peptide (Aβ) aggregation in Alzheimer's disease. J Phys Chem B 125(21):5559–5571

Jeyakumar M, Sathya S, Gandhi S, Tharra P, Suryanarayanan V, Singh SK, Devi KP (2019) α-bisabolol β-D-fucopyranoside as a potential modulator of β-amyloid peptide induced neurotoxicity: an in vitro & in silico study. Bioorg Chem 88:102935

Jeyakumar M, Sathya S, Gandhi S, Tharra P, Aarthy M, Balan DJ, Devi KP (2022) α-bisabolol β-D-fucopyranoside inhibits β-amyloid (Aβ) 25–35 induced oxidative stress in Neuro-2a cells via antioxidant approaches. Process Biochem 121:493–503

Karimi SA, Noorbakhsh M, Komaki H, Reza Nikoo M, Hasanein P, Shahidi S, Komaki A (2022) The interactive effects of verapamil and CB1 cannabinoid receptor antagonist/inverse agonist, AM251 on passive avoidance learning and memory in rat. Behav Pharmacol 33(2–3):222–229

Lao K, Ji N, Zhang X, Qiao W, Tang Z, Gou X (2019) Drug development for Alzheimer's disease. J Drug Target 27(2):164–173

Long JM, Holtzman DM (2019) Alzheimer disease: an update on pathobiology and treatment strategies. Cell 179(2):312–339

Majdi A, Sadigh-Eteghad S, Aghsan SR, Farajdokht F, Vatandoust SM, Namvaran A, Mahmoudi J (2020) Amyloid-β, tau, and the cholinergic system in Alzheimer's disease: seeking direction in a tangle of clues. Rev Neurosci 31(4):391–413

Matuszyk MM, Garwood CJ, Ferraiuolo L, Simpson JE, Staniforth RA, Wharton SB (2022) Biological and methodological complexities of beta-amyloid peptide: implications for Alzheimer's disease research. J Neurochem 160(4):434–453

Michaels TC, Saric A, Curk S, Bernfur K, Arosio P, Meisl G, Knowles TP (2020) Dynamics of oligomer populations formed during the aggregation of Alzheimer's Aβ42 peptide. Nat Chem 12(5):445–451

Misrani A, Tabassum S, Yang L (2021) Mitochondrial dysfunction and oxidative stress in Alzheimer's disease. Front Aging Neurosci 13:617588

Nguyen K, Hoffman H, Chakkamparambil B, Grossberg GT (2021) Evaluation of rivastigmine in Alzheimer's disease. Neurodegener Dis Manag 11(1):35–48

Nirale P, Paul A, Yadav KS (2020) Nanoemulsions for targeting the neurodegenerative diseases: Alzheimer's, Parkinson's and Prion's. Life Sci 245:117394

Okello EJ, Mather J (2020) Comparative kinetics of acetyl-and butyryl-cholinesterase inhibition by green tea catechins| relevance to the symptomatic treatment of Alzheimer's disease. Nutrients 12(4):1090

Ovais M, Zia N, Ahmad I, Khalil AT, Raza A, Ayaz M, Shinwari ZK (2018) Phyto-therapeutic and nanomedicinal approaches to cure Alzheimer's disease: present status and future opportunities. Front Aging Neurosci 10:284

Pei X, Hu F, Luo F, Huang X, Li X, Xing S, Long D (2022) The neuroprotective effects of alpha-lipoic acid on an experimental model of Alzheimer's disease in PC12 cells. J Appl Toxicol 42(2):285–294

Peng Y, Tao H, Wang S, Xiao J, Wang Y, Su H (2021) Dietary intervention with edible medicinal plants and derived products for prevention of Alzheimer's disease: a compendium of time-tested strategy. J Funct Foods 81:104463

Piemontese L, Tomas D, Hiremathad A, Capriati V, Candeias E, Cardoso SM, Santos MA (2018) Donepezil structure-based hybrids as potential multifunctional anti-Alzheimer's drug candidates. J Enzyme Inhib Med Chem 33(1):1212–1224

Prabhu R, Anjali R, Archunan G, Prabhu NM, Pugazhendhi A, Suganthy N (2019) Ecofriendly one pot fabrication of methyl gallate@ ZIF-L nanoscale hybrid as pH responsive drug delivery system for lung cancer therapy. Process Biochem 84:39–52

Raju P, Natarajan S (2021) Anticancer, anti-biofilm and antimicrobial activity of fucoidan-loaded zeolitic imidazole framework fabricated by one-pot synthesis method. Appl Nanosci 13:1919–1937

Raju P, Arivalagan P, Natarajan S (2020) One-pot fabrication of multifunctional catechin@ ZIF-L nanocomposite: Assessment of antibiofilm, larvicidal and photocatalytic activities. J Photochem Photobiol B 203:111774

Raju P, Balakrishnan K, Mishra M, Ramasamy T, Natarajan S (2022) Fabrication of pH responsive FU@ Eu-MOF nanoscale metal organic frameworks for lung cancer therapy. J Drug Deliv Sci Technol 70:103223

Roy J, Tsui KC, Ng J, Fung ML, Lim LW (2021) Regulation of Melatonin and Neurotransmission in Alzheimer's Disease. Int J Mol Sci 22(13):6841

Sinha K, Sun C, Kamari R, Bettermann K (2020) Current status and future prospects of pathophysiology-based neuroprotective drugs for the treatment of vascular dementia. Drug Discov Today 25(4):793–799

Stanciu GD, Luca A, Rusu RN, Bild V, Beschea Chiriac SI, Solcan C, Ababei DC (2019) Alzheimer's disease pharmacotherapy in relation to cholinergic system involvement. Biomolecules 10(1):40

Sun Y, Kakinen A, Wan X, Moriarty N, Hunt CP, Li Y, Ding F (2021) Spontaneous formation of β-sheet nano-barrels during the early aggregation of Alzheimer's amyloid beta. Nano Today 38:101125

Tan SJ, Ismail IS (2020) Potency of selected berries, grapes, and citrus fruit as neuroprotective agents. Evid Based Complement Altern Med 2020:1

Terao I, Honyashiki M, Inoue T (2022) Comparative efficacy of lithium and aducanumab for cognitive decline in patients with mild cognitive impairment or Alzheimer's disease: a systematic review and network meta-analysis. Ageing Res Rev 81:101709

Thakur A, Chun YS, October N, Yang HO, Maharaj V (2019) Potential of South African medicinal plants targeting the reduction of Aβ42 protein as a treatment of Alzheimer's disease. J Ethnopharmacol 231:363–373

Thoe ES, Fauzi A, Tang YQ, Chamyuang S, Chia AYY (2021) A review on advances of treatment modalities for Alzheimer's disease. Life Sci 276:119129

Tripathi SS, Singh S, Garg G, Kumar R, Verma AK, Singh AK, Rizvi SI (2022) Metformin ameliorates acetaminophen-induced sub-acute toxicity via antioxidant property. Drug Chem Toxicol 45(1):52–60

Wang Q, Yu X, Li L, Zheng J (2014) Inhibition of amyloid-β aggregation in Alzheimer's disease. Curr Pharm Des 20(8):1223–1243

Wang CF, Song CY, Wang X, Huang LY, Ding M, Yang H, Bi JZ (2019) Protective effects of melatonin on mitochondrial biogenesis and mitochondrial structure and function in the HEK293-APPswe cell model of Alzheimer's disease. Eur Rev Med Pharmacol Sci 23(8):3542–3550

Wong KH, Riaz MK, Xie Y, Zhang X, Liu Q, Chen H, Yang Z (2019) Review of current strategies for delivering Alzheimer's disease drugs across the blood-brain barrier. Int J Mol Sci 20(2):381

Yan Y, Yang H, Xie Y, Ding Y, Kong D, Yu H (2020) Research progress on Alzheimer's disease and resveratrol. Neurochem Res 45(5):989–1006

Yiannopoulou KG, Papageorgiou SG (2020) Current and future treatments in Alzheimer disease: an update. J Cent Nerv Syst Dis 12:1179573520907397

Yu N, Huang Y, Jiang Y, Zou L, Liu X, Liu S, Zhu Y (2020) Ganoderma lucidum triterpenoids (GLTs) reduce neuronal apoptosis via inhibition of ROCK signal pathway in APP/PS1 transgenic Alzheimer's disease mice. Oxid Med Cell Longev 2020:1–11

Zhang X, Fu Z, Meng L, He M, Zhang Z (2018) The early events that initiate β-amyloid aggregation in Alzheimer's disease. Front Aging Neurosci 10:359

Zhang Y, Ding C, Li C, Wang X (2021) Advances in fluorescent probes for detection and imaging of amyloid-β peptides in Alzheimer's disease. Adv Clin Chem 103:135–190

Zhao J, Xu N, Yang X, Ling G, Zhang P (2022) The roles of gold nanoparticles in the detection of amyloid-β peptide for Alzheimer's disease. Coll Interface Sci Commun 46:100579