



Neuroprotective effects of magnolol in aluminium chloride and D-galactose induced Alzheimer's disease model

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ABSTRACT

Alzheimer's disease (AD) is a disorder associated with continuing deterioration of neurons in the brain and is characterised by aggregation of neurotoxic amyloid beta (A β) plaques. It is also accompanied by increased oxidative stress, cholinergic impairment, and remarkably reduced brain-derived neurotrophic factor (BDNF). The net result is progressive loss of memory and cognitive abilities. The study aims to explore the anti-AD profile of magnolol. AD was induced in adult Swiss albino mice by simultaneously administering aluminium chloride at a dose of 5 mg/kg/day; p.o. and D-galactose at a dose of 60 mg/kg/day; i.p. for a period of 90 days. The dose dependent ameliorative effect of magnolol (20 and 40 mg/kg/day; p.o.) was assessed with the help of behavioural, biochemical and Enzyme-Linked Immunosorbent Assay (ELISA) analyses. Magnolol was shown to ameliorate memory and cognitive insufficiencies as assessed from Morris Water Maze, Elevated Plus Maze and Novel Object Recognition paradigms. It also elicited a remarkable antioxidant effect, as evidenced by analysing various parameters like thiobarbituric acid reactive substances, glutathione, and catalase. Magnolol also had an inhibitory activity on acetylcholinesterase, β -secretase and caspase-3. Disintegration of A β plaques and improvement of BDNF levels were also reported in the present study. Magnolol presents a multifactorial approach, beyond its conventional antioxidant mechanism, against experimentally induced AD in mice.

1. INTRODUCTION

According to The Centers for Disease Control and Prevention in the United States, Alzheimer's disease (AD) is listed among the top 10 primary reasons for mortality. It represents the most prevalent variant of dementia, marked by diminishing levels of cortical and hippocampal acetylcholine, increased acetylcholinesterase (AChE) expression, buildup of amyloid beta (A β) plaques, tau neurofibrillary tangles (NFTs), and accompanying neuroinflammation [1] (Fig. 1). In AD, the journey begins with minor lapses in short-term memory and

progresses to a state where the individual becomes reliant on others for daily functioning, ultimately leading to mortality. Oxidative stress plays a crucial role in both the initiation and development of AD owing to its capacity for causing neurodegeneration [2]. The pharmacological treatments utilised to address AD mostly include drugs like donepezil, galantamine, and rivastigmine (AChE inhibitors), as well as memantine (NMDA glutamate antagonist). However, these medications offer only symptomatic alleviation [3,4]. Crucially, these agents do not halt or reverse the underlying neurodegenerative processes, leaving patients to face continued cognitive decline and progression of the disease. There is a strong pursuit for reliable, disease-altering and safer agents to treat and/or manage AD across various stages of the disease. Many traditional medicinal plants and herbs have been used historically for cognitive enhancement and neuroprotection. Scientific studies

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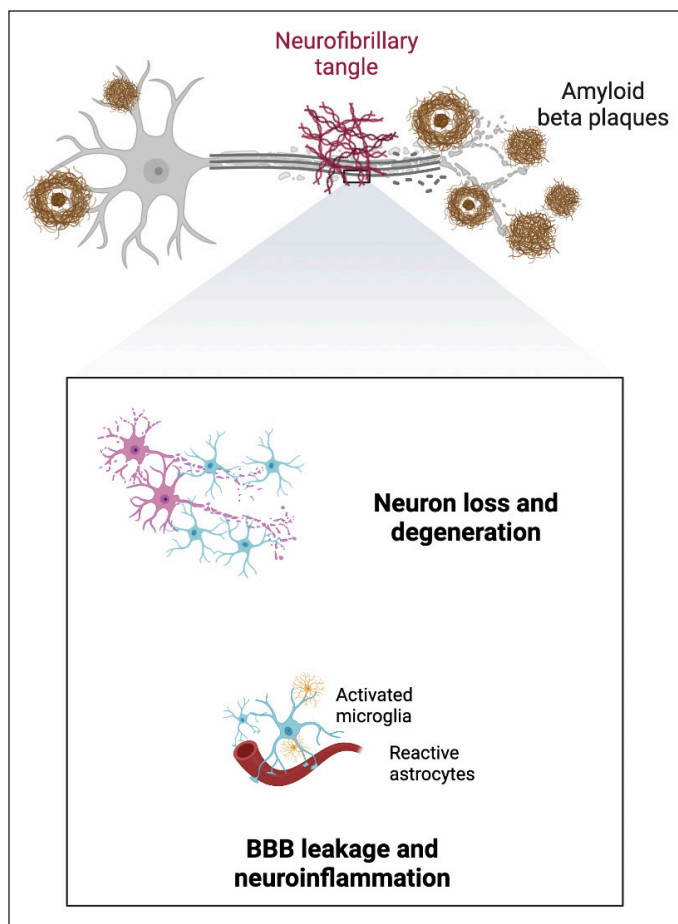


Figure 1. Pathophysiological hallmarks of AD.

have confirmed that components from these plants share physicochemical properties with approved drugs and exert beneficial effects in preclinical models of AD. Examples include *Ginkgo biloba*, *Bacopa monnieri*, and compounds like rosmarinic acid with demonstrated efficacy in experimental and limited clinical settings [5–8]. Natural compounds, such as magnolol, which is a polyphenol extracted from *Magnolia officinalis*, have shown promise because of their wide ranging pharmacological characteristics. This study examines magnolol in an established aluminium chloride (AlCl_3) + D-galactose induced AD mice model.

In this research study, AD was simulated in rodents (Swiss albino mice) by concurrent administration of AlCl_3 and D-galactose. Aluminium easily penetrates the brain, disturbing the slow axonal transport, prompting inflammation, causing morphological and synaptic irregularities, eventually leading to neuronal aberrations [9]. D-galactose, classified as a reducing sugar, swiftly undergoes chemical reactions with free amines to produce Advanced Glycation End Products. Extended administration of D-galactose induces alterations mimicking natural ageing in animals, such as impaired cognition, oxidative stress, reduced immunological function, and genetic modifications. In addition, it results in dysfunction of the mitochondria and an increase in brain AChE levels [9–11]. Simultaneous dosing of AlCl_3 and D-galactose for a

duration of 90 days has been documented to induce AD related features in rodents. These characteristics include increased levels of β -secretase, aggregation of $\text{A}\beta$ plaques, and activation of caspase-3, while simultaneously decreasing brain-derived neurotrophic factor (BDNF) [12–15].

Magnolol is a polyphenol (neolignan) obtained from the extract of *M. officinalis* bark. The trees are mainly found in Eastern and South–Eastern regions of Asia [16]. There are several traditional Chinese and Japanese herbal formulas that contain *Magnolia* such as Banxia Houpo Tang and Saiboku-To [17,18]. *Magnolia officinalis* has been used in Asian traditional medicine for treating anxiety, sleep disorders, nervousness, and so on. It implies that magnolia bark extract holds the capability to elicit central effects. It has been listed in the Japanese Pharmacopoeia XIV (English Edition, 2001) and Pharmacopoeia of the People’s Republic of China (English Edition, 2005) [19]. In an *in vitro* study involving PC12 cell lines magnolol was reported to reduce $\text{A}\beta$ induced cell death [20]. Magnolol was also reported to attenuate an AD-like pathology in a transgenic *C. elegans* model. It was also reported to reduce $\text{A}\beta$ deposition and enhance the phagocytosis and breakdown of $\text{A}\beta$ in microglia cells [21]. In addition, magnolol has been reported to have an oral LD_{50} value of 2,200 mg/kg (in mouse). Several *in silico* models have also predicted appreciable anti-AD effects of magnolol [22]. In this study, the doses of magnolol, i.e., 20 mg/kg (p.o.) and 40 mg/kg (p.o.), have been based on established literature [23,24].

According to the established endpoints in well-established literature sources, transfer latency (TL) in the Elevated Plus Maze paradigm [25], escape latency (EL) in the Morris Water Maze (MWM) paradigm [26,27], and novel object recognition (NOR) [28,29] behavioural assessments were utilised to evaluate the effects of magnolol on memory enhancement and disease amelioration. To assess the potential of magnolol in mitigating markers of oxidative stress, thiobarbituric acid reactive substances (TBARS), glutathione (GSH), and Catalase (CAT) assays were performed [30,31]. Moreover, to comprehend the impact of magnolol on different pathological pathways associated with AD, its potential was evaluated for inhibitory effect against enzymes, including AChE, β -secretase, and caspase-3. In addition, the capacity of magnolol to decrease the buildup of $\text{A}\beta_{1-42}$ and increase BDNF levels was examined.

Although earlier research has reported antioxidant, anti-amyloid, and caspase-3 inhibitory effects of magnolol based on *in vitro* studies and has predicted potential effects using *in silico* models, the efficacy of magnolol has not been validated in a comprehensive *in vivo* model of AD. To the best of our knowledge, this study presents the first report to assess the effect of magnolol in a long-term, AlCl_3 + D-galactose model of AD in mice that exhibits a close similarity to the multifactorial AD pathology. Furthermore, this work simultaneously studies key mechanistic endpoints such as AChE activity, β -secretase and caspase-3 inhibition, $\text{A}\beta_{1-42}$ aggregation, oxidative stress markers, and BDNF levels to give an integrated *in vivo* evaluation of magnolol’s disease-modifying potential.

2. MATERIALS AND METHODS

This study hypothesises that magnolol will ameliorate memory and cognitive deficits, reduce oxidative stress, inhibit key AD-related enzymes (AChE, β -secretase, caspase-3), decrease A β aggregation, and restore BDNF levels in a long-term AlCl₃ + D-galactose-induced AD mouse model.

2.1. Experimental animals

NIPER, SAS Nagar, Punjab (a CCSEA-registered-registered breeding facility) provided adult Swiss albino mice (either sex; weighing 20–30 g each). The animals were accommodated in the central animal house facility at Lovely Institute of Technology (Pharmacy), Lovely Professional University (Phagwara, Punjab), which is registered with the CCSEA. Food and water were available *ad libitum*. All procedures were conducted in compliance with regulations laid down by CCSEA. The study protocol was approved by the Institutional Animal Ethics Committee of Lovely Professional University, India (Approval No.: LPU/IAEC/2020/75).

2.2. Chemicals and drugs

AlCl₃ was sourced from Molychem, and D-galactose was sourced from LobaChemie. Magnolol was purchased from Xi'an Pincredit Bio-tech Company Ltd., Shaanxi, China. Donepezil hydrochloride was procured *ex gratia* from Sun Pharmaceutical Industries Ltd., Gurugram (India).

2.3. ELISA kits

Mouse A β ₁₋₄₂ and Mouse Caspase-3 ELISA kits were procured from Cusabio Biotech Company Ltd. (Wuhan, China), while Shanghai Korain Biotech Company Ltd. (Shanghai, China) provided Mouse Beta Secretase and Mouse BDNF kits. In India, the procurement of all ELISA kits was facilitated by Everon Lifesciences.

2.4. Design of experiment

The rodents were segregated into six groups, each consisting of six animals ($n = 6$). The protocol outlined in [Figure 2](#) was adhered to accordingly. Magnolol was formulated into a suspension using 0.5% w/v sodium-carboxymethyl cellulose. Groups 1, 3, 4, and 5 were included in a similar study

Group	Treatment
Group 1: Vehicle Control (VC)	Vehicle (10 ml/kg; p.o., 5 ml/kg; i.p.)
Group 2: Magnolol <i>per se</i>	Normal saline (5 ml/kg; i.p.), Magnolol (40 mg/kg; p.o.)
Group 3: Disease Control (DC)	Aluminium chloride (5 mg/kg; p.o.), D-galactose (60 mg/kg; i.p.)
Group 4 Standard Treatment (DT)	Aluminium chloride (5 mg/kg; p.o.), D-galactose (60 mg/kg; i.p.), Donepezil (5mg/kg, p.o.)
Group 5: Magnolol Low Dose (MLD) Treated Group	Aluminium chloride (5 mg/kg; p.o.), D-galactose (60 mg/kg; i.p.), Magnolol (20 mg/kg; p.o.)
Group 6: Magnolol High Dose (MHD) Treated Group	Aluminium chloride (5 mg/kg; p.o.), D-galactose (60 mg/kg; i.p.), Magnolol (40 mg/kg; p.o.)

Day 0	Day 1	Day 60	Day 61	Day 85	Day 86	Day 87	Day 88	Day 89	Day 90	Day 91	
	Aluminium chloride + D-galactose										
	Donepezil and Magnolol (as per protocol)										
	Morris Water Maze (Acquisition trials)							Morris Water Maze (Probe Trial)			
								Transfer Latency (Acquisition Trial)		Transfer Latency (Retention Trial)	
								Habituation Day (Novel Object Recognition)		Training Day (Novel Object Recognition)	Testing Day (Novel Object Recognition)
Locomotor Activity										Locomotor Activity	

Figure 2. Study protocol.

to evaluate the potential anti-AD effects of vanillin [32] and a commonly used probiotic bacteria (*Lactobacillus rhamnosus*), either individually or in different combinations, following the same protocol (data not yet published) so as to reduce the number of animals to be sacrificed. Parallel studies were conducted to achieve three objectives, i.e., a. Evaluation of the neuroprotective effect of vanillin, b. Evaluation of the neuroprotective effect of magnolol, c. Evaluation of the neuroprotective effect of *L. rhamnosus* and various combinations of the above-mentioned three interventions. In line with the principles of 3R, data were shared among the studies for certain overlapping groups. The doses were chosen on the basis of previously established literature—AlCl₃ and D-galactose [15], magnolol [23,24], and donepezil [30,33].

2.4.1. Disease induction

The simultaneous dosing of AlCl₃ and D-galactose has been profoundly used to mimic non-transgenic AD. Separately, these agents were known to mimic certain parameters of AD pathogenesis. D-galactose was known to induce subacute senescence [34] while Aluminium was known to be a neurotoxin [35]. Both of these agents express the effect due to oxidative damage, mitochondrial dysfunction, and elevation of AChE. After oral administration of AlCl₃, xanthine oxidase and GSH peroxidase activities have been demonstrated to be increased and diminished, respectively, resulting in aggregation of intermediate neurotoxic products like H₂O₂ and OH⁻ radicals, which might be essential for Aluminium toxicity [36]. Following AlCl₃ injection (intracerebroventricular), immune activity in phagocytic microglia and astrocytes, determined by estimation of glial fibrillary acidic protein and ED1, respectively, exhibited a greater inflammation in brains of rats. Increase in inflammatory parameters and interactions with cholinergic neurons may contribute to the Aluminium caused cognitive and memory lapses [37]. Aluminium causes specific toxicity to cytoskeletal structures of brain neurons [38]. Aluminium can bind to Aβ-protein and form cross-links between them, which causes the proteins to aggregate into oligomers. These oligomers are toxic to neurons [39]. Exposure to aluminium leads to advancement of amyloidogenic pathway via activation of β-secretase and gamma secretase. In addition, it leads to inhibition of alpha secretase thereby retarding the non-amyloidogenic pathway [40]. Administration of AlCl₃ has also been reported to enhance caspase-3 mediated neuronal apoptosis in brains of rodents [41]. AlCl₃ has also been linked to an increase in oxido-inflammatory burden while reducing the levels of BDNF [42]. D-galactose can replicate the effects of natural senescence in mice and Aluminium can stimulate the expression of Amyloid Precursor Protein in neurons, which may promote generation of Aβ. The individual effects of Aluminium and D-galactose were well established by several experiments. However, the combined effect was still not observed. A modified protocol made the use of simultaneous administration of AlCl₃ (p.o.) and D-galactose (s.c.) for a period of 10 weeks. This method used for Kunming mice resulted in development of AD like lesions. Formation of structures similar to senile plaques and NFTs was also reported. The behavioural and pathological

changes lasted for a minimum of 6 weeks post withdrawal of AlCl₃ and D-galactose administration [13]. Another modified protocol made use of D-galactose (60 mg/kg day, i.p.) and AlCl₃ (5 mg/kg day, p.o.) once daily for 90 days to develop AD like condition [15]. The concurrent use of these substances is regarded as a simple and cost-effective method for creating an animal model of AD [43].

2.5. Behavioural parameters

2.5.1. Locomotor activity

Locomotor activity of all mice was assessed using an actophotometer on the 0th and 91st day of the study. It was assessed to analyse the potential effects of various treatments on the central nervous system. The protocol was followed as established in previously published literature [29,44].

2.5.2. Transfer latency

The methodology, procedure, and endpoint were followed by previously published studies [29,45]. The apparatus used for the test was the Elevated Plus Maze. It consists of four arms, two enclosed and two open, elevated from the ground, and measures the rodent's inclination to explore the open (well lit) arms versus remaining in the closed (dark) arms. The test measured the time the animal took (TL) to traverse inside either of the enclosed arms of the apparatus. The decrease in TL during the retention test, as compared to the TL on the previous day (acquisition), indicated improvement in memory and cognition.

2.5.3. Novel object recognition

This examination relies on the natural inclination of mice to allocate greater time in the exploration of an unfamiliar/novel object when it is presented alongside both a known and an unknown/novel object. The evaluation spans 3 days (habituation, training, and testing). The methodology, procedure, and outcome measures were derived from earlier investigations [27,28,46]. Investigation time was determined by employing the formula given below:

$$(T_n - T_f / T_t) * 100$$

where T_n = time taken for exploration of the unfamiliar object, T_f = time taken for exploration of the known object, and T_t = the total time taken by the animals to explore both objects.

An exploration time <50% signifies a stronger inclination of the rodent towards the familiar object, whereas an exploration time of 50% indicates a comparable inclination for both the known and unknown objects. A percentage >50% indicates a greater inclination for the unknown/unfamiliar/novel object.

2.5.4. Escape latency

Estimation of EL in the MWM paradigm was implemented as a tool to evaluate the memory of test animals, characterized as the duration that a rodent takes in the MWM for swimming to the concealed platform and ascending onto it [47]. The MWM test usually consists of trials conducted over 6 days and is characterised by its simplicity. This approach offers advantages such as the effective differentiation

between “spatial” situations involving a hidden platform and “non-spatial” situations with a visible platform, along with a reduction in interference from odour trails. The methods, procedures, and endpoints were derived from previous studies [27,29,48]. The inclination of mice to escape from water typically does not depend on the variations in activity level or body mass, rendering it applicable across various experimental models involving animals [27,48].

2.6. Biochemical parameters

At the conclusion of the protocol, euthanasia of all animals was conducted through decapitation subsequent to cervical dislocation. Following brain extraction, both the cortical and hippocampal tissues were extracted and submerged in ice-cold PBS, i.e., phosphate-buffered saline with a pH of 7.4, having the final concentration of 10%w/v. Subsequently, the cortical and hippocampal regions underwent homogenisation in Phosphate-Buffered Saline, after which the homogenate was subjected to centrifugation (10,000g for 15 minutes at 4°C). The supernatant was retrieved and utilised for the estimation of TBARS [49], GSH [50], CAT [51], and AChE [52]. The Biuret kit method was employed to estimate total protein. All observations were recorded in triplicate.

2.7. ELISA

Molecular markers were assessed using ELISA. The assays were performed using the commercially available kits, following the manufacturer’s general framework. The double enzyme sandwich method was used for all assays. Each sample was run in triplicate to ensure analytical reliability. Standard curves were generated for every assay using the kit-provided calibrators, and only curves with an $R^2 \geq 0.99$ were accepted.

2.7.1. β -secretase

β -secretase has emerged as a highly sought-after focus for the screening of potential drugs aimed at combating AD. This enzyme is responsible for cleaving the amyloid precursor protein, initiating the creation of $A\beta$ [53]. The capability to inhibit β -secretase activity is greatly desirable in the development of potential drugs for AD, as it may lead to a modification in the progression of the disease. In the assay, standard points were prepared by serially diluting the standard stock solution (800 pg/ml) 1:2 with the provided diluent to produce 400, 200, 100 and 50 pg/ml solutions. Standard diluent served as the zero standard (0 pg/ml). The content of β -secretase for all test groups was assessed using the protocol available in the package insert [54].

2.7.2. Caspase-3

Caspase-3 plays a role in facilitating the apoptosis pathway of cell death, which not only contributes to neuronal degeneration but also accelerates the advancement of the key pathological processes in AD. Numerous investigations have documented elevated levels of caspase-3 in synapses of AD patients. The inhibition of the caspase-3 enzyme presents a promising and novel direction for research in the development of anti-AD drugs development [55]. In the assay, standard points were prepared by serially diluting the standard stock

solution (20 ng/ml) 1:2 with the provided diluent to produce 10, 5, 2.5, 1.25, 0.625, and 0.312 ng/ml solutions. Standard diluent served as the zero standard (0 ng/ml). The content of caspase-3 for all test groups was assessed using the protocol available in the package insert [56].

2.7.3. $A\beta_{1-42}$

The buildup of $A\beta_{1-42}$ in the brain results in the disruption of synaptic plasticity, subsequently exacerbating the decline in memory function [57]. The accumulation of $A\beta$ also induces mitochondrial dysfunction, contributing to the advancement of AD [58]. The anti- $A\beta$ aggregatory effect is ardently sought after for any potential anti-AD drug candidate. In the assay, the standard solution of 1,000 pg/ml concentration was diluted using the provided diluent. 2-fold dilution series were prepared to achieve final concentrations of 500, 250, 125, 62.5, 31.2, and 15.6 pg/ml. Standard diluent served as the zero standard (0 pg/ml). The content of $A\beta_{1-42}$ for all test groups was assessed using the protocol available in the package insert [59].

2.7.4. Brain-derived neurotrophic factor

BDNF plays a crucial role in maintaining mature cortical neurons in adults, and its early dysfunction is linked to the emergence of initial short-term memory deficits in AD. A scarcity of BDNF is implicated in the progression of neuronal degeneration in AD. Any prospective anti-AD treatment capable of modulating BDNF levels could prove to be a significant asset in therapeutic strategies [60]. In the assay, standard points were prepared by serially diluting the standard stock solution (6.4 ng/ml) 1:2 with the provided diluent to produce 3.2, 1.6, 0.8, and 0.4 ng/ml solutions. Standard diluent served as the zero standard (0 ng/ml). The content of BDNF for all test groups was assessed using the protocol available in the package insert [61].

2.8. Statistical tools

A two-way analysis of variance (ANOVA) was employed to evaluate the data pertaining to locomotor activity and EL (acquisition trials), whereas the remaining parameters, including behavioural tests, biochemical assays, and ELISA based tests, were analysed using one-way ANOVA. Statistical significance was assessed at the levels of $p < 0.001$, $p < 0.01$, and $p < 0.05$, utilizing *post-hoc* Tukey’s test. All statistical analyses were performed using GraphPad Prism.

3. RESULTS

3.1. Presentation of results

The findings were presented as mean \pm SEM. The statistical significance in comparison with the group receiving only the vehicle [vehicle control (VC)] has been denoted as *** ($p < 0.001$), ** ($p < 0.01$), and * ($p < 0.05$). The comparison with the disease inflicted [disease control (DC)] group has been represented as ^^ ($p < 0.001$), ^ ($p < 0.01$), and ^ ($p < 0.05$), respectively. ### ($p < 0.001$), ## ($p < 0.01$), and # ($p < 0.05$), respectively, have been used to denote the comparison with standard drug (donepezil) treated group.

3.2. Effect of magnolol on locomotor activity

Figure 3 illustrates the influence of magnolol and the comparative treatments on locomotor behaviour. Across all experimental groups, the total crossings recorded on both the 0th day and the 91st day showed no appreciable variation. Likewise, when each group's performance on day 91 was compared with its own baseline values, no meaningful change in the number of crossings was detected.

3.3. Effect of magnolol on TL

Figure 4 shows the effects of magnolol and the comparative treatments on TL. During retention test, magnolol showed a significant decrease in TL ($p < 0.05$), as compared to VC group, which indicated a potential improvement in cognitive performance. TL was significantly ($p < 0.001$) higher in the DC group compared to the VC group. Every therapeutic intervention that included donepezil, low-dose magnolol, and high-dose magnolol reported a significant reduction in TL ($p < 0.001$) relative

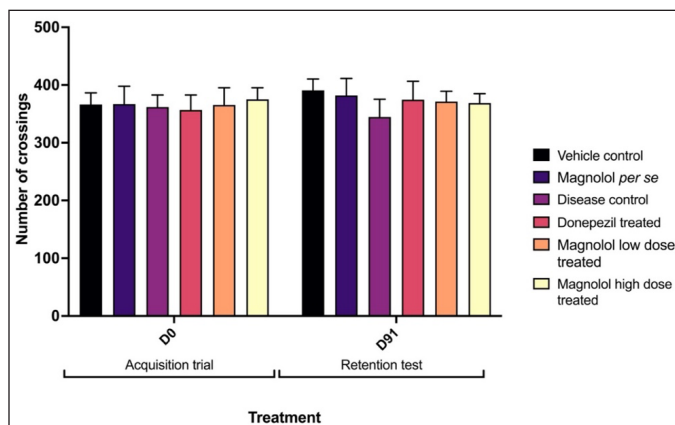


Figure 3. Impact of magnolol and other interventions on locomotor activity (mean \pm SEM).

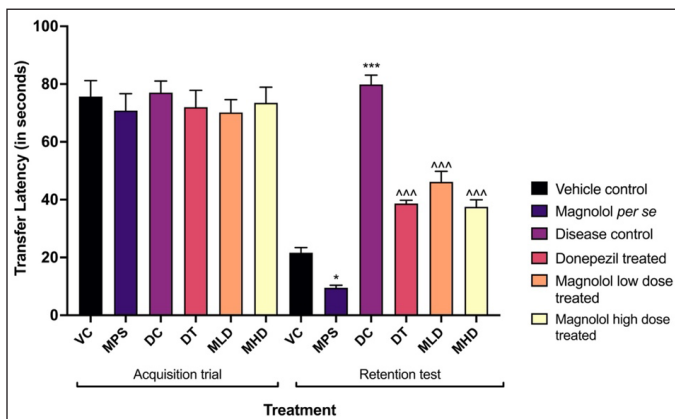


Figure 4. Effect of magnolol and other interventions on TL (mean \pm SEM). ***, ** and * have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$ in comparison with the VC group, respectively. The comparisons with DC group have been represented as ^^^, ^^ and ^ which signify $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. To represent comparisons with the donepezil treated group, ###, ## and # have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

to the DC group. Nevertheless, there was no significant difference in TL between the animals that were treated with donepezil and those that were treated with either dose of magnolol.

3.4. Effect of magnolol on investigation time

Figure 5 shows the effect of the different regimens of magnolol and other treatments used on the time of investigation. In the NOR task, the animals that were treated with magnolol alone showed a significant increase in investigation time ($p < 0.05$), as compared to the VC group, and this is indicative of the fact that magnolol may be acting as a memory booster. The DC group showed a significantly ($p < 0.001$) lower investigation time as compared to the VC group. The therapeutic interventions, including donepezil, low-dose magnolol, and high-dose magnolol, caused a significant enhancement of the investigation time ($p < 0.001$) compared with the DC group.

3.5. Effect of magnolol on EL

Figure 6 shows the effects of magnolol and the other treatments on acquisition time for the MWM and performance of test animals during the probe trial. On Day 1, the EL values between all the groups were similar, with no significant differences. By Day 2, the DC group presented with a significantly elevated EL ($p < 0.001$) compared with the VC group along with a significant increase in comparison with its own baseline on Day 1 ($p < 0.01$). In contrast, the donepezil group had a meaningful reduction in EL ($p < 0.05$) as compared with the DC group, and the magnolol high-dose group had an even greater reduction ($p < 0.001$).

On Day 3, magnolol per se showed a significant reduction in EL ($p < 0.05$) as compared with the VC group. The DC group again had much higher EL value ($p < 0.001$) than did the VC group. All treatment groups - donepezil, magnolol low dose, and magnolol high dose - had significantly lower EL values compared to the DC group ($p < 0.001$ for all).

On Day 4, the VC group showed a significant reduction in EL, as compared to its Day 2 reading ($p < 0.01$). The DC group continued to perform poorly with EL being markedly higher than

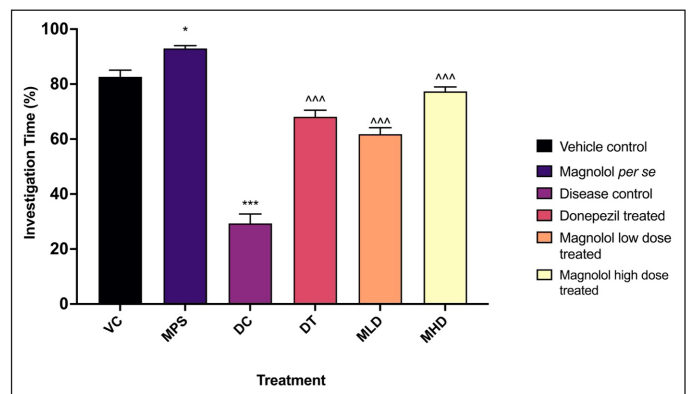


Figure 5. Effect of magnolol and other interventions on investigation time (mean \pm SEM). ***, ** and * have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$ in comparison with the VC group, respectively. The comparisons with DC group have been represented as ^^^, ^^ and ^ which signify $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. To represent comparisons with the donepezil treated group, ###, ## and # have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

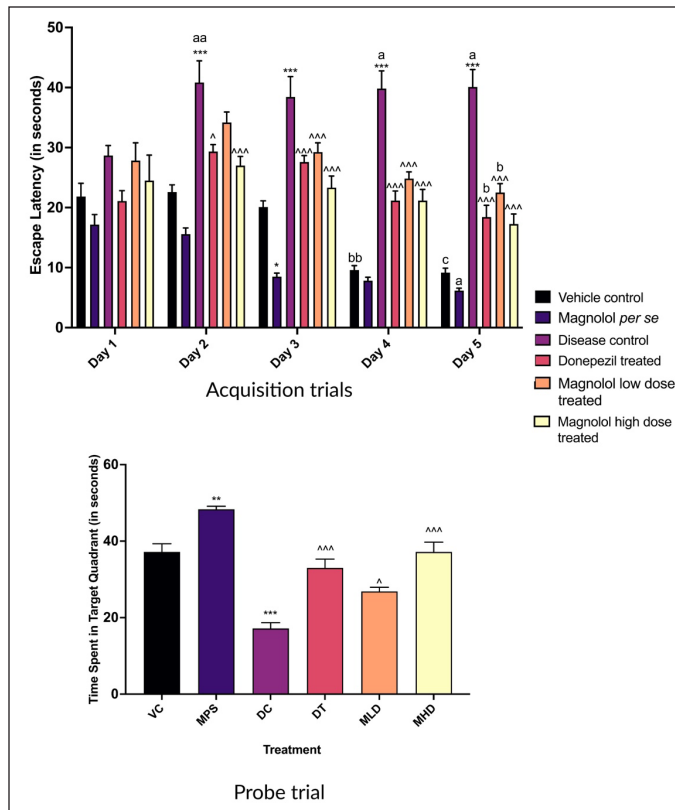


Figure 6. Effect of magnolol and other interventions on EL during acquisition trials and time spent in the target quadrant during probe trial (mean \pm SEM). ***, ** and * have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$ in comparison with the VC group, respectively. The comparisons with DC group have been represented as ^^^, ^^ and ^ which signify $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. To represent comparisons with the donepezil treated group, ###, ## and # have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. a represents $p < 0.05$ concerning the same group on day 1, aa represents $p < 0.01$ concerning the same group on day 1. b represents $p < 0.05$ concerning the same group on day 2, bb represents $p < 0.01$ concerning the same group on day 2. c represents $p < 0.05$ concerning the same group on day 3.

both the VC group ($p < 0.001$) and its own Day 1 value ($p < 0.05$). All the drug treated groups showed significantly lower EL values compared to the DC group.

By Day 5, the VC group demonstrated an additional decrease in EL as compared to its own counterpart on Day 3 ($p < 0.05$). The DC group still had significantly higher EL values than both the VC group ($p < 0.001$) and its Day 1 value ($p < 0.05$). As shown above, donepezil treatment, magnolol low-dose, and magnolol high-dose all had excellent reductions in EL compared to the DC group ($p < 0.001$). In addition, both the donepezil and magnolol low-dose groups had significantly lower EL values than their own Day 2 values ($p < 0.05$).

During the probe trial, magnolol per se caused a significant increase in time spent in the target quadrant when compared with the VC group ($p < 0.01$). On the other hand, the DC group spent significantly less time in the target quadrant than the VC group ($p < 0.001$). Treatment with either donepezil or magnolol high dose resulted in a substantial increase in time spent in the target quadrant compared with

the DC group ($p < 0.001$ for both). Similarly, magnolol low dose was able to yield a meaningful improvement compared to the DC group ($p < 0.05$). No significant differences were found between the donepezil group and each of the magnolol-treated groups.

3.6. Effect of magnolol on TBARS, GSH, CAT, and AChE

The effects of magnolol and other treatments on TBARS, GSH, CAT, and AChE have been illustrated in Figure 7. In the cortex, the group treated with magnolol alone showed a consequential reduction in TBARS levels ($p < 0.001$) as compared to the VC group. Conversely, the DC group displayed a consequential increase in TBARS levels contrasted with the VC group ($p < 0.001$). All treatments, including donepezil, low-dose magnolol, and high-dose magnolol, resulted in a consequential reduction in TBARS levels contrasted with the DC group ($p < 0.001$ for all treatments). Specifically, the low-dose magnolol treatment led to a consequential reduction in TBARS levels contrasted with the group treated with donepezil ($p < 0.01$), and the high-dose magnolol treatment resulted in a consequential reduction in TBARS levels contrasted with the group which received the standard drug, i.e., donepezil ($p < 0.001$). Comparable effect was observed in the hippocampus. However, in the hippocampal tissue, both of the magnolol-treated groups demonstrated a consequential reduction in TBARS levels contrasted with the group treated with Donepezil ($p < 0.001$).

In the cortex, treatment with magnolol itself resulted in a consequential ($p < 0.001$) elevation in GSH levels when contrasted with the control group that received the vehicle. Conversely, the DC group exhibited a consequential ($p < 0.001$) reduction in GSH levels, contrasted with the VC group. While treatment with donepezil demonstrated a consequential ($p < 0.05$) increase in GSH levels, both the magnolol treatment groups showed a consequential ($p < 0.001$) rise in GSH levels, contrasted with the DC group. Notably, the GSH levels in the low-dose magnolol-treated group were remarkably ($p < 0.01$) higher than those in the standard drug treated group, and the magnolol treatment (high-dose) resulted in a consequential ($p < 0.001$) elevation in GSH levels contrasted with the donepezil-treated group. In the hippocampus, treatment with magnolol itself led to a consequential ($p < 0.01$) rise in GSH levels contrasted with the VC group. The DC group, on the other hand, exhibited a consequential ($p < 0.001$) reduction in GSH levels contrasted with the VC group. Donepezil treatment remarkably ($p < 0.05$) increased GSH levels contrasted with the DC group. Both the magnolol treatment groups showed a consequential ($p < 0.001$) elevation in GSH levels contrasted with the DC group. When contrasted with the donepezil treated group, the increase in GSH levels was consequential for both the magnolol-treated groups ($p < 0.05$; low-dose) and ($p < 0.001$; high-dose).

In the cortex, the CAT in the magnolol per se treated group showed an inconsequential increase when compared with the VC group. Conversely, the DC group had a notably lower CAT ($p < 0.001$) contrasted with the VC group. While the increase in CAT with donepezil treatment contrasted with the DC group, this increase did not reach statistical significance.

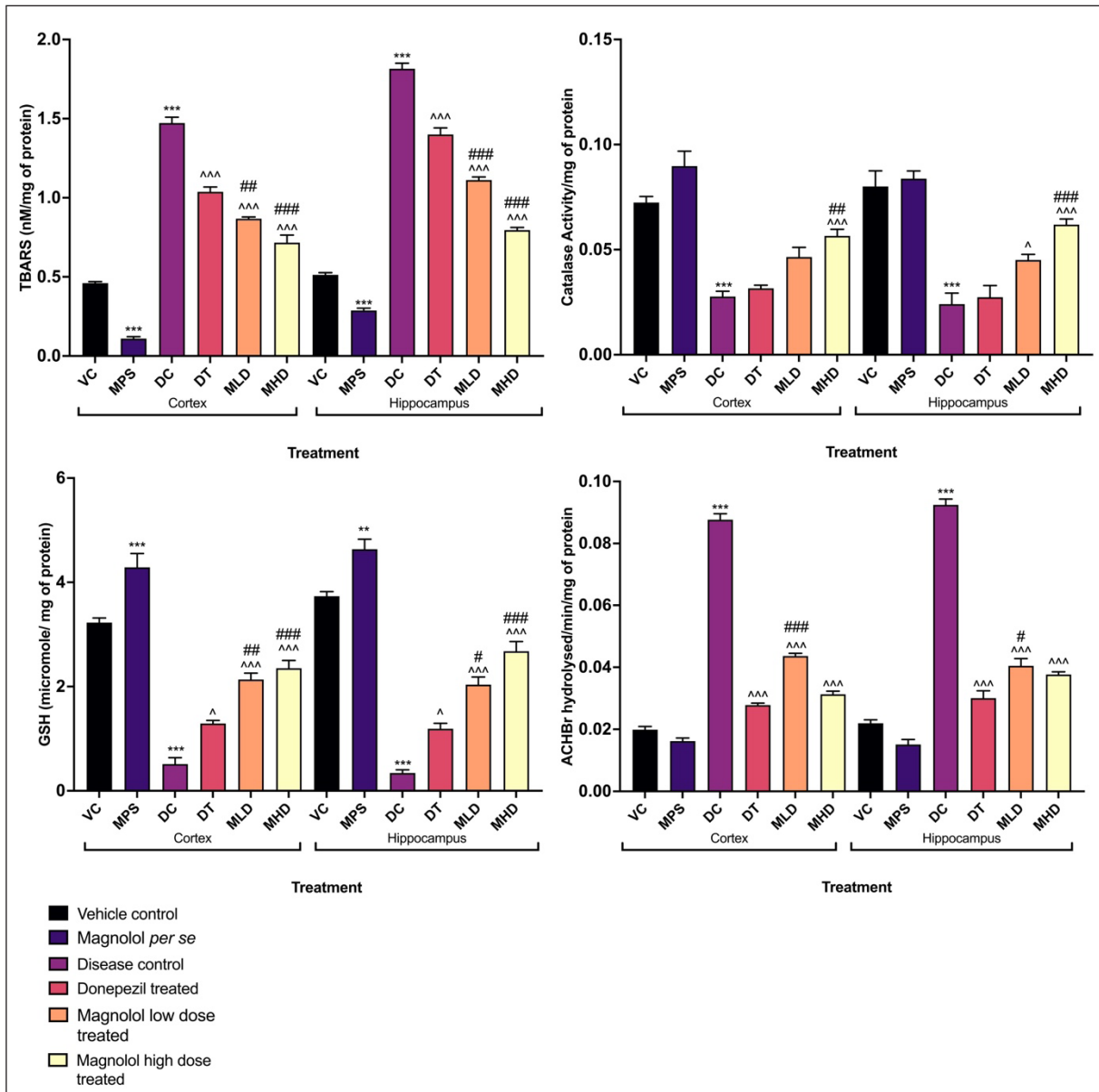


Figure 7. Effect of magnolol and other treatments on TBARS, GSH, CAT, and AChE expressed as mean \pm SEM. ***, ** and * have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$ in comparison with the VC group, respectively. The comparisons with DC group have been represented as ^^^, ^^ and ^ which signify $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. To represent comparisons with the donepezil treated group, ###, ## and # have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

The low-dose magnolol treatment resulted in an inconsequential elevation in CAT, whereas the magnolol treatment (high-dose) yielded a consequential ($p < 0.001$) increase in CAT, in contrast to the DC group. Notably, the magnolol-treated group (high-dose) exhibited a consequential ($p < 0.01$) rise in CAT contrasted with the group treated with the standard drug. In the hippocampal tissue, the CAT in the magnolol per se treated group showed an inconsequential increase contrasted with the VC group. The DC group had a remarkably ($p < 0.001$) reduced CAT contrasted with the VC group. Treatment with donepezil resulted in an inconsequential increase in CAT contrasted with the DC group. In contrast, the magnolol treatment (low-dose) yielded a consequential ($p < 0.05$) growth

in CAT, and the magnolol treatment (high-dose) resulted in a consequential ($p < 0.001$) increase in CAT contrasted with the DC group. When contrasted with the standard drug treated group, the magnolol-treated group (high-dose) exhibited a remarkably ($p < 0.001$) higher CAT.

In the cortex, the administration of magnolol alone did not result in a consequential change in AChE activity when contrasted with the VC group. However, the DC group exhibited a consequential elevation in AChE activity ($p < 0.001$), contrasted with the VC group. Treatment with donepezil remarkably decreased AChE activity ($p < 0.001$) contrasted with the DC group. The low-dose magnolol treatment led to a consequential downregulation in AChE

activity ($p < 0.001$), contrasted with the DC group, although it was remarkably higher than the AChE activity observed in the donepezil-treated group. On the other hand, the magnolol treatment (high-dose) resulted in a consequential downregulation in AChE activity ($p < 0.001$), contrasted with the DC group, and it exhibited AChE activity comparable to that of the donepezil-treated group. In the hippocampus, the administration of magnolol alone showed an inconsequential reduction in AChE activity contrasted with the VC group. In contrast, the DC group had a consequential uptick in AChE activity ($p < 0.001$), contrasted with the VC group. All treatments, including the standard drug (donepezil) and magnolol (low-dose and high-dose, both), remarkably decreased AChE activity ($p < 0.001$), contrasted

with the DC group. The low-dose magnolol treatment group had a remarkably higher AChE activity ($p < 0.05$) contrasted with the standard drug-treated group, whereas the magnolol treatment group (high-dose) exhibited AChE activity comparable to that of the donepezil-treated group.

3.7. Effect of magnolol on β -secretase, caspase-3, $A\beta_{1-42}$ and BDNF

Figure 8 presents the influence of magnolol and the other treatment groups on β -secretase, caspase-3, $A\beta_{1-42}$, and BDNF levels. In the cortex, magnolol per se did not produce a meaningful inhibition of β -secretase relative to the VC group. The DC group, however, showed a pronounced elevation in β -secretase

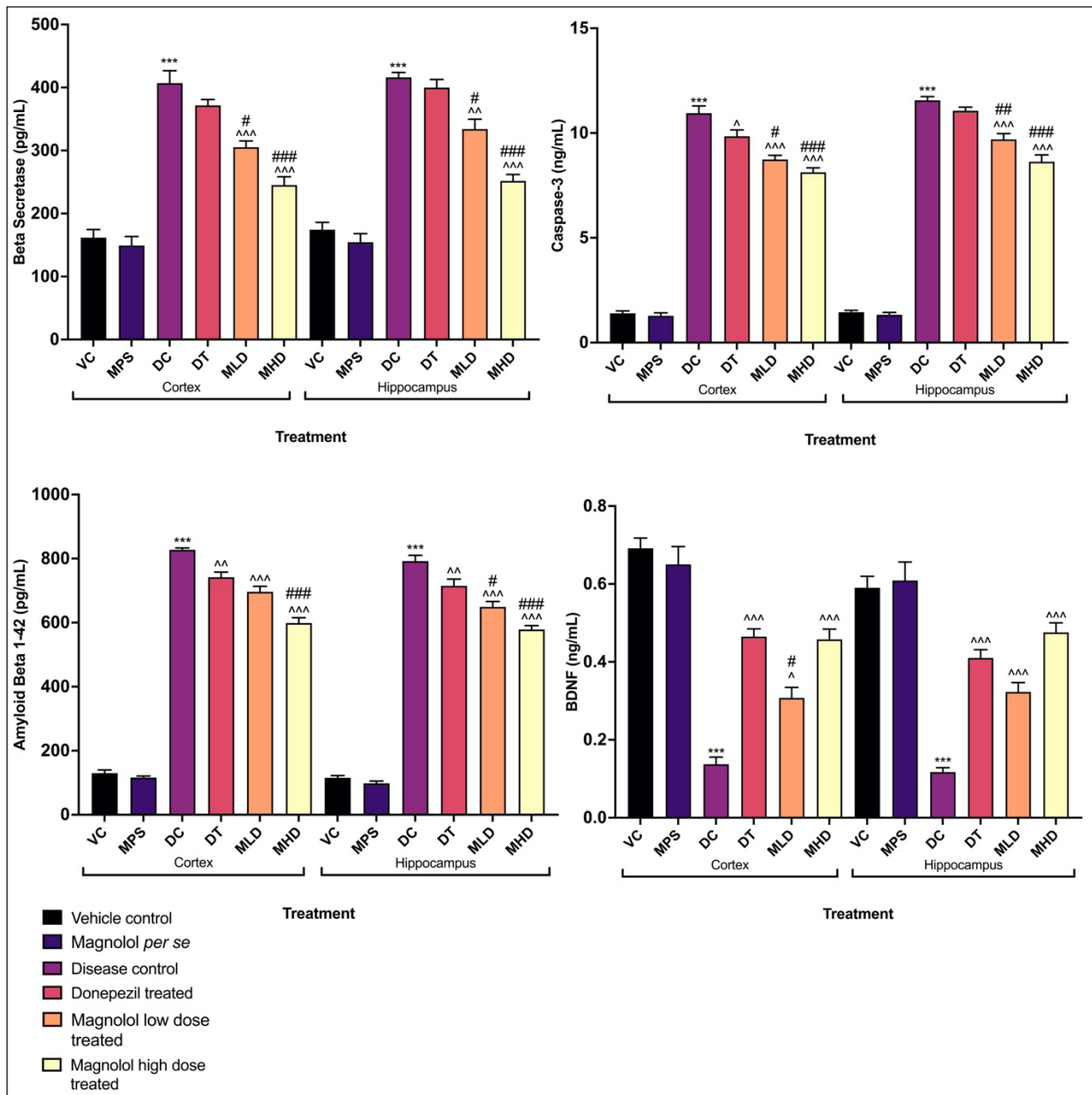


Figure 8. Effect of magnolol and other treatments on β -secretase, caspase-3, $A\beta_{1-42}$ and BDNF expressed as mean \pm SEM. ***, ** and * have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$ in comparison with the VC group, respectively. The comparisons with DC group have been represented as ^^, ^^ and ^ which signify $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively. To represent comparisons with the donepezil treated group, ###, ## and # have been used to represent $p < 0.001$, $p < 0.01$ and $p < 0.05$, respectively.

activity ($p < 0.001$) compared with VC group. Donepezil did not markedly alter β -secretase levels, whereas both magnolol doses produced strong inhibition of the enzyme ($p < 0.001$) as compared to the DC group. When compared with the donepezil group, β -secretase activity was significantly lower in the magnolol low-dose group ($p < 0.05$) and even further reduced in the high-dose group ($p < 0.001$). A similar pattern was seen in the hippocampus, though the low-dose magnolol treatment produced a significant reduction relative to the DC group ($p < 0.01$).

For caspase-3 levels in the cortex, magnolol per se resulted in a small, statistically non-significant decrease compared with VC. The DC group showed a strong elevation in Caspase-3 ($p < 0.001$) as compared to the VC group. Donepezil significantly reduced caspase-3 ($p < 0.05$), and both magnolol doses produced marked decreases ($p < 0.001$) as compared to the DC group. Relative to donepezil, the low dose of magnolol further reduced caspase-3 ($p < 0.05$), while the high dose produced a much stronger suppression ($p < 0.001$). In the hippocampus, magnolol alone caused a non-significant reduction in caspase-3 as compared to the VC group. DC group showed a marked ($p < 0.001$) elevation in caspase-3 as compared to the VC group. Donepezil also produced a non-significant decline as compared to the DC group. In contrast, both magnolol doses significantly lowered caspase-3 ($p < 0.001$) when compared to the DC group. Both magnolol treated groups showed a significant ($p < 0.01$ for low-dose) and ($p < 0.001$ for high-dose) decrease in caspase-3 levels as compared to the donepezil treated group.

Regarding $A\beta_{1-42}$ levels in the cortex, magnolol per se produced a decrease that did not reach significance compared with VC group. The DC group exhibited a sharp rise in $A\beta_{1-42}$ ($p < 0.001$) as compared to the VC group. Donepezil significantly lowered $A\beta_{1-42}$ relative to DC ($p < 0.01$), and both magnolol doses produced even stronger reductions ($p < 0.001$). Notably, the high-dose magnolol group showed significantly lower $A\beta_{1-42}$ levels than the donepezil group ($p < 0.001$). The hippocampus demonstrated similar trends, though the magnolol low-dose group produced a significant decline in $A\beta_{1-42}$ compared with donepezil treated group ($p < 0.05$).

For BDNF levels in the cortex, magnolol alone produced no meaningful change compared with VC. The DC group showed a marked reduction in BDNF ($p < 0.001$) as compared to the VC group. Donepezil significantly increased BDNF relative to DC ($p < 0.001$). The low-dose magnolol treatment also elevated BDNF ($p < 0.05$) as compared to DC group, though levels remained significantly lower than those observed with donepezil ($p < 0.05$). The high-dose magnolol group demonstrated a strong increase in BDNF ($p < 0.001$) as compared to the DC group, comparable to the group that was treated with the standard drug. In the hippocampus, magnolol per se led to a small, non-significant uptick in BDNF compared with VC, whereas the DC group again showed a marked reduction ($p < 0.001$) as compared to the VC group. Donepezil, magnolol low dose, and magnolol high dose all significantly enhanced BDNF levels relative to DC ($p < 0.001$).

4. DISCUSSION

AD evolves and advances through various pathways, with key pathological features including elevated expression of AChE (resulting in decreased ACh levels), accumulation of

$A\beta$ plaques (causing neuronal mitochondrial dysfunction), and heightened levels of caspase-3 (triggering neuronal apoptotic cell death) [2,62,63]. Oxidative stress remarkably contributes to the onset and advancement of AD [64,65]. Present pharmacological treatments fail to address the fundamental pathological mechanisms of AD, offering only symptomatic alleviation. Antioxidant compounds are being increasingly recognised for their potential in managing the pathology of AD. Magnolol (as a part of Magnolia bark extract) has been used by humans for ages under Chinese and Japanese traditional medicine systems. Magnolol has been reported to have a neuroprotective effect *in vitro* by its antioxidant and caspase-3 inhibitory activities [17,20]. In the present study, magnolol treatment has been shown to have a remarkable *in vivo* antioxidant activity. The expression of caspase-3 in cortical and hippocampal tissues of the mouse brain was also found to be reduced with magnolol treatment. Furthermore, magnolol has been reported to have an inhibitory effect on the deposition of $A\beta$ in a transgenic *C. elegans* [21]. In our study, it was reported that magnolol decreases the production of neurotoxic $A\beta$ by inhibiting β -secretase enzyme and helps in the disintegration of $A\beta_{1-42}$ oligomers. This research reveals that magnolol mitigates amnesia and cognitive insufficiencies by affecting various pathological pathways associated with AD. As a phenolic compound, its notable antioxidant properties play a crucial role in alleviating AD symptoms. Its ability to scavenge free radicals protects neurons from degradation, thereby preserving their integrity. In addition, it inhibits the AChE enzyme, leading to increased levels of acetylcholine in the cortex and hippocampus. This neuronal protection, particularly for cholinergic neurons, is enhanced by improved acetylcholine levels, resulting in improved memory and cognitive function. Moreover, magnolol's effects on the amyloid route of pathogenesis may offer disease-altering relief in AD-affected brains. It also prevents neuronal apoptosis by inhibiting caspase-3 and remarkably elevates BDNF levels, indicating its role in restoration of BDNF-dependent synaptic plasticity and neuronal cell survival. The multifactorial effects observed with magnolol are consistent with those reported in the pharmacological profile of other neuroprotective compounds from plant sources. For example, naringenin [9] and hesperidin [66] have been shown to reduce AChE activity, reducing the markers of oxidative stress and lower the $A\beta$ burden in $AlCl_3/D$ -galactose models in rodents, and vanillin [30,32,67] and epigallocatechin gallate [68,69] possess $A\beta$ disaggregating behaviour and caspase-3 inhibition in a fashion similar to those observed with magnolol. Compared with these compounds, magnolol shows a greater convergence of action (including inhibition of β -secretase, restoration of BDNF, disintegration of $A\beta$ aggregates, and attenuation of caspase-3), potentially suggesting that magnolol may have effects on upstream pathways as well as downstream pathways relating to neurodegeneration. This suggests magnolol as a potential multi-target natural agent in this expanding class of anti-AD candidates derived from plants. The restoration of BDNF levels observed in this study also parallels reports from other polyphenols and neolignans, many of which enhance synaptic plasticity through CREB-BDNF signalling. However, the concurrent reduction in β -secretase activity and $A\beta_{1-42}$ aggregation distinguishes magnolol from several natural compounds that predominantly act as antioxidants. This dual impact on amyloid processing

and neurotrophic support provides a mechanistic rationale for magnolol's ability to improve cognitive performance more comprehensively than single-pathway agents. This study underscores magnolol's potential as an effective, safe, and cost-efficient pharmacotherapeutic option for AD.

The present study corroborates the previously established findings pertaining to magnolol, such as antioxidant effect [70,71], AChE inhibition [72,73], β -secretase inhibitory activity [74], anti-A β aggregatory activity [21], BDNF upregulation activity [75,76], and overall neuroprotective action by eliciting reduction of apoptosis by inhibition of caspase-3 [20,77,78]. This investigation has some limitations that should be acknowledged. First, the sample size ($n = 6$ per group) was adequate for the behavioural and biochemical analyses but limits the statistical power of the research to detect subtle changes. Second, the absence of histological or immunohistochemical confirmation reduces the ability to visualise region-specific neurodegeneration. Consequently, future studies that include histopathological assessment, other molecular endpoints, and other models of AD will be indispensable in the validation and extension of these findings.

5. SUMMARY AND CONCLUSION

This study presents a comprehensive evaluation of the pharmacological potential of magnolol (a bioactive neolignan isolated from *M. officinalis*) and highlights its variety of pharmacological effects such as neuroprotective, and antioxidant activities. Through detailed assessment, including studies of behavioural parameters, biochemical parameters, and molecular markers, magnolol consistently demonstrated its potential anti-AD effect. It exhibited ability to mitigate oxidative stress, subdue key factors of neurodegeneration, and improve functional outcomes in relevant experimental models. These experimental observations corroborate its acceptance in traditional medicine systems and are further supported by its high LD₅₀ value, which reassures its favourable safety profile. On mechanistic basis, the neolignan exerts multi-targeted actions which are relevant to AD, such as inhibition of AChE, β -secretase, and caspase-3, reduction of A β aggregation, mitigating oxidative stress, and restoration of BDNF levels. This goes on to illustrate its capacity to target several connected pathological pathways simultaneously. In the context of the persistent scarcity of effective disease-altering therapeutic interventions for AD, the multi-targeted and biologically robust nature of magnolol indicates its promise as a natural therapeutic candidate that could meaningfully supplement the existing anti-AD armamentarium. Collectively, these findings provide compelling justification for further exploration of magnolol for development of safer, more comprehensive interventions for neurodegenerative disorders.

6. KEY TAKEAWAYS

- Magnolol showed clear neuroprotective benefits in the AICl₃ + D-galactose AD model, with noticeable improvements in learning and memory.
- Its effects seem to come from several pathways working together, i.e., strong antioxidant action, along with lowering AChE, β -secretase, and caspase-3 activity.
- Magnolol helped in the breakdown of A β ₁₋₄₂ plaques.

- Treatment restored BDNF levels, supporting healthier synaptic function and better neuronal survival.
- Behavioural performance in MWM, EPM, and NOR improved.
- Magnolol has a favourable safety background, supported by long-standing traditional use.
- This work marks the first comprehensive *in vivo* study to show magnolol influencing several AD-related pathways at once.
- The main limitations were the small sample size and the absence of histopathological evidence, which future studies should address.
- Overall, magnolol represents a promising, multi-target, natural compound candidate for AD therapy with potential advantages over current symptomatic treatments.

7. LIST OF ABBREVIATIONS

AChE, Acetylcholinesterase; AD, Alzheimer's disease; A β , Amyloid beta; BDNF, Brain-derived neurotrophic factor; CAT, Catalase activity; CCSEA, Committee for Control and Supervision of Experiments on Animals; DC, Disease control; EL, Escape latency; ELISA, Enzyme-Linked Immunosorbent Assay; GSH, Glutathione; IAEC, Institutional Animal Ethics Committee; MWM, Morris Water Maze; NOR, Novel object recognition; TBARS, Thiobarbituric acid reactive substances; TL, Transfer latency; VC, Vehicle Control.

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9. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

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11. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

12. ETHICAL APPROVALS

Ethical approval details are given in the 'Materials and Methods' section.

13. DATA AVAILABILITY

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

14. PUBLISHER'S NOTE

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15. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCES

- Anand A, Khurana N, Kumar R, Sharma N. Food for the mind: the journey of probiotics from foods to anti-Alzheimer's disease therapeutics. *Food Biosci.* 2023;51:102323. doi: <https://doi.org/10.1016/J.FBIO.2022.102323>
- Anand A, Patience AA, Sharma N, Khurana N. The present and future of pharmacotherapy of Alzheimer's disease: a comprehensive review. *Eur J Pharmacol.* 2017;815:364–75. doi: <https://doi.org/10.1016/j.ejphar.2017.09.043>
- Reisberg B, Doody R, Stoffler A, Schmitt F, Ferris S, Mobius HJ. Memantine in moderate-to-severe Alzheimer's disease. *N Engl J Med.* 2003;348:1333–41. doi: <https://doi.org/10.1056/NEJMoa013128>
- Anand A, Khurana P, Chawla J, Sharma N, Khurana N. Emerging treatments for the behavioral and psychological symptoms of dementia. *CNS Spectr.* 2017;23:361–9. doi: <https://doi.org/10.1017/S1092852917000530>
- Maurer K, Ihl R, Dierks T, Frölich L. Clinical efficacy of *Ginkgo biloba* special extract EGB 761 in dementia of the Alzheimer type. *J Psychiatr Res.* 1997;31:645–55. doi: [https://doi.org/10.1016/S0022-3956\(97\)00022-8](https://doi.org/10.1016/S0022-3956(97)00022-8)
- Saraf MK, Prabhakar S, Khanduja KL, Anand A. *Bacopa monniera* attenuates scopolamine-induced impairment of spatial memory in mice. *Evidence-Based Complementary Alternative Med.* 2011;2011:1–19. doi: <https://doi.org/10.1093/ecam/neaq038>
- Habtemariam S. The therapeutic potential of rosemary (*Rosmarinus officinalis*) diterpenes for Alzheimer's disease. *Evidence-Based Complementary Alternative Med.* 2016;2016:1–4. doi: <https://doi.org/10.1155/2016/2680409>
- Da Rosa MM, De Amorim LC, Alves JV de O, Aguiar IF da S, Oliveira FG da S, Da Silva MV, *et al.* The promising role of natural products in Alzheimer's disease. *Brain Disord.* 2022;7:100049. doi: <https://doi.org/10.1016/J.DSCB.2022.100049>
- Chiroma SM, Mohd Moklas MA, Mat Taib CN, Baharuldin MTH, Amon Z. D-galactose and aluminium chloride induced rat model with cognitive impairments. *Biomed Pharmacother.* 2018;103:1602–8. doi: <https://doi.org/10.1016/J.BIOPHA.2018.04.152>
- Li H, Zheng L, Chen C, Liu X, Zhang W. Brain senescence caused by elevated levels of reactive metabolite methylglyoxal on D-galactose-induced aging mice. *Front Neurosci.* 2019;13:1004. doi: <https://doi.org/10.3389/FNINS.2019.01004/BIBTEX>
- Shwe T, Pratchayasakul W, Chattipakorn N, Chattipakorn SC. Role of D-galactose-induced brain aging and its potential used for therapeutic interventions. *Exp Gerontol.* 2018;101:13–36. doi: <https://doi.org/10.1016/J.EXGER.2017.10.029>
- Haider S, Liaquat L, Ahmad S, Batool Z, Siddiqui RA, Tabassum S, *et al.* Naringenin protects A β 13/D-galactose induced neurotoxicity in rat model of AD via attenuation of acetylcholinesterase levels and inhibition of oxidative stress. *PLoS One.* 2020;15:227631. doi: <https://doi.org/10.1371/JOURNAL.PONE.0227631>
- Xing Z, He Z, Wang S, Yan Y, Zhu H, Gao Y, *et al.* Ameliorative effects and possible molecular mechanisms of action of fibrauretin from *Fibraurea recisa* Pierre on D-galactose/A β 13-mediated Alzheimer's disease. *RSC Adv.* 2018;8:31646–57. doi: <https://doi.org/10.1039/C8RA05356A>
- Xiao F, Li XG, Zhang XY, Hou JD, Lin LF, Gao Q, *et al.* Combined administration of D-galactose and aluminium induces Alzheimer-like lesions in brain. *Neurosci Bull.* 2011;27:143–55. doi: <https://doi.org/10.1007/s12264-011-1028-2>
- Gao L, Peng XM, Huo SX, Liu XM, Yan M. Memory enhancement of Acteoside (Verbascoside) in a senescent mice model induced by a combination of d-gal and A β 13. *Phytotherapy Res.* 2015;29:1131–6. doi: <https://doi.org/10.1002/ptr.5357>
- Poivre M, Duez P. Biological activity and toxicity of the Chinese herb *Magnolia officinalis* Rehder & E. Wilson (Houpo) and its constituents. *J Zhejiang Univ Sci B.* 2017;18:194. doi: <https://doi.org/10.1631/JZUS.B1600299>
- Kuribara H, Kishi E, Hattori N, Okada M, Maruyama Y. The anxiolytic effect of two oriental herbal drugs in Japan attributed to honokiol from magnolia bark. *J Pharm Pharmacol.* 2000;52:1425–9. doi: <https://doi.org/10.1211/0022357001777432>
- Iwasaki K, Wang Q, Seki H, Satoh K, Takeda A, Arai H, *et al.* The effects of the traditional Chinese medicine, “Banxia Houpo Tang (Hange-Koboku To)” on the swallowing reflex in Parkinson's disease. *Phytomedicine.* 2000;7:259–63. doi: [https://doi.org/10.1016/S0944-7113\(00\)80042-2](https://doi.org/10.1016/S0944-7113(00)80042-2)
- Koetter U, Barrett M, Lacher S, Abdelrahman A, Dolnick D. Interactions of Magnolia and *Ziziphus* extracts with selected central nervous system receptors. *J Ethnopharmacol.* 2009;124:421–5. doi: <https://doi.org/10.1016/J.JEP.2009.05.040>
- Hoi CP, Ho YP, Baum L, Chow AHL. Neuroprotective effect of honokiol and magnolol, compounds from *Magnolia officinalis*, on beta-amyloid-induced toxicity in PC12 cells. *Phytother Res.* 2010;24:1538–42. doi: <https://doi.org/10.1002/ptr.3178>
- Xie Z, Zhao J, Wang H, Jiang Y, Yang Q, Fu Y, *et al.* Magnolol alleviates Alzheimer's disease-like pathology in transgenic *C. elegans* by promoting microglia phagocytosis and the degradation of beta-amyloid through activation of PPAR- γ . *Biomed Pharmacother.* 2020;124:109886. doi: <https://doi.org/10.1016/J.BIOPHA.2020.109886>
- Anand A, Nuthan Kumar Babu V, Giriyaam R, Turan S, Sharma N, Khurana N, *et al.* The relationship of magnolol, an important phytoconstituent, with neurological disorders: an *In silico* evaluation. *Plant Cell Biotechnol Mol Biol.* 2021;22:389–97.
- Cheng J, Dong S, Yi L, Geng D, Liu Q. Magnolol abrogates chronic mild stress-induced depressive-like behaviors by inhibiting neuroinflammation and oxidative stress in the prefrontal cortex of mice. *Int Immunopharmacol.* 2018;59:61–7. doi: <https://doi.org/10.1016/J.INTIMP.2018.03.031>
- Xian YF, Qu C, Liu Y, Ip SP, Yuan QJ, Yang W, *et al.* Magnolol ameliorates behavioral impairments and neuropathology in a transgenic mouse model of Alzheimer's disease. *Oxidative Med Cellular Longevity.* 2020;2020:5920476. doi: <https://doi.org/10.1155/2020/5920476>
- Itoh J, Nabeshima T, Kameyama T. Utility of an elevated plus-maze for the evaluation of memory in mice: effects of nootropics, scopolamine and electroconvulsive shock. *Psychopharmacology (Berl).* 1990;101:27–33. doi: <https://doi.org/10.1007/BF02253713>
- Vorhees CV, Williams MT. Morris water maze: procedures for assessing spatial and related forms of learning and memory. *Nat Protoc.* 2006;1:848–58. doi: <https://doi.org/10.1038/nprot.2006.116>
- Bromley -Brits K, Deng Y, Song W. Morris Water Maze test for learning and memory deficits in Alzheimer's disease model mice. *J Visualized Experiments.* 2011; 13: e2920. doi: <https://doi.org/10.3791/2920>
- Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. *Cogn Process.* 2012;13:93–110. doi: <https://doi.org/10.1007/s10339-011-0430-z>
- Lueptow LM. Novel object recognition test for the investigation of learning and memory in mice. *J Visualized Experiments.* 2017;2017:55718. doi: <https://doi.org/10.3791/55718>

30. Anand A, Khurana N, Ali N, AlAsmari AF, Alharbi M, Waseem M, *et al.* Ameliorative effect of vanillin on scopolamine-induced dementia-like cognitive impairment in a mouse model. *Front Neurosci.* 2022;0:1748. doi: <https://doi.org/10.3389/FNINS.2022.1005972>
31. Habibyar AF, Sharma N, Khurana N. PASS assisted prediction and pharmacological evaluation of hesperidin against scopolamine induced amnesia in mice. *Eur J Pharmacol.* 2016;789:385–94. doi: <https://doi.org/10.1016/j.ejphar.2016.07.013>
32. Anand A, Khurana N, Kaur S, Ali N, Alasmari AF, Waseem M, *et al.* The multifactorial role of vanillin in amelioration of aluminium chloride and D-galactose induced Alzheimer's disease in mice. *Eur J Pharmacol.* 2023;954:175832. doi: <https://doi.org/10.1016/J.EJPHAR.2023.175832>
33. Su D, Zhao Y, Wang B, Xu H, Li W, Chen J, *et al.* Isoflurane-induced spatial memory impairment in mice is prevented by the acetylcholinesterase inhibitor donepezil. *PLoS One.* 2011;6:e27632. doi: <https://doi.org/10.1371/JOURNAL.PONE.0027632>
34. Gao J, He H, Jiang W, Chang X, Zhu L, Luo F, *et al.* Salidroside ameliorates cognitive impairment in a d-galactose-induced rat model of Alzheimer's disease. *Behavioural Brain Res.* 2015;293:27–33. doi: <https://doi.org/10.1016/J.BBR.2015.06.045>
35. Kawahara M, Kato-Negishi M. Link between Aluminum and the pathogenesis of Alzheimer's disease: the integration of the Aluminum and amyloid cascade hypotheses. *Int J Alzheimers Dis.* 2011;2011:276393. doi: <https://doi.org/10.4061/2011/276393>
36. Moumen R, Ait-Oukhatar N, Bureau F, Fleury C, Bouglé D, Arhan P, *et al.* Aluminium increases xanthine oxidase activity and disturbs antioxidant status in the rat. *J Trace Elements Med Biol.* 2001;15:89–93. doi: [https://doi.org/10.1016/S0946-672X\(01\)80049-3](https://doi.org/10.1016/S0946-672X(01)80049-3)
37. Platt B, Fiddler G, Riedel G, Henderson Z. Aluminium toxicity in the rat brain: histochemical and immunocytochemical evidence. *Brain Res Bull.* 2001;55:257–67. doi: [https://doi.org/10.1016/s0361-9230\(01\)00511-1](https://doi.org/10.1016/s0361-9230(01)00511-1)
38. Boni UD, Otvos A, Scott JW, Crapper DR. Neurofibrillary degeneration induced by systemic aluminum. *Acta Neuropathol.* 1976;35:285–94.
39. Shunan D, Yu M, Guan H, Zhou Y. Neuroprotective effect of Betalain against AlCl₃-induced Alzheimer's disease in Sprague Dawley Rats via putative modulation of oxidative stress and nuclear factor kappa B (NF-κB) signaling pathway. *Biomed Pharmacother.* 2021;137:111369. doi: <https://doi.org/10.1016/J.BIOPHA.2021.111369>
40. Skalny A, Aschner M, Jiang Y, Gluhcheva Y, Tizabi Y, Lobinski R, *et al.* Molecular mechanisms of aluminum neurotoxicity: update on adverse effects and therapeutic strategies. *Adv Neurotoxicol.* 2021;10:1–34. doi: <https://doi.org/10.1016/bs.ant.2020.12.001i>
41. Mesole SB, Alfred OO, Yusuf UA, Lukubi L, Ndhlovu D. Apoptotic inducement of neuronal cells by aluminium chloride and the neuroprotective effect of Eugenol in Wistar Rats. *Oxid Med Cell Longev.* 2020;2020:8425643. doi: <https://doi.org/10.1155/2020/8425643>
42. Abbas F, Eladl MA, El-Sherbiny M, Abozied N, Nabil A, Mahmoud SM, *et al.* Celastrol and thymoquinone alleviate aluminum chloride-induced neurotoxicity: behavioral psychomotor performance, neurotransmitter level, oxidative-inflammatory markers, and BDNF expression in rat brain. *Biomed Pharmacother.* 2022;151:113072. doi: <https://doi.org/10.1016/J.BIOPHA.2022.113072>
43. Wei Y, Liu D, Zheng Y, Li H, Hao C, Ouyang W. Protective effects of kinetin against aluminum chloride and D-galactose induced cognitive impairment and oxidative damage in mouse. *Brain Res Bull.* 2017;134:262–72. doi: <https://doi.org/10.1016/j.brainresbull.2017.08.014>
44. Nazari QA, Kume T, Takada-Takatori Y, Izumi Y, Akaike A. Protective effect of luteolin on an oxidative-stress model induced by microinjection of sodium nitroprusside in mice. *J Pharmacol Sci.* 2013;122:109–17. doi: <https://doi.org/10.1254/jphs.13019FP>
45. Parle M, Dhingra D, Kulkarni SK. Memory-strengthening activity of *Glycyrrhiza glabra* in exteroceptive and interoceptive behavioral models. *J Med Food.* 2004;7:462–6. doi: <https://doi.org/10.1089/jmf.2004.7.462>
46. Leger M, Quiedeville A, Bouet V, Haelewyn B, Boulouard M, Schumann-Bard P, *et al.* Object recognition test in mice. *Nature Protocols.* 2013;8(12):2531–47. doi: <https://doi.org/10.1038/nprot.2013.155>
47. Morris RGM. Spatial localization does not require the presence of local cues. *Learn Motiv.* 1981;12:239–60. doi: [https://doi.org/10.1016/0023-9690\(81\)90020-5](https://doi.org/10.1016/0023-9690(81)90020-5)
48. Vorhees CV, Williams MT. Assessing spatial learning and memory in rodents. *ILAR J.* 2014;55:310–2. doi: <https://doi.org/10.1093/ilar/ilu013>
49. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–8. doi: [https://doi.org/10.1016/0003-2697\(79\)90738-3](https://doi.org/10.1016/0003-2697(79)90738-3)
50. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med.* 1963;61:882–8.
51. Aebi H. Catalase in vitro. *Methods Enzymol.* 1984;105:121–6. doi: [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
52. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem Pharmacol.* 1961;7:88–95. doi: [https://doi.org/10.1016/0006-2952\(61\)90145-9](https://doi.org/10.1016/0006-2952(61)90145-9)
53. Vassar R, Kovacs DM, Yan R, Wong PC. Symposium: the β-Secretase Enzyme BACE in health and Alzheimer's disease: regulation, cell biology, function, and therapeutic potential. *J Neurosci.* 2009;29:12787. doi: <https://doi.org/10.1523/JNEUROSCI.3657-09.2009>
54. Bioassay Technology Laboratory. Protocols - BT LAB. Shanghai Korain Biotech, Shanghai, China. [cited 2022 Nov 8]. 2022. Available from: <https://www.bt-laboratory.com/index.php/Shop/Index/protocols.html>
55. D'Amelio M, Cavallucci V, Middei S, Marchetti C, Pacioni S, Ferri A, *et al.* Caspase-3 triggers early synaptic dysfunction in a mouse model of Alzheimer's disease. *Nature Neurosci.* 2010;14(1):69–76. doi: <https://doi.org/10.1038/nn.2709>
56. CUSABIO. Mouse caspase 3, Casp-3 ELISA Kit – Cusabio. CUSABIO, Wuhan, China; 2022 [cited 2022 Nov 9]. Available from: <https://www.cusabio.com/ELISA-Kit/Mouse-Caspase-3-Casp-3-ELISA-Kit-69045.html>
57. Sadigh-Eteghad S, Sabermarouf B, Majdi A, Talebi M, Farhoudi M, Mahmoudi J. Amyloid-beta: a crucial factor in Alzheimer's disease. *Med Princ Pract.* 2015;24:1–10. doi: <https://doi.org/10.1159/000369101>
58. Anand A, Chawla J, Mahajan A, Sharma N, Khurana N. Therapeutic potential of epigallocatechin gallate. *Int J Green Pharm.* 2017;11:S364–70. doi: <https://doi.org/10.22377/ijgp.v11i03.1143>
59. CUSABIO. Mouse amyloid beta peptide 1–42 (Aβ1–42) ELISA Kit - CUSABIO CUSABIO, Wuhan, China. [cited 2022 Nov 9]. 2022. Available from: <https://www.cusabio.com/ELISA-Kit/Mouse-amyloid-beta-peptide-1-42A%CE%B21-42-ELISA-Kit-66283.html>
60. Giuffrida ML, Copani A, Rizzarelli E. A promising connection between BDNF and Alzheimer's disease. *Aging (Albany NY).* 2018;10:1791. doi: <https://doi.org/10.18632/AGING.101518>
61. Bioassay Technology Laboratory. Mouse brain derived neurotrophic factor, BDNF ELISA Kit - BT LAB [cited 2022 Nov 9]. 2022. Available from: https://www.bt-laboratory.com/index.php/Shop/Index/productShijieDetail/p_id/2202.html
62. Heneka MT, Carson MJ, Khoury J el, Landreth GE, Brosseron F, Feinstein DL, *et al.* Neuroinflammation in Alzheimer's disease. *Lancet Neurol.* 2015;14:388–405. doi: [https://doi.org/10.1016/S1474-4422\(15\)70016-5](https://doi.org/10.1016/S1474-4422(15)70016-5)
63. Alzheimer's Association. What is Alzheimer's? Alzheimer's Association, Chicago, IL; 2017.
64. Betteridge DJ. What is oxidative stress?. *Metabolism.* 2000;49:3–8. doi: [https://doi.org/10.1016/s0026-0495\(00\)80077-3](https://doi.org/10.1016/s0026-0495(00)80077-3)

65. Huang WJ, Zhang X, Chen WW. Role of oxidative stress in Alzheimer's disease. *Biomed Rep.* 2016;4:519–22. doi: <https://doi.org/10.3892/br.2016.630>
66. Chauhan P, Vadia N, Ballal S, Joshi KK, Maharana L, Chauhan AS, *et al.* Decoding the neuroprotective potential of hesperidin: insights into Alzheimer's disease. *Neuroscience.* 2025;589:205–20. doi: <https://doi.org/10.1016/J.NEUROSCIENCE.2025.09.028>
67. Song S, Ma X, Zhou Y, Xu M, Shuang S, Dong C. Studies on the interaction between vanillin and β -Amyloid protein via fluorescence spectroscopy and atomic force microscopy. *Chem Res Chin Univ.* 2016;32:172–7. doi: <https://doi.org/10.1007/s40242-016-5347-8>
68. He M, Zhao L, Wei MJ, Yao WF, Zhao HS, Chen FJ. Neuroprotective effects of (–)-epigallocatechin-3-gallate on aging mice induced by D-galactose. *Biol Pharm Bull.* 2009;32:55–60. doi: <https://doi.org/10.1248/BPB.32.55>
69. Singh NA, Bhardwaj V, Ravi C, Ramesh N, Mandal AKA, Khan ZA. EGCG nanoparticles attenuate aluminum chloride induced neurobehavioral deficits, beta amyloid and Tau Pathology in a rat model of Alzheimer's disease. *Front Aging Neurosci.* 2018;10:244. doi: <https://doi.org/10.3389/FNAGI.2018.00244>
70. Amorati R, Zotova J, Baschieri A, Valgimigli L. Antioxidant activity of magnolol and Honokiol: kinetic and mechanistic investigations of their reaction with peroxy radicals. *J Organic Chem.* 2015;80:10651–9. doi: <https://doi.org/10.1021/ACS.JOC.5B01772>
71. Shen JL, Man KM, Huang PH, Chen WC, Chen DC, Cheng YW, *et al.* Honokiol and magnolol as multifunctional antioxidative molecules for dermatologic disorders. *Molecules.* 2010;15:6452. doi: <https://doi.org/10.3390/MOLECULES15096452>
72. Lee YK, Yuk DY, Kim TI, Kim YH, Kim KT, Kim KH, *et al.* Protective effect of the ethanol extract of *Magnolia officinalis* and 4-O-methylhonokiol on scopolamine-induced memory impairment and the inhibition of acetylcholinesterase activity. *J Natural Med.* 2009;63(3):274–82. doi: <https://doi.org/10.1007/S11418-009-0330-Z>
73. Hou YC, Lee Chao PD, Chen SY. Honokiol and magnolol increased hippocampal acetylcholine release in freely-moving rats. *Am J Chin Med (Gard City N Y).* 2000;28:379–84. doi: <https://doi.org/10.1142/S0192415X00000441>
74. Santos J, Quimque MT, Liman RA, Agbay JC, Macabeo APG, Corpuz MJA, *et al.* Computational and experimental assessments of magnolol as a neuroprotective agent and utilization of UiO-66(Zr) as its drug delivery system. *ACS Omega.* 2021;6:24382. doi: <https://doi.org/10.1021/ACSOMEGA.1C02555>
75. Liu Z, Xie J, Lin K, Qi L. Influencing mechanism of magnolol on expression of BDNF and Bax in rats with cerebral ischemic stroke. *Exp Ther Med.* 2018;16:4423. doi: <https://doi.org/10.3892/ETM.2018.6807>
76. Li LF, Lu J, Li XM, Xu CL, Deng JM, Qu R, *et al.* Antidepressant-like effect of magnolol on BDNF up-regulation and serotonergic system activity in unpredictable chronic mild stress treated rats. *Phytother Res.* 2012;26:1189–94. doi: <https://doi.org/10.1002/PTR.3706>
77. Zhu S, Liu F, Zhang R, Xiong Z, Zhang Q, Hao L, *et al.* Neuroprotective potency of neolignans in *Magnolia officinalis* cortex against brain disorders. *Front Pharmacol.* 2022;13:857449. doi: <https://doi.org/10.3389/fphar.2022.857449>
78. Li H, Liu X, Zhu Y, Liu Y, Wang Y. Magnolol derivative 002C-3 protects brain against ischemia–reperfusion injury via inhibiting apoptosis and autophagy. *Neurosci Lett.* 2015;588:178–83. doi: <https://doi.org/10.1016/J.NEULET.2015.01.007>

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