



Ficus deltoidea Preserves Hippocampal Neuronal Integrity and Redox Balance in Oxidative Stress-Driven Alzheimer's Disease-Like Rat Model

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Abstract

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia worldwide, with oxidative stress playing a central role in its pathogenesis. *Ficus deltoidea* (FD), a medicinal plant rich in flavonoids vitexin and isovitexin, possesses potent antioxidant and anti-inflammatory properties, yet its neuroprotective efficacy in AD remains incompletely characterized. This study investigated the protective effects of FD in a D-galactose- and aluminum chloride (AlCl₃)-induced oxidative stress-driven AD-like rat model using behavioral, histological, ultrastructural, and biochemical approaches. Fifty-four male Wistar rats were assigned to six groups: control, AD-like model, donepezil (1 mg/kg), and FD-treated groups (50, 100, and 200 mg/kg) for 10 weeks. Anxiety-like behavior and spatial working memory were assessed using the elevated plus maze (EPM) and T-maze tests, respectively. Hippocampal neuronal integrity was evaluated by hematoxylin and eosin (H&E) staining and transmission electron microscopy (TEM), while oxidative stress biomarkers (MDA, CAT, T-SOD, CuZn-SOD, and HO-1) were quantified using ELISA. FD treatment, particularly at 200 mg/kg, significantly improved spatial working memory and normalized anxiety-related behavior, with treatment responses approaching those observed in the donepezil-treated group. Histological analyses revealed preservation of pyramidal neurons across CA1, CA2, CA3, and dentate gyrus subregions, while ultrastructural studies demonstrated marked protection of mitochondrial integrity, myelin sheath organization, and smooth endoplasmic reticulum morphology. Biochemically, FD significantly reduced lipid peroxidation and enhanced endogenous antioxidant defenses. In conclusion, FD exerted significant neuroprotective effects characterized by preservation of hippocampal structure, maintenance of neuronal ultrastructure, and restoration of redox homeostasis in an oxidative stress-driven AD-like model. These findings demonstrate that FD mitigates oxidative stress-associated neuronal injury and cognitive impairment in a D-galactose and AlCl₃-induced AD-like rat model, supporting its potential as a phytotherapeutic candidate for oxidative stress-related neurodegeneration. However, further studies are required to determine its effects on canonical Alzheimer's disease pathologies, including amyloid and tau abnormalities.

Keywords *Ficus deltoidea* · Alzheimer's disease · Oxidative stress · Hippocampus · Neuroprotection · Cognitive dysfunction · Therapy

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia, accounting for approximately 60–80% of all cases worldwide [1]. It is clinically characterized by progressive memory impairment and cognitive decline, which are primarily attributed to extensive neuronal loss and synaptic dysfunction within the cortex and hippocampus [2, 3]. The global prevalence of AD is projected to reach 106.8 million individuals by 2050, posing an escalating socioeconomic and public health burden [4]. AD is broadly classified into early-onset AD, which occurs between 30 and 60 years of age and represents approximately 5–6% of cases, and late-onset AD, which manifests after 60 years of age and accounts for over 90% of cases [5, 6]. Early-stage AD typically presents with short-term memory deficits, whereas advanced stages are associated with severe cognitive deterioration, mood disturbances, disorientation, social withdrawal, and long-term memory loss, accompanied by widespread neuronal degeneration and synaptic loss [7].

The neuropathological mechanisms of AD include extracellular β -amyloid plaque deposition and intracellular neurofibrillary tau tangles accumulation, chronic neuroinflammation, oxidative stress, and cholinergic dysfunction [8]. β -Amyloid and hyperphosphorylated tau trigger sustained microglial activation and pro-inflammatory cytokine release, which in turn worsen tau pathology and synaptic damage [9]. Oxidative stress, arising from an imbalance between excess reactive oxygen species (ROS) and weakened antioxidant defenses, leads to lipid peroxidation, protein oxidation, and mitochondrial failure, accelerating neuronal death. Loss of cholinergic neurons reduces acetylcholine signaling in the hippocampus and neocortex, impairing memory and learning while further promoting β -amyloid accumulation and tau hyperphosphorylation [10]. Mitochondrial dysfunction compounds these effects by depleting synaptic energy supply and activating stress kinases that disrupt synaptic plasticity, ultimately resulting in the loss of glutamatergic synapses, the strongest structural correlate of cognitive decline in AD [11]. These mechanisms reinforce one another in a vicious cycle, and rodent models capture this complexity through behavioral, molecular, and histological endpoints, underscoring the need for multi-target therapeutic strategies in AD [12].

Furthermore, lipid peroxidation products, particularly malondialdehyde (MDA), are widely used indicators of oxidative membrane damage and are consistently elevated in AD brains and experimental models [13–15]. Conversely, endogenous antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) constitute the

primary enzymatic defense against superoxide radicals and hydrogen peroxide, respectively, and their reduced activity has been linked to heightened neuronal vulnerability in AD [16, 17]. In addition, heme oxygenase-1 (HO-1), an inducible stress-responsive enzyme regulated by redox signaling pathways, plays a critical role in cellular defense, mitochondrial protection, and neuroadaptive responses under oxidative conditions [18–20]. Dysregulation of these oxidative stress biomarkers reflects impaired redox homeostasis and contributes to hippocampal neurodegeneration and cognitive decline in AD [21].

The experimental animal models play a pivotal role in elucidating AD pathophysiology and evaluating potential therapeutic strategies. Among these, rat models offer a particularly robust and translationally relevant platform, given their well-characterized neuroanatomy, conserved cognitive and behavioral phenotypes, and superior feasibility for controlled, reproducible experimental manipulation [22]. Importantly, the combined use of D-galactose-induced accelerated aging and aluminum chloride ($AlCl_3$)-mediated neurotoxicity is widely employed as an oxidative stress-driven AD-like model. This paradigm reproduces several features associated with neurodegeneration, including oxidative stress, mitochondrial dysfunction, hippocampal neuronal injury, and cognitive impairment. However, it does not fully replicate the complex pathobiology of human AD, particularly progressive amyloid- β deposition, tau hyperphosphorylation, genetic risk factors, and the prolonged temporal evolution of the disorder. Consequently, findings derived from this model should be interpreted as reflecting neuroprotective effects in oxidative stress-associated neurodegeneration rather than definitive therapeutic effects against Alzheimer's disease itself [19, 23]. Meanwhile, behavioral paradigms such as the elevated plus maze (EPM) and T-maze are widely employed to assess anxiety-like behavior and hippocampal-dependent learning and memory, both of which are profoundly affected in AD [24].

Ficus deltoidea (FD), a medicinal plant native to Southeast Asia, has gained increasing attention for its neuroprotective potential, largely attributed to its high flavonoid content, including vitexin and isovitexin [25]. These bioactive compounds exhibit potent antioxidant and anti-inflammatory activities, effectively scavenging ROS and enhancing endogenous antioxidant defense systems such as SOD and glutathione peroxidase [26]. Polyphenolic constituents of FD further contribute to cellular protection by mitigating oxidative damage and preserving neuronal integrity [27]. Despite growing evidence supporting the antioxidant and neuroprotective properties of FD, its efficacy in experimental models combining D-galactose mediated aging and $AlCl_3$ -induced neurotoxicity remains insufficiently explored. In particular, the effects of FD on hippocampal neuronal viability,

ultrastructural integrity, and redox homeostasis in AD-like conditions have not been comprehensively investigated [28].

Therefore, this study hypothesized that FD confers neuroprotection by attenuating oxidative stress, enhancing endogenous antioxidant defenses, and preserving hippocampal neuronal and ultrastructural integrity. To test this hypothesis, the present work systematically evaluated the behavioral performance, hippocampal histopathology, ultrastructural alterations, and oxidative stress biomarkers in a D-galactose and AlCl_3 -induced oxidative stress-driven AD-like rat model. Elucidating these effects may provide valuable insights into the therapeutic potential of plant-derived compounds for neurodegenerative disorders characterized by cognitive decline [29].

Of note, a related study by Usman et al. [28], conducted by the same research group, investigated FD neuroprotection in a partially overlapping AD-like model system with a focus on behavioral outcomes, hippocampal histology, and amyloid pathology. The present study is substantively differentiated by three key elements: (i) systematic transmission electron microscopy (TEM) assessment of hippocampal neuronal ultrastructure, including mitochondrial integrity, myelin sheath organization, and smooth endoplasmic reticulum morphology; (ii) region-specific histomorphometric quantification of pyknotic neurons and pyramidal cell diameter across all four hippocampal subfields (CA1, CA2, CA3, and dentate gyrus), providing a more granular structural analysis; and (iii) comprehensive redox biomarker profiling inclusive of HO-1, which was not reported in the prior study. These complementary but non-overlapping analytical endpoints establish the novelty and independent scientific contribution of the present work.

Materials and Methods

Chemicals and Reagents

D-galactose and aluminum chloride (AlCl_3) were purchased from Evergreen Engineering and Resources (Selangor, Malaysia), while donepezil hydrochloride of European Pharmacopeia grade was purchased from Labchem Sdn. Bhd. (Selangor, Malaysia). Commercial ELISA kits for oxidative stress biomarkers were purchased from Prima Nexus Sdn. Bhd. (Selangor, Malaysia). All other chemicals and reagents used in this study were of analytical grade and prepared according to the manufacturer's instructions.

The standardized ethanol extract of *Ficus deltoidea* (FD) leaves was supplied by Platinum Herb Sdn. Bhd., a certified company specializing in standardized herbal extracts [28, 30]. The extract was standardized using vitexin and isovitexin as marker compounds [31], quantified by high-performance liquid chromatography (HPLC). Batch consistency

certification and quality control parameters, including moisture content, heavy metal screening, and microbial limits, were documented by the supplier. However, full in-house phytochemical validation was beyond the scope of the present study and is acknowledged as a limitation. Future investigations should incorporate detailed phytochemical profiling using validated chromatographic techniques such as HPLC, liquid chromatography-mass spectrometry (LC-MS), or gas chromatography-mass spectrometry (GC-MS) prior to in vivo testing.

Experimental Animals

Fifty-four (54) male Wistar rats (6 weeks old; 100–200 g) were obtained from Takrif Bistari Enterprise Sdn. Bhd. (Selangor, Malaysia). Animals were housed in polycarbonate cages under controlled environmental conditions ($25 \pm 2^\circ\text{C}$, 12 h light/dark cycle) at the Universiti Putra Malaysia (UPM) animal facility. Rats had ad libitum access to standard chow and water and were acclimatized for two weeks prior to experimentation. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (UPM/IACUC/AUP-R051/2022) and conducted in accordance with international guidelines for the ethical use of laboratory animals.

Experimental Design and Drug Administration

Rats were randomly assigned to six groups ($n=9$ per group) (Table 1). The sample size ($n=9$ animals per group) was selected based on previous studies employing similar D-galactose and AlCl_3 -induced neurodegeneration models and comparable behavioral, histological, and biochemical outcome measures. No formal a priori power analysis was performed. The selected group size was considered sufficient for exploratory evaluation of treatment effects while minimizing unnecessary animal use in accordance with the principles of reduction in animal experimentation.

Randomization was performed using a random number table to ensure allocation concealment. Behavioral assessments, histological evaluations, ultrastructural analyses, and biochemical measurements were conducted using coded samples, and investigators remained blinded to treatment allocation throughout data acquisition and analysis whenever feasible. Behavioral scoring during the EPM and T-maze tests, as well as histological analyses, were conducted by investigators who were blinded to treatment allocation. For biochemical assays, sample labeling and processing were managed by a designated team member who was not involved in analytical measurements, thereby maintaining blinding throughout the biochemical assessment pipeline.

D-galactose, AlCl_3 , donepezil, and FD extract were dissolved in distilled water. The selected FD doses (50, 100,

Table 1 Experimental groups, treatments, and routes of administration

Group	Experimental condition	Treatment regimen	Route of administration
Group 1 (Control)	Normal control	Normal saline (1 mL/kg)	i.p.
Group 2 (AD model)	AD-like model	D-gal (60 mg/kg) + AlCl ₃ (200 mg/kg)	i.p. (D-gal); oral (AlCl ₃)
Group 3 (donepezil)	Positive control	D-gal (60 mg/kg) + AlCl ₃ (200 mg/kg) + Donepezil (1 mg/kg)	i.p. (D-gal); oral (AlCl ₃ , Donepezil)
Group 4 (FD 50)	Low-dose FD	D-gal (60 mg/kg) + AlCl ₃ (200 mg/kg) + FD (50 mg/kg)	i.p. (D-gal); oral (AlCl ₃ , FD)
Group 5 (FD 100)	Medium-dose FD	D-gal (60 mg/kg) + AlCl ₃ (200 mg/kg) + FD (100 mg/kg)	i.p. (D-gal); oral (AlCl ₃ , FD)
Group 6 (FD 200)	High-dose FD	D-gal (60 mg/kg) + AlCl ₃ (200 mg/kg) + FD (200 mg/kg)	i.p. (D-gal); oral (AlCl ₃ , FD)

AlCl₃ aluminum chloride, AD Alzheimer's disease, dH₂O distilled water, D-gal D-galactose, FD *Ficus deltoidei*, i.p. intraperitoneal

and 200 mg/kg) were based on previously published in vivo studies demonstrating biological activity and neuroprotective efficacy of standardized FD extracts without evidence of overt toxicity [28, 30, 32]. These doses were chosen to represent a low-, intermediate-, and high-dose range for evaluating potential dose-dependent effects. The selection was therefore guided primarily by prior efficacy and safety observations reported in the literature rather than by dedicated pharmacokinetic, bioavailability, or dose-optimization studies. No formal pharmacokinetic analyses or maximum tolerated dose assessments were performed before the present study. All treatments were administered once daily in the morning for ten consecutive weeks. Behavioral assessments were conducted between weeks 9 and 10 and at least 2 hours after the daily treatment dose to minimize acute pharmacological confounds on behavioral performance. At study termination, the rats were euthanized by decapitation without prior anesthesia to prevent anesthetic-induced biochemical alterations in hippocampal oxidative stress markers and tissue morphology. This method is recognized as a valid euthanasia technique for neurochemical rodent studies [33]. The brains were rapidly excised, rinsed in ice-cold phosphate-buffered saline, and either fixed in 10% neutral buffered formalin (NBF) for histological analysis or dissected to isolate hippocampi, which were stored at -80 °C for biochemical assays. The overall study protocol is depicted in Fig. 1.

Neurobehavioral Assessments: Elevated Plus Maze (EPM) and T-Maze Tests

Behavioral testing was performed in a dedicated room under controlled lighting and noise conditions. Rats were acclimated to the testing environment for one hour prior to each assessment. Anxiety-like behavior and spatial working memory were evaluated using the EPM and T-maze, respectively. Behavioral scoring was conducted by investigators blinded to treatment allocation, and automated analyses were performed using ANY-maze software where applicable.

The EPM apparatus consisted of two open arms (50 × 10 cm) and two enclosed arms (50 × 10 × 40 cm) connected by a central platform (10 × 10 cm) and elevated 50 cm above the floor. Testing was conducted under dim red lighting (60 lx). Each rat was placed on the central platform, facing an open arm, and placed on the central platform facing an open arm for an initial 20-s orientation period, followed by 5 min (300 s) of free exploration. The primary measure was the cumulative time spent in enclosed arms, defined as the total duration (in seconds) across the entire 5-min session during which all four paws of the animal were within an enclosed arm [34], as tracked by ANY-maze software. This parameter was recorded independently for each testing day and was not averaged across sessions. The EPM was conducted prior to the T-maze, with a minimum 48-h inter-test interval between the two paradigms to minimize carry-over stress effects; however, this testing order is acknowledged as a potential source of behavioral sensitization. The apparatus was cleaned with 70% ethanol between trials.

The T-maze consisted of a start arm (50 × 16 cm) and two choice arms (50 × 10 cm) arranged in a T-configuration, equipped with guillotine doors and a central partition [35, 36]. Each session comprised a single sample phase followed by a choice phase, with one session conducted per testing day. During the sample phase, one choice arm was blocked, and the rat was confined to the other (designated) arm for 30 s. The rat was then returned to the start arm for a 30-s inter-trial interval before the guillotine doors were raised for the 2-min choice phase. Time spent in each arm was recorded to assess spontaneous alternation behavior and spatial working memory using ANY-maze software. Preferential exploration of the previously unvisited (novel) arm during the choice phase was used as the criterion for intact working memory function.

Histopathological Analysis of the Hippocampus

Following fixation in 10% NBF, brain tissues were processed, embedded in paraffin, and sectioned coronally at 6-μm thickness. Sections were deparaffinized, rehydrated,

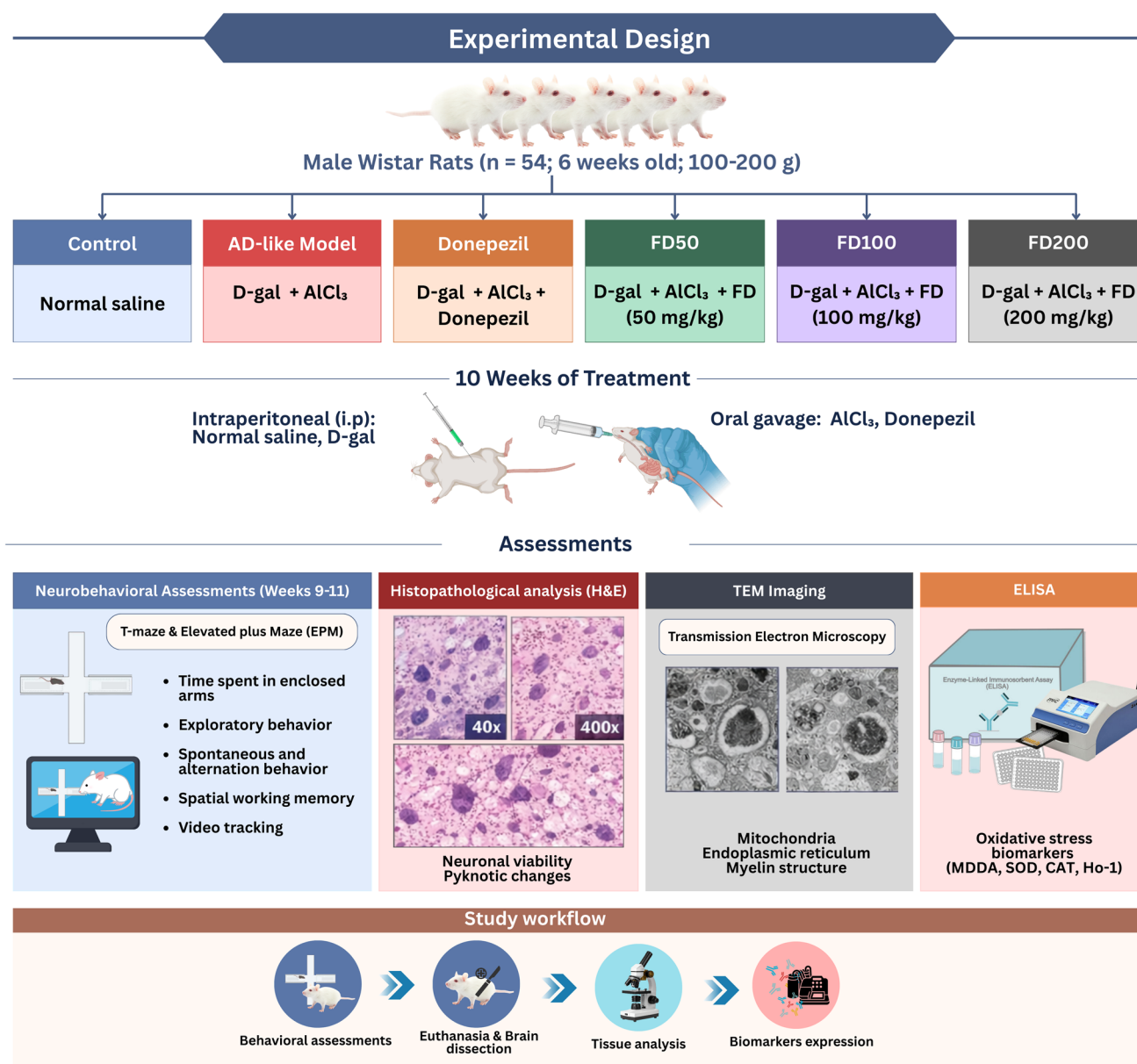


Fig. 1 Experimental design, treatment protocol, and outcome assessments in the D-galactose and aluminum chloride (AlCl₃)-induced oxidative stress-driven AD-like rats

and stained with H&E. Slides were examined under light microscopy to assess hippocampal cytoarchitecture. Hippocampal subregions (CA1, CA2, CA3, and dentate gyrus) were analyzed at 400 $\times$ magnification using a bright-field microscope (Olympus BX51). Neuronal viability, pyknotic changes, and pyramidal neuron diameter were evaluated using a semi-quantitative scoring system based on established criteria [37]. For histological and ultrastructural analyses, three rats per group were selected based on high-quality tissue preservation [38]. Rats with incomplete transcardial perfusion or visible tissue artefacts during processing were excluded [39]. Selection was performed by a

histologist blinded to group assignment. For each rat, three non-overlapping coronal sections were analyzed (giving a total of nine sections per group), consistent with published protocols for rodent hippocampal morphometry [40].

Ultrastructural Analysis of the Hippocampus

Small hippocampal tissue blocks ($\approx 1 \text{ mm}^3$) were fixed in 4% glutaraldehyde at 4 $^{\circ}\text{C}$ for 12 h, post-fixed in osmium tetroxide, dehydrated through graded acetone, and embedded in resin. Semi-thin sections were used to identify regions of interest, followed by preparation of ultrathin

sections stained with uranyl acetate and lead citrate. Ultrastructural features were examined using a transmission electron microscope (JEOL JEM 2100F FETEM) to assess mitochondrial integrity, myelin sheath organization, and smooth endoplasmic reticulum morphology [41]. Ultrastructural analyses were performed using coded specimens, and investigators conducting image acquisition and morphological evaluation were blinded to treatment allocation until completion of data interpretation.

For quantitative histomorphometric analysis, pyknotic neuron counts and neuronal diameter measurements were performed in the CA1, CA2, CA3, and dentate gyrus regions using standardized microscopic fields at 400 $\times$ magnification. Three non-overlapping fields were analyzed per section, and three coronal sections were evaluated per animal. Analyses were performed by investigators blinded to treatment allocation. Mean values obtained from all analyzed fields were used for statistical analysis.

Quantification of Oxidative Stress Biomarkers in the Hippocampus

Hippocampal tissue homogenates were analyzed for oxidative stress biomarkers (MDA, SOD, CAT, and HO-1) using sandwich ELISA kits according to manufacturers' protocols. Absorbance was measured at 450 nm using a microplate reader (VersaMax, USA), and concentrations were calculated from standard curves. Strict adherence to incubation times, washing steps, and control inclusion ensured assay reliability [42]. Although commercially validated ELISA kits were used, independent analytical validation of assay performance characteristics, including sensitivity, specificity, recovery, linearity, and inter-assay reproducibility, was not performed within the present study. Future investigations should incorporate comprehensive assay validation and reporting in accordance with current recommendations for biomarker research.

Statistical Analysis

Data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. For model induction confirmation via one-way ANOVA, the overall significance threshold was set at $p < 0.001$. For post-hoc pairwise comparisons using Tukey's test, conventional significance thresholds were applied: $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$. Analyses were conducted using GraphPad Prism version 9.3.1 (GraphPad Software, USA).

Results

Effects of *Ficus deltoidea* (FD) on Anxiety-Like Behavior in the EPM Test

Anxiety-like behavior was assessed using the EPM by quantifying the time spent in the enclosed arms across three testing days. One-way ANOVA revealed a significant effect of treatment on enclosed-arm duration on day 1 ($F(5, 48) = 5.1127$, $\eta^2 = 0.35$, $p < 0.001$). Compared with the AD-like rat model group, significantly longer enclosed-arm durations were observed in the donepezil group ($p < 0.001$) and all FD-treated groups (FD50: $p = 0.005$; FD100: $p = 0.025$; FD200: $p = 0.022$), as well as in the control group (Table 2 and Fig. 2A).

At 24 h post-testing (day 2), the duration of the enclosed arm remained significantly different across groups ($F(5, 48) = 6.259$, $\eta^2 = 0.39$, $p < 0.001$). Rats treated with donepezil and FD (particularly FD200) exhibited significantly increased enclosed-arm time compared with the AD-like rat model group (Table 2, Fig. 2B). Similarly, on day 7, one-way ANOVA demonstrated a robust group effect ($F(5, 48) = 8.027$, $\eta^2 = 0.46$, $p < 0.001$), with all FD-treated groups showing significantly greater enclosed-arm duration than the AD-like rat model group, approaching control and donepezil values (Fig. 2C).

Trajectory analysis obtained using ANY-maze software on day 7 revealed that the AD-like rat model exhibited prolonged, disorganized paths before entering the enclosed arms, whereas FD-treated and donepezil-treated rats displayed shorter, more direct trajectories similar to the control group (Fig. 2D).

Effects of *Ficus deltoidea* (FD) on Spatial Working Memory in the T-Maze Test

Spatial working memory was evaluated using the T-maze test by measuring the time spent in the left and right arms. One-way ANOVA revealed significant group differences in time spent in the left arm ($F(5, 48) = 6.360$, $\eta^2 = 0.40$, $p < 0.001$). Compared with the AD-like rat model group, significantly increased left-arm exploration was observed in control, donepezil, FD100, and FD200 groups, whereas FD50 did not differ significantly (Table 3 and Fig. 3A). Similarly, time spent in the right arm differed significantly across groups ($F(5, 48) = 7.048$, $\eta^2 = 0.42$, $p < 0.001$). The AD-like rat model group spent significantly less time in the right arm compared with control, donepezil, and FD-treated groups, with the most pronounced increases observed in the donepezil and FD200 groups (Fig. 3B).

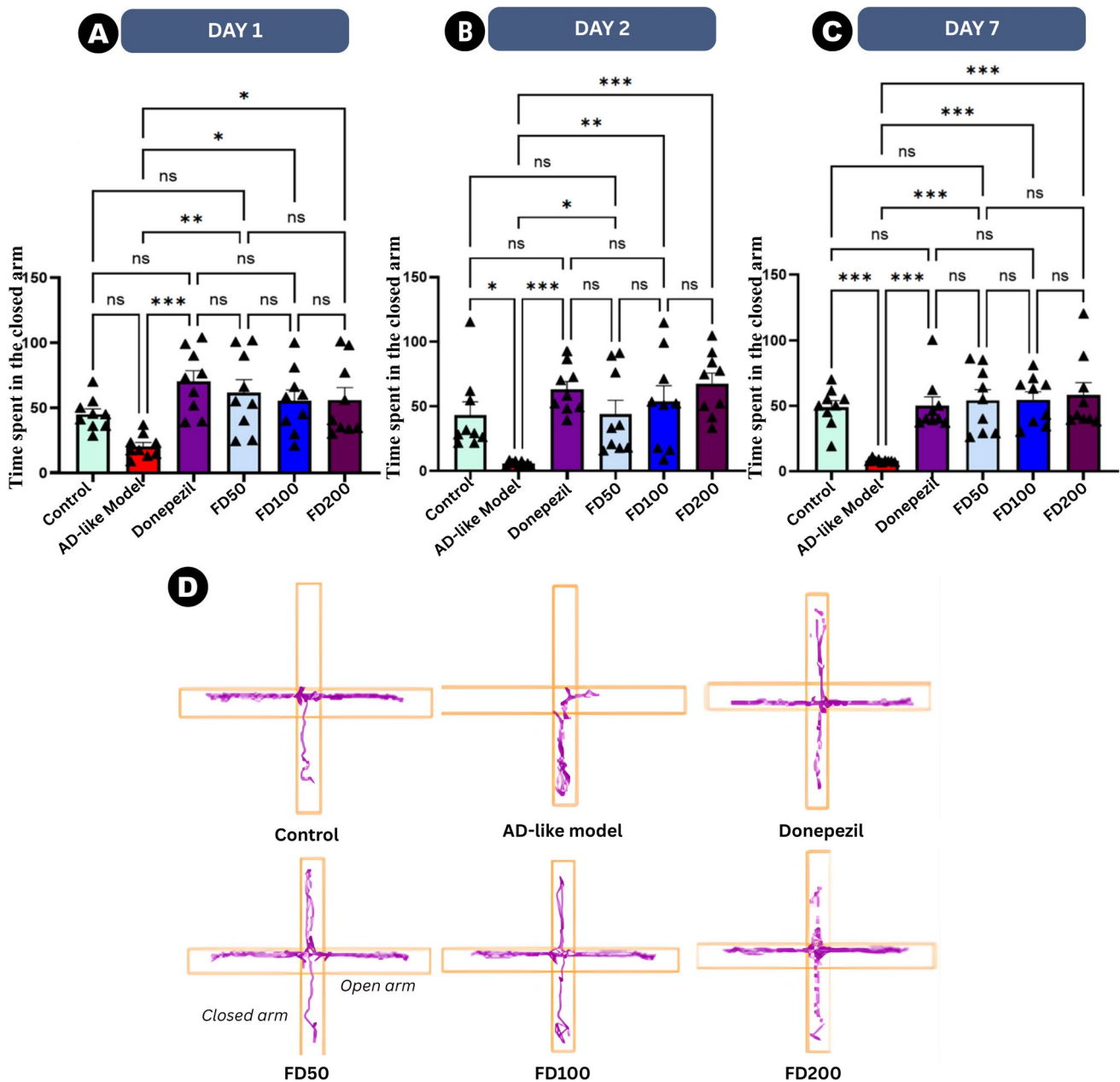


Fig. 2 *Ficus deltoidea* (FD) alleviates anxiety-like behavior in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. **A–C** Quantitative analysis of time spent in the closed arms on day 1, day 2, and day 7, respectively. A significant reduction in time spent in closed arms was observed in the AD-like rat model group compared to the control. Treatment with

FD (50, 100, and 200 mg/kg) dose-dependently restored this behavior, mirroring the effects of the positive control, donepezil. **D** Representative ANY-maze track plots from day 7. Data are presented as mean $\pm$ standard error of the mean (SEM) ($n=9$ per group). Statistical significance is indicated as $*p<0.05$, $**p<0.01$, and $***p<0.001$; “ns” denotes non-significance

ANY-maze trajectory plots demonstrated erratic and reduced arm exploration in the AD-like rat model group, whereas FD-treated rats exhibited structured exploration patterns resembling those observed in the control and donepezil-treated groups, indicating preserved spatial working memory (Fig. 3 C, D for left and right arms, respectively).

Effects of *Ficus deltoidea* (FD) on Hippocampal Histopathology

Cornu Ammonis-1 (CA1) Region

H&E staining of the CA1 subregion in control rats revealed a densely packed pyramidal cell layer with well-defined

Table 2 Time spent in enclosed arms (seconds) in D-galactose and AlCl_3 -induced AD-like rats

Group	Control	AD-like model	Donepezil (1 mg/kg)	FD (50 mg/kg)	FD (100 mg/kg)	FD (200 mg/kg)
Day 1 (acquisition)	45.24 ± 4.07	20.59 ± 2.96	70.54 ± 8.11	61.82 ± 10.03	55.69 ± 8.32	56.21 ± 9.53
Cohen's $d^\dagger$	2.03	—	3.12	2.55	2.16	2.20
p -value	0.224	—	< 0.001	0.005	0.025	0.022
Day 2 (24 h retention)	43.22 ± 10.1	20.59 ± 2.96	63.06 ± 6.11	43.98 ± 10.67	53.69 ± 12.26	67.58 ± 8.15
Cohen's $d^\dagger$	1.82	—	2.78	1.86	2.22	3.01
p -value	0.046	—	< 0.001	0.04	0.005	< 0.001
Day 7 (long-term retention)	49.19 ± 4.90	8.02 ± 0.61	50.17 ± 6.82	54.17 ± 8.13	54.69 ± 6.16	58.32 ± 9.45
Cohen's $d^\dagger$	4.21	—	4.36	4.74	4.82	5.13
p -value	< 0.001	—	< 0.001	< 0.001	< 0.001	< 0.001

Data are expressed as mean ± standard error of the mean (SEM) ($n=9$ per group). p vs. Alzheimer's disease (AD)-like rats' model
 AlCl_3 aluminum chloride

† Cohen's d was calculated relative to the AD-like rats' model. Effect size interpretation: 0.2 (small), 0.5 (moderate), and ≥ 0.8 (large)

Table 3 Time spent in left and right arms (seconds) in D-galactose and AlCl_3 -induced AD-like rats

Group	Left arm (s)	Cohen's $d^\dagger$	p -value	Right arm (s)	Cohen's $d^\dagger$	p -value
Control	11.30 ± 0.80	1.83	0.013	11.00 ± 1.36	2.02	0.009
AD-like model	5.09 ± 0.49	—	—	4.19 ± 0.35	—	—
Donepezil (1 mg/kg)	14.60 ± 2.10	2.41	< 0.001	14.20 ± 2.16	2.56	< 0.001
FD 50 mg/kg	9.64 ± 0.98	1.07	ns	14.20 ± 0.86	2.61	0.025
FD 100 mg/kg	11.50 ± 0.94	1.92	0.01	11.90 ± 1.18	2.08	0.002
FD 200 mg/kg	11.90 ± 1.55	1.85	0.005	12.20 ± 1.36	2.14	0.001

Data are expressed as mean ± standard error of the mean (SEM) ($n=9$ per group). p vs. Alzheimer's disease (AD)-like rats' model

AlCl_3 aluminum chloride, *ns* not significant

† Cohen's d was calculated relative to the AD-like rats' model. Effect size interpretation: 0.2 (small), 0.5 (moderate), and ≥ 0.8 (large)

neuronal morphology, characterized by large, round nuclei and evenly distributed cytoplasm (Fig. 4A). In contrast, the AD-like rat model group exhibited marked cytoarchitectural disruption in the CA1 region, including reduced neuronal density, increased numbers of pyknotic neurons, shrunken cell bodies, and loss of laminar organization (Fig. 4B). Quantitative analysis demonstrated a significant increase in pyknotic neuron counts in the CA1 region across experimental groups ($F(5, 12) = 10.09$, $\eta^2 = 0.81$, $p < 0.001$). The AD-like rat model group showed the highest number of pyknotic neurons (Fig. 5H), whereas FD treatment significantly reduced neuronal degeneration in a dose-dependent manner (Fig. 4D-F). Notably, the FD100 and FD200 groups exhibited substantial preservation of neuronal integrity, with pyknotic neuron counts approaching those observed in the control and donepezil-treated groups (Fig. 4H and I). Analysis of pyramidal neuron diameter further revealed significant group differences in the CA1 region ($F(5, 12) = 8.81$, $\eta^2 = 0.79$, $p = 0.001$). The AD-like rat model group demonstrated a pronounced reduction in neuronal diameter

(Fig. 4I), while FD treatment, particularly at 100 and 200 mg/kg, significantly preserved neuronal size and morphology (Fig. 4H and I).

Cornu Ammonis-2 (CA2) Region

In the CA2 subregion, control hippocampi exhibited compact pyramidal neurons with intact nuclei and minimal evidence of cellular degeneration (Fig. 5A). In contrast, the AD-like rat model group displayed moderate but consistent neuronal pathology, including increased pyknotic cells, irregular nuclear contours, and partial disorganization of the pyramidal layer (Fig. 5B). Statistical analysis revealed significant differences in pyknotic neuron counts among groups in the CA2 region ($F(5, 12) = 7.02$, $\eta^2 = 0.75$, $p = 0.001$). The AD-like rat model group showed significantly elevated pyknotic neuron counts compared with controls. FD administration resulted in a significant reduction in neuronal degeneration, with the FD100 and FD200 groups demonstrating greater preservation of pyramidal

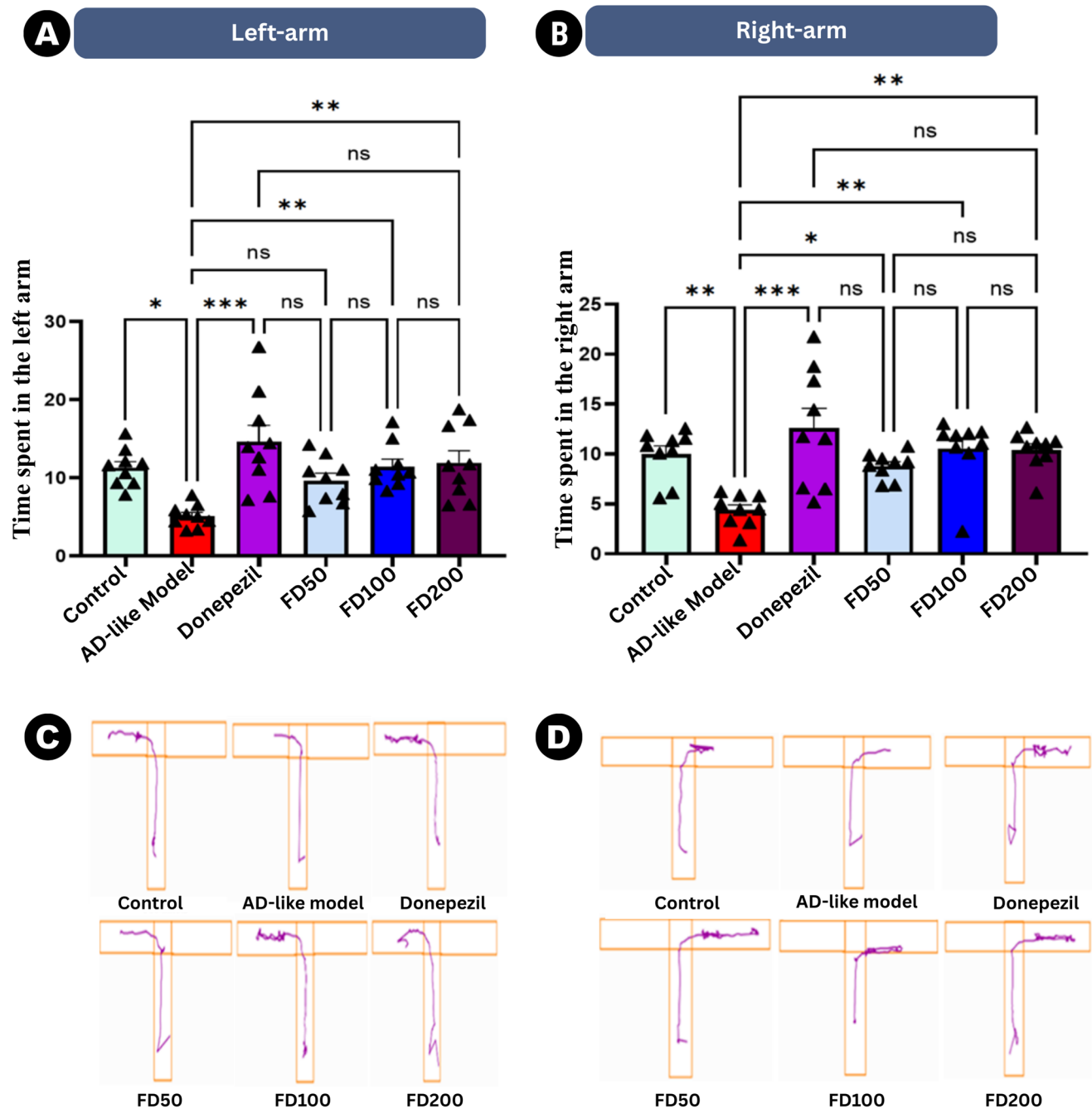


Fig. 3 *Ficus deltoidea* (FD) improves spatial working memory in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. **A, B** Quantitative analysis of time spent in the left and right arms, respectively. The AD-like rat model group exhibited a significant decrease in exploration time for both arms compared to the control, signaling impaired spatial memory. Treatment with FD (50, 100, and 200 mg/kg) or donepezil significantly

reversed these deficits, with FD showing dose-dependent improvements in arm exploration. **C, D** Representative ANY-maze track plots illustrating navigation patterns toward the left and right arms. Data are presented as mean $\pm$ standard error of the mean (SEM) ($n=9$ per group). Statistical significance is indicated as * $p<0.05$, ** $p<0.01$, and *** $p<0.001$; “ns” denotes non-significance

neurons compared with the FD50 (Fig. 5E, F). Similarly, pyramidal neuron diameter differed significantly across treatment groups in the CA2 region ($F(5, 12)=7.02$,

$\eta^2=0.75$, $p=0.003$). The reduction in neuronal diameter observed in the AD-like rat model group was attenuated by FD treatment in a dose-dependent manner, with higher

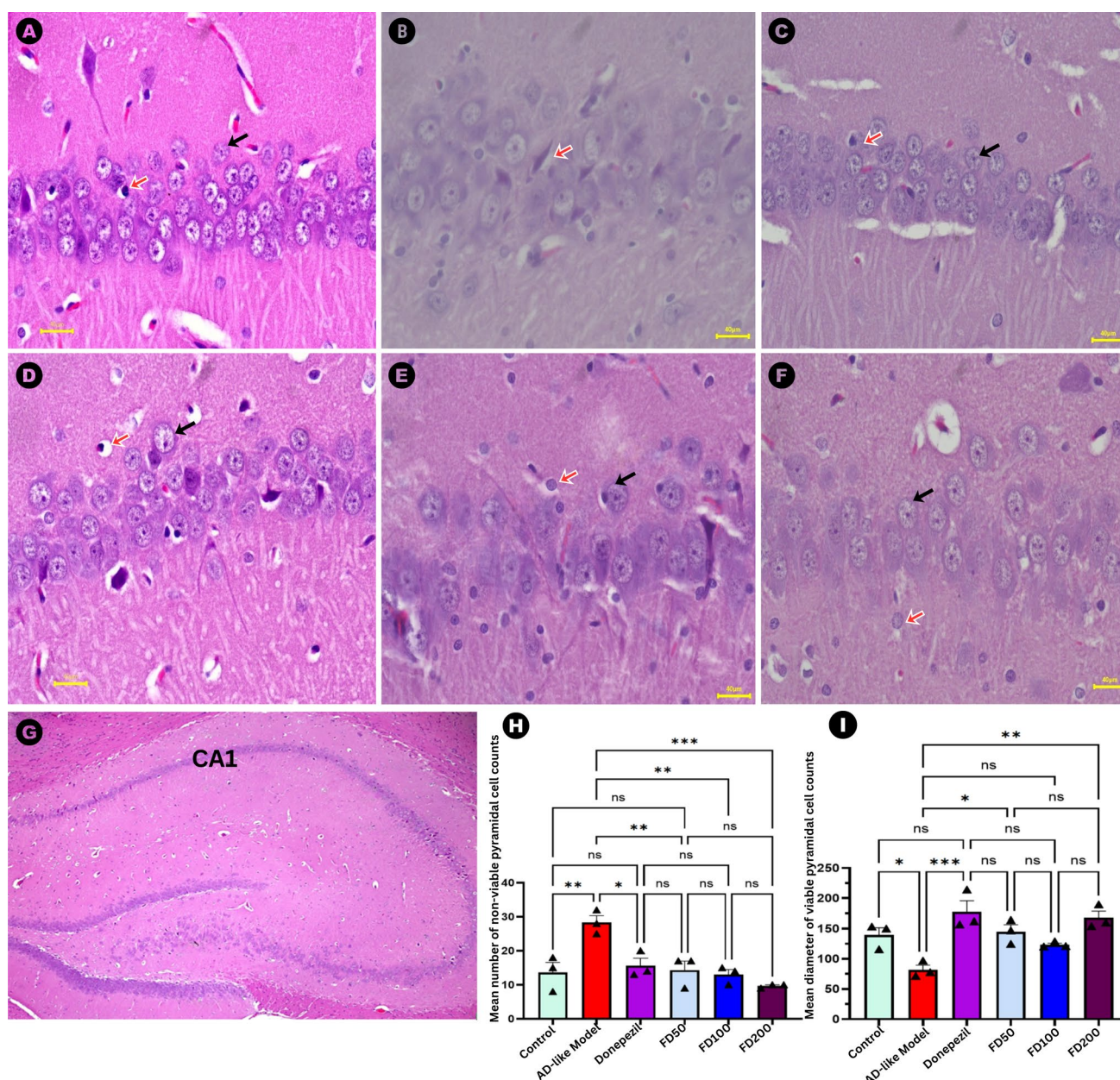


Fig. 4 Neuroprotective effects of FD on CA1 hippocampal pyramidal cells in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. **A–F** Representative H&E-stained photomicrographs (400 $\times$ magnification) of the hippocampal CA1 subfield. **A** Control group shows a densely packed layer of healthy pyramidal neurons with distinct nuclei and normal morphology (black arrows). **B** AD-like model group displays significant neurodegeneration characterized by extensive vacuolation, neuronal necrosis, and prominent pericellular spaces (red arrows) compared to the control. **C** Donepezil (1 mg/kg) shows a reduction in neuronal death and vacuolation, though some disorganization of the pyramidal layer persists. **D–F** FD-treated groups (50, 100, and 200 mg/kg): dose-dependent restoration of the pyramidal cell layer; FD 200; **F** The most robust neuroprotection, with abundant viable neurons and a near-absence of

neuropil vacuolation. **G** A low-magnification (40 $\times$) anatomical overview of the whole hippocampus, highlighting the subregion cornu ammonis (CA1) used for assessment. **H, I** Morphometric quantification of the CA1 subregion. **H** Mean number of non-viable pyramidal cell counts: the AD-like rat model group showed a significant increase in cell death, which was significantly attenuated by FD treatment, particularly at the 200 mg/kg dose. **I** Mean diameter of viable pyramidal cell counts: the reduction in cell size/viability observed in the AD-like model was reversed by donepezil and FD treatments. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n=3$). Statistical significance is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; “ns” indicates non-significance. Black arrows indicate viable pyramidal neurons; red arrows indicate non-viable/degenerate neurons. Scale bars = 40 μm

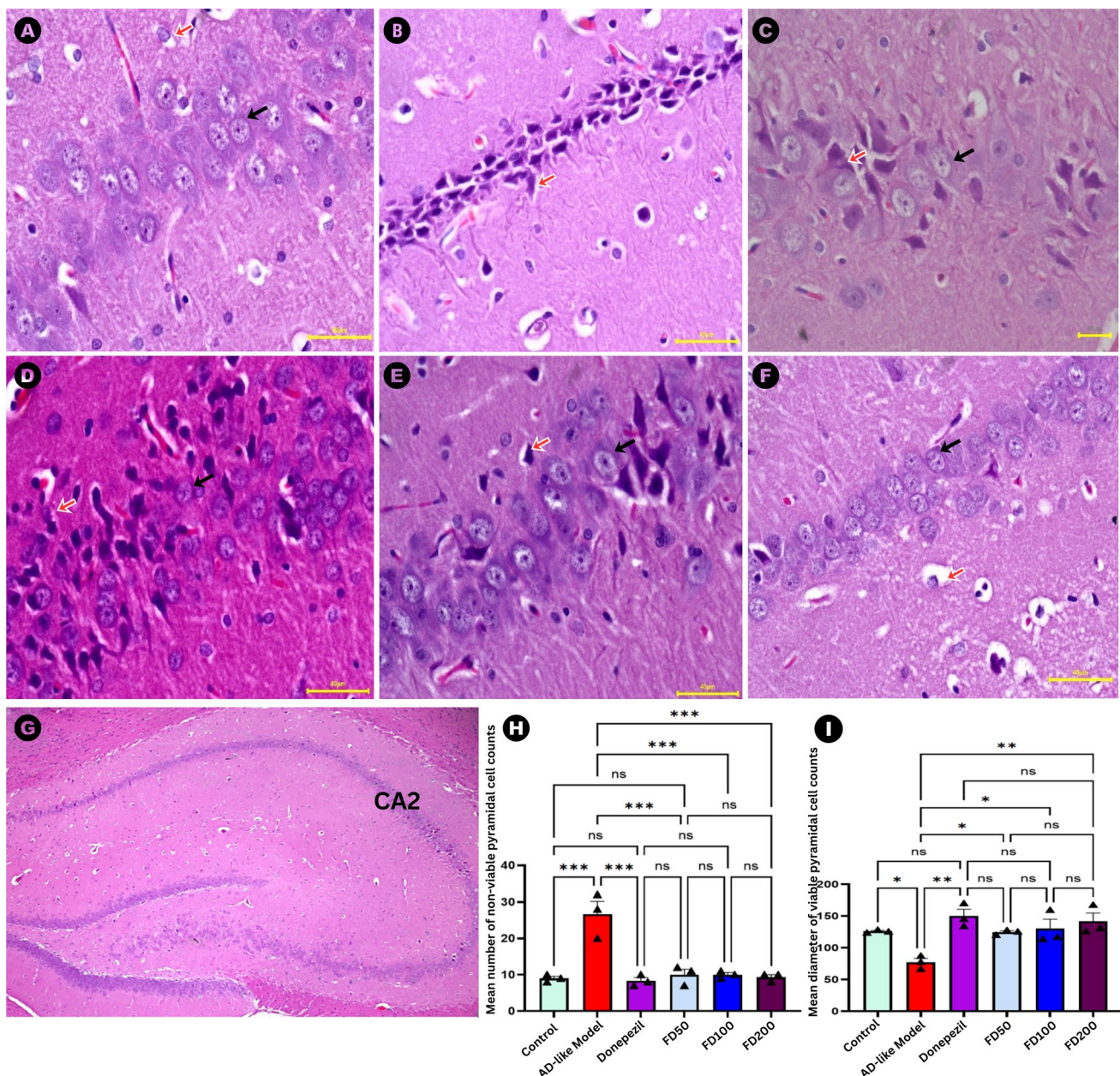


Fig. 5 Neuroprotective effects of FD on CA2 hippocampal pyramidal cells in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. **A–F** Representative H&E-stained photomicrographs (400 $\times$ magnification) of the hippocampal CA2 subfield. **A** Control group shows normal histological architecture with densely packed, viable pyramidal neurons (black arrows). **B** AD-like rat model group exhibits severe neurodegeneration characterized by necrosis, extensive vacuolation, and widened pericellular spaces (red arrows). **C** Donepezil (1 mg/kg) shows partial recovery with a reduction in necrotic neurons, though some misalignment of the pyramidal layer remains. **D–F** FD-treated groups (50, 100, and 200 mg/kg) demonstrate dose-dependent neuroprotection with numerous viable pyramidal neurons (black arrows) and a significant reduction in vacuolation and neuronal damage (red arrows) compared to the AD-like

rat model group. **G** A low-magnification (40 $\times$) anatomical overview of the whole hippocampus, highlighting the subregion cornu ammonis (CA2) used for assessment. **H, I** Morphometric quantification of the CA2 subregion. **H** Mean number of non-viable pyramidal cell counts: the significant increase in neuronal death in the AD-like rat model group and the restorative effect of FD across all doses. **I** Mean diameter of viable pyramidal cell counts: FD treatment significantly prevented the shrinkage of neuronal cell bodies observed in the AD-like rat model group. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n=3$). Statistical significance is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; “ns” indicates non-significance. Black arrows indicate viable pyramidal neurons; red arrows indicate non-viable/degenerate neurons. Scale bars = 40 μm

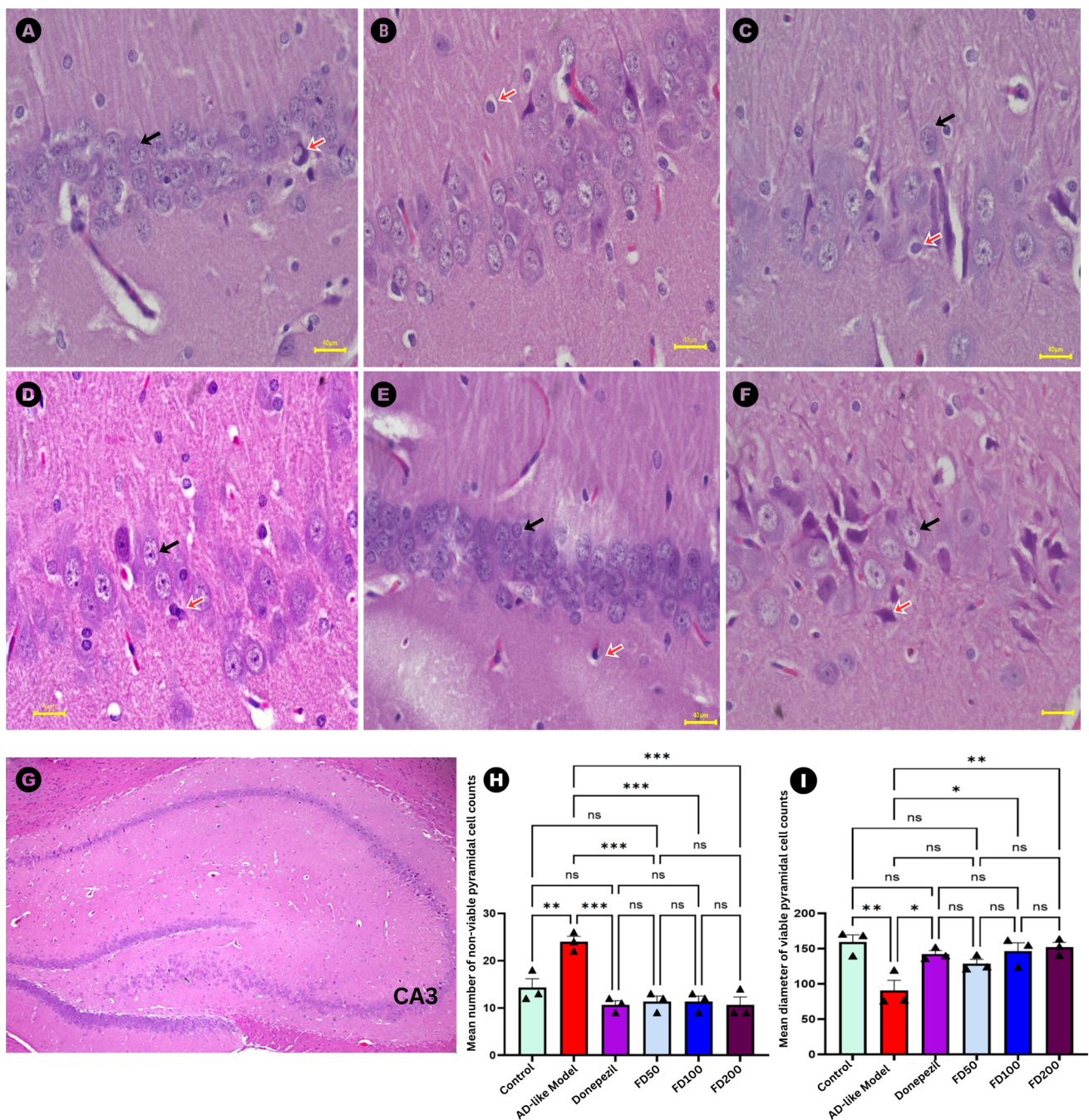


Fig. 6 Neuroprotective effects of FD on CA3 hippocampal pyramidal cells in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. **A–F** Representative H&E-stained photomicrographs (400 $\times$ magnification) of the hippocampal CA3 subfield. **A** Control group features healthy pyramidal neurons with distinct cellular boundaries and nuclei (black arrows). **B** AD-like rat model group demonstrates severe neurodegeneration characterized by necrosis, prominent vacuolations, and expanded pericellular spaces (red arrows). **C** Donepezil (1 mg/kg) shows partial restoration with reduced necrotic neurons, though some misalignment and decreased cell density remain visible. **D–F** FD-treated groups (50, 100, and 200 mg/kg) reveal a dose-dependent neuroprotective effect, evidenced by numerous regenerating pyramidal neurons and a marked absence of neuropil vacuolation compared to the AD-like rat model group. **G**

A low-magnification (40 $\times$) anatomical overview of the whole hippocampus, highlighting the subregion cornu ammonis (CA3) used for assessment. **H, I** Morphometric quantification of the CA3 subregion. **H** Mean number of non-viable pyramidal cells: the AD-like rat model group showed a significant increase in non-viable cells, which was significantly attenuated in all FD-treated groups. **I** Mean diameter of viable pyramidal cells: FD treatment successfully prevented the significant reduction in neuronal diameter (atrophy) observed in the AD-like rat model group. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n=3$). Statistical significance is indicated by asterisks: * $p<0.05$, ** $p<0.01$, and *** $p<0.001$; “ns” indicates non-significant differences. Black arrows indicate viable pyramidal neurons; red arrows indicate non-viable/degenerate neurons. Scale bars = 40 μm

FD doses restoring neuronal dimensions toward control levels (Fig. 5H–I).

Cornu Ammonis-3 (CA3) Region

The CA3 subregion of control rats displayed robust pyramidal neurons with prominent nuclei and intact cytoplasmic structure (Fig. 6A). In contrast, the AD-like rat model group exhibited pronounced neuronal degeneration in CA3, characterized by extensive pyknotic nuclei, reduced pyramidal neuron density, and disrupted cellular alignment (Fig. 6B). Quantitative assessment revealed a significant increase in pyknotic neuron counts in the CA3 region ($F(5, 12) = 11.61$, $\eta^2 = 0.83$, $p < 0.001$). FD treatment significantly mitigated neuronal degeneration, with the FD100 and FD200 groups demonstrating marked reductions in pyknotic neurons compared with the AD-like rat model group (Fig. 6E and F). Pyramidal neuron diameter analysis also showed significant group differences in CA3 ($F(5, 12) = 6.72$, $\eta^2 = 0.74$, $p = 0.003$). The AD-like rat model group exhibited significantly reduced neuronal diameter, whereas FD administration preserved neuronal morphology in a dose-dependent fashion, particularly at higher doses (Fig. 6H and I).

Dentate Gyrus (DG) Region

H&E staining of the DG in control rats revealed a well-organized granule cell layer with tightly packed neurons, intact nuclear membranes, and minimal evidence of cellular degeneration (Fig. 7A). Granule cells displayed uniform morphology with clearly defined nuclei, reflecting preserved hippocampal neuroarchitecture. In contrast, the DG of the AD-like rats exhibited marked histopathological alterations, including thinning of the granule cell layer, increased numbers of pyknotic granule neurons, nuclear condensation, and disruption of the normal laminar organization (Fig. 7B). Quantitative analysis demonstrated a significant increase in the number of pyknotic neurons in the DG across experimental groups ($F(5, 12) = 8.59$, $\eta^2 = 0.78$, $p = 0.001$). The AD-like rat model group exhibited the highest pyknotic neuron counts compared to the control group. FD treatment significantly attenuated DG neuronal degeneration in a dose-dependent manner, with the FD100 and FD200 groups showing marked reductions in pyknotic neuron counts, comparable to those observed in the donepezil-treated group (Fig. 7D–F). Assessment of granule neuron diameter further revealed significant inter-group differences in the DG ($F(5, 12) = 31.16$, $\eta^2 = 0.93$, $p < 0.001$). The AD-like rat model group demonstrated a pronounced reduction in granule neuron diameter, reflecting severe structural compromise. In contrast, FD administration, particularly at 100

and 200 mg/kg, significantly preserved neuronal size and cytoarchitecture, restoring granule cell dimensions toward control values (Fig. 7H and I).

Effects of *Ficus deltoidea* (FD) on Hippocampal Ultrastructure

TEM analysis revealed compact, tightly wrapped myelin sheaths in control rats (Fig. 8A). In contrast, the AD-like rat model group displayed marked myelin disruption, detachment, and bulging (Fig. 8B). FD treatment, particularly at 100 and 200 mg/kg, preserved myelin sheath compaction and reduced splitting, resembling control morphology (Fig. 8E, F). Moreover, control hippocampi exhibited mitochondria with intact double membranes and organized cristae (Fig. 8G). Meanwhile, the AD-like rat model group showed swollen mitochondria with fragmented cristae and electron-lucent matrices (Fig. 8H). FD200 treatment markedly preserved mitochondrial morphology, with near-normal cristae and membrane integrity (Fig. 8L). Finally, the SER appeared as an interconnected tubular network in control rats (Fig. 8M). However, the AD-like rat model group demonstrated fragmented and scattered SER structures (Fig. 8N). FD treatment, particularly at higher doses, restored SER organization and continuity (Fig. 8P–R).

Effects of *Ficus deltoidea* (FD) on Oxidative Stress Biomarkers

Hippocampal total SOD (T-SOD) activity differed significantly among groups ($F(5, 30) = 5.662$, $\eta^2 = 0.49$, $p < 0.001$), with the AD-like rat model group showing reduced activity and FD treatment significantly elevated T-SOD activity, with the highest increase observed in the FD200 group (Table 4 and Fig. 9A). Similarly, copper-zinc-SOD (CuZn-SOD) activity was significantly reduced in the AD-like rat model group ($F(5, 30) = 4.532$, $\eta^2 = 0.60$, $p = 0.003$), whilst FD treatment significantly increased CAT activity across all doses, with FD200 exhibiting the highest restoration (Table 4 and Fig. 9C). Besides, MDA levels were significantly elevated in the AD-like rat model group ($F(5, 30) = 10.66$, $\eta^2 = 0.64$, $p < 0.001$). Meanwhile, FD treatment significantly reduced MDA levels in a dose-dependent manner, indicating reduced lipid peroxidation (Table 4 and Fig. 9D). Finally, HO-1 levels differed significantly among groups ($F(5, 30) = 25.82$, $\eta^2 = 0.81$, $p < 0.001$), with marked suppression observed in the AD-like rat model group. However, FD treatment significantly upregulated HO-1 expression,

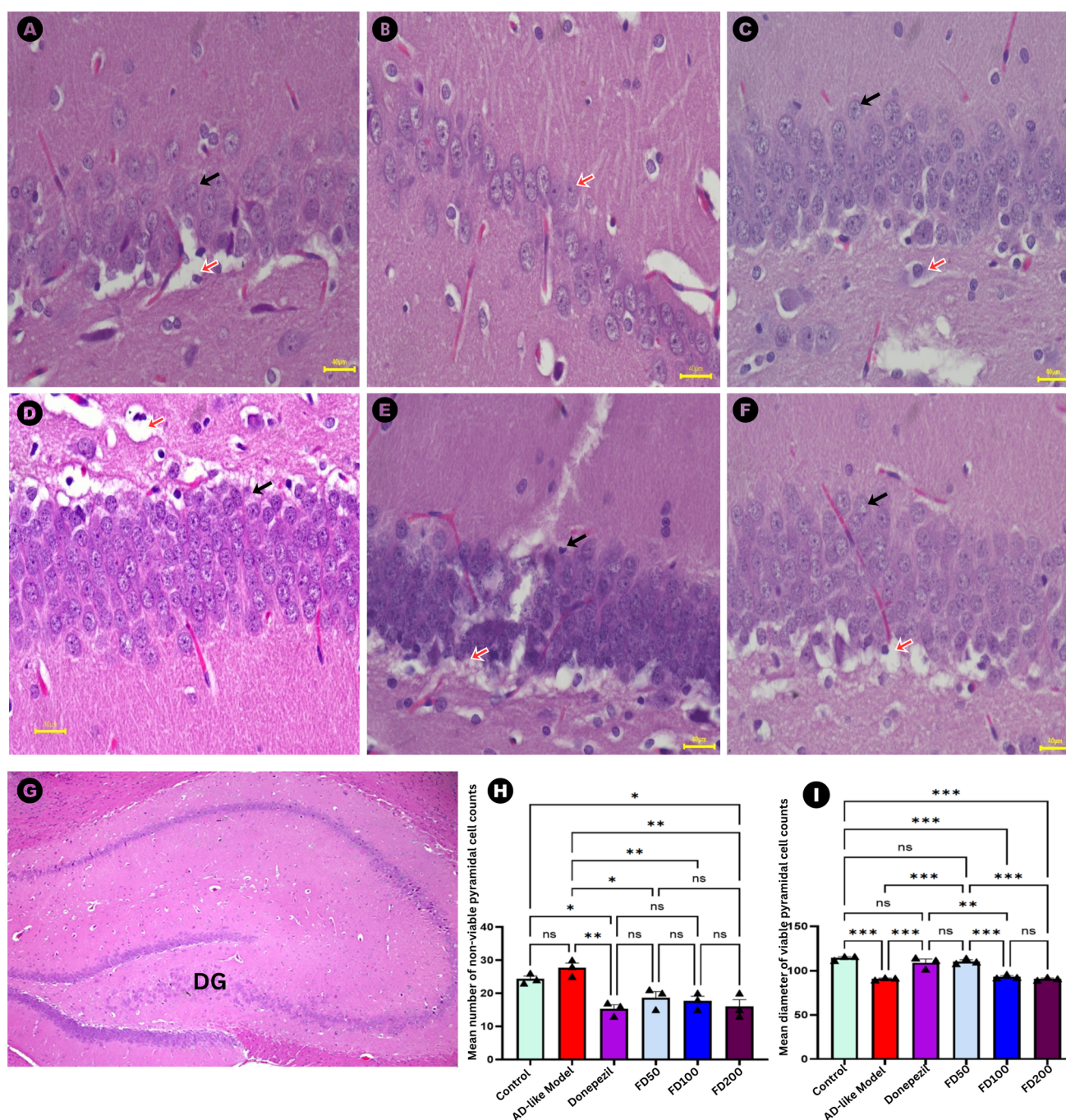


Fig. 7 Neuroprotective effects of FD on the DG hippocampal subfield in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. **A–G** Representative H&E-stained photomicrographs (400 $\times$ magnification) of the hippocampal DG subfield. **A** Control group shows normal histological architecture with healthy pyramidal neurons (black arrows). **B** AD-like rat model group exhibits severe neuronal damage, including vacuolations, necrosis, and wide pericellular spaces around non-viable neurons (red arrows). **C** Donepezil (1 mg/kg) displays a reduction in necrotic neurons and vacuolation compared to the AD-like rat model group, though some misalignment of the pyramidal layer remains. **D–F** FD-treated groups (50, 100, and 200 mg/kg) show dose-dependent recovery with numerous healthy pyramidal neurons (black arrows) and a significant reduction in vacuolation (red arrows) compared to the

AD-like rat model group. **G** A low-magnification (40 $\times$) anatomical overview of the whole hippocampus, highlighting the DG hippocampal subregion used for assessment. **H, I** Morphometric quantification of the DG subregion. **H** Mean number of non-viable pyramidal cell counts shows a significant increase in neuronal death in the AD-like rat model, which was mitigated by donepezil and FD treatments. **I** Mean diameter of viable pyramidal cell counts illustrates the preservation of neuronal size in treated groups compared to the shrinkage (atrophy) observed in the AD-like rat model group. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n=3$). Statistical significance is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; “ns” indicates non-significant differences. Black arrows indicate normal/viable pyramidal neurons; red arrows indicate neuronal damage/necrosis. Scale bars = 40 μm

particularly at 200 mg/kg, approaching control levels (Table 4 and Fig. 9E).

Discussion

The present study provides multi-level evidence that *Ficus deltoidea* (FD) confers significant neuroprotection in a D-galactose and AlCl_3 -induced oxidative stress-driven AD-like rat model. By integrating behavioral assessments, hippocampal histopathology, ultrastructural analyses, and oxidative stress profiling, this work demonstrates that FD mitigates anxiety-like behavior, preserves spatial working memory, maintains hippocampal cytoarchitecture, and restores antioxidant defense systems. Importantly, this study extends current knowledge by systematically linking FD-mediated behavioral recovery with region-specific hippocampal neuronal preservation and modulation of redox-sensitive biomarkers, thereby addressing critical gaps in FD-based neuroprotection research.

Anxiety-related symptoms often precede overt cognitive decline in AD and are increasingly recognized as early neuropsychiatric manifestations of disease progression [43]. Consistent with clinical and experimental observations, the AD-like rat model in this study exhibited disrupted anxiety-related behavior in the EPM [44, 45]. Specifically, the AD-like rat model group spent significantly greater time in the closed arms compared to the control group across days 1, 2, and 7 ($p < 0.001$), indicating an anxiety-like phenotype. Interestingly, administration of FD at doses of 50, 100, and 200 mg/kg led to a progressive, dose-dependent reduction in closed-arm time spent, with values gradually aligning with those of the donepezil reference group by day 7. Of note, the 200 mg/kg FD dose yielded a closed-arm duration of 58.32 ± 9.45 s, approximating the donepezil group's value of 62.15 ± 8.73 s and representing a meaningful improvement over the AD-like rat model group. These findings suggest that FD treatment normalized the time spent in enclosed arms and may have contributed to the restoration of adaptive anxiety responses rather than nonspecific alterations in locomotion. Although several FD-treated groups demonstrated outcome measures that approached those observed in the donepezil-treated group, the present study was not designed to determine therapeutic equivalence or non-inferiority between treatments. Formal equivalence analyses were not performed, and therefore the findings should not be interpreted as demonstrating that FD is equivalent to donepezil. Rather, the results indicate that FD produced substantial neuroprotective effects within the experimental model and warrant further comparative investigation.

This behavioral improvement aligns with previous reports demonstrating that plant-derived antioxidants

ameliorate anxiety-like behavior in the AD-like rat models by stabilizing hippocampal and limbic circuitry [19, 24, 46, 47]. Moreover, the directional trends seen in the EPM findings and the hippocampal structural outcomes lend further support to a consistent dose–response relationship across measures. The open field test and Morris water maze outcomes obtained from the same experimental model have been published separately elsewhere [30, 48] and are therefore not repeated in the present study. Nevertheless, because locomotor activity was not independently assessed within the present study, the observed differences in enclosed arm time could in part reflect alterations in general motor activity rather than purely anxiety-like behavior. Notably, no significant group differences in total arm entries were observed during the EPM, providing an indirect indication that locomotor capacity was not substantially impaired across groups; however, a partial contribution of motor-related factors to EPM metric variability cannot be ruled out.

Spatial working memory deficits assessed using the T-maze further corroborated the neuroprotective efficacy of FD. The AD-like rat model group displayed impaired decision-making and reduced arm exploration, reflecting hippocampal dysfunction, whereas FD-treated groups, particularly at 100 and 200 mg/kg, showed marked improvement comparable to the control and donepezil-treated animals. Given that T-maze performance depends heavily on hippocampal integrity [49], these findings strongly suggest that FD preserves hippocampal-dependent cognitive processing. This behavioral recovery parallels with earlier work demonstrating that flavonoid-rich botanicals enhance synaptic plasticity and memory by reducing oxidative stress and neuroinflammation [4, 50]. Although the Morris water maze test is considered a gold-standard paradigm for hippocampal-dependent spatial learning and memory, it was not included in the present study because the T-maze test offered lower stress burden and better practical compatibility with the treatment timeline. While the EPM and T-maze findings indicate significant improvements in anxiety-related behavior and hippocampal-dependent working memory, these paradigms evaluate only selected aspects of behavioral function. AD affects multiple cognitive domains, including spatial learning, reference memory, recognition memory, and executive processing, which were not comprehensively assessed in the present study. Therefore, the behavioral findings should be interpreted as evidence of improvement in specific functional domains rather than complete restoration of cognitive performance.

Furthermore, the histopathological evaluation revealed that FD robustly protected hippocampal neurons across CA1, CA2, CA3, and DG subregions. The AD-like rat model group exhibited hallmark features of neurodegeneration, including pyramidal neuron loss, pyknosis, and

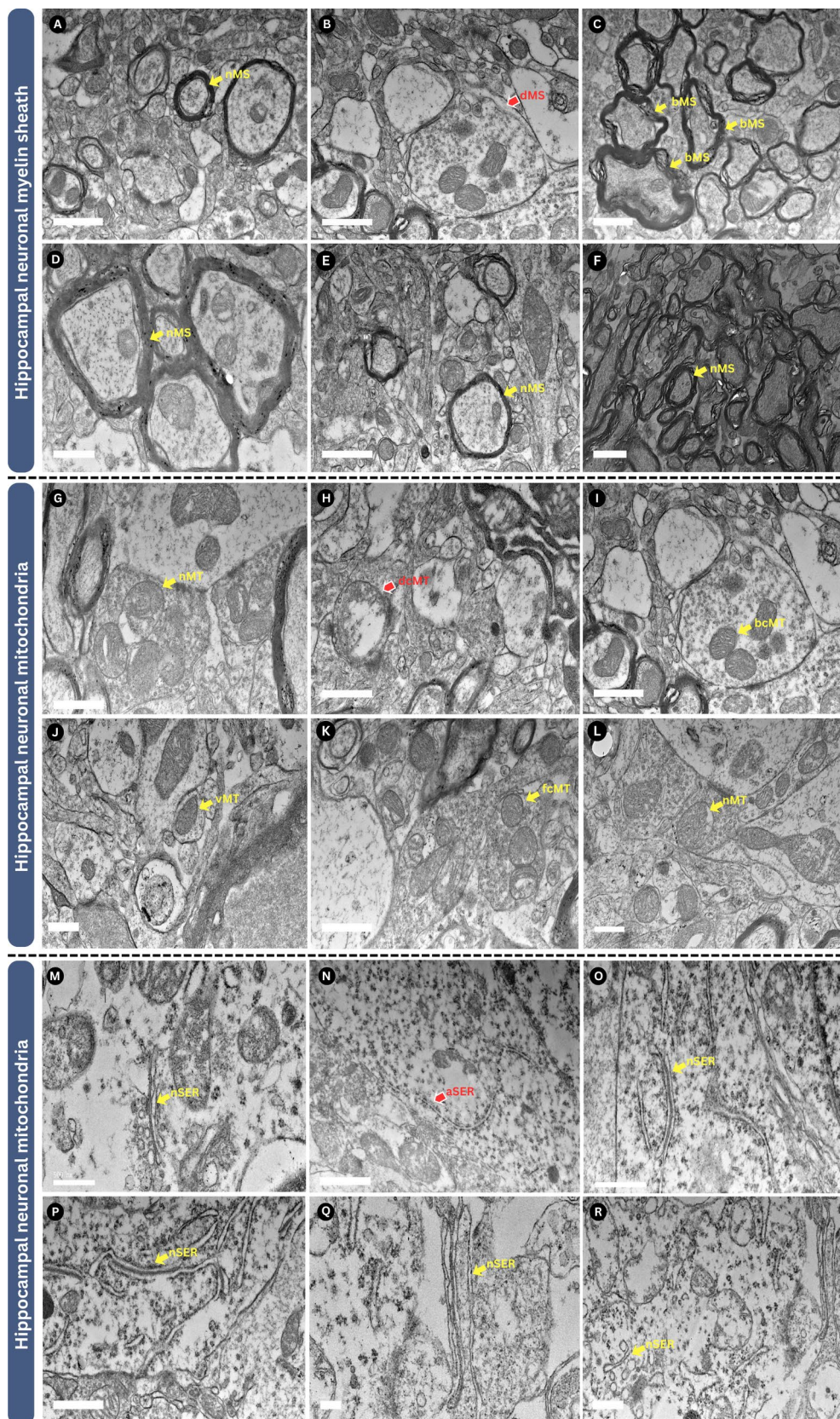


Fig. 8 Neuroprotective effects of FD on ultrastructural changes in hippocampal neuronal organelles in D-galactose and aluminum chloride (AlCl_3)-induced oxidative stress-driven AD-like rats. Representative transmission electron micrographs showing the ultrastructure of the myelin sheath, mitochondria, and smooth endoplasmic reticulum (SER) in the hippocampus. Groups: **A, G, M** control; **B, H, N** AD-like model; **C, I, O** donepezil (1 mg/kg); **D, J, P** FD50; **E, K, Q** FD100; **F, L, R** FD200. **A–F** Hippocampal neuronal myelin sheath: **A, D, F** The control, FD50, and FD200 groups displayed normal myelin sheaths (nMS), characterized by thick, electron-dense layers tightly wrapped around the axons. **B** The AD-like rat model group exhibited significant detached myelin sheaths (dMS), separating from the underlying axon. **C** The donepezil group showed “myelin balloons” (bMS), where the sheath bulges away from the axon despite areas of intact myelin. **E** The FD100 group revealed mild disruption of the myelin sheath with regions of normal attachment (scale bar: 0.5 μm). **G–L** Hippocampal neuronal mitochondria: **G, L** The control and FD200 groups showed normal mitochondria (nMT) with distinct, intact cristae. **H** The AD-like rat model group displayed degenerated cristae (dcMT) and mitochondrial swelling. **I** The donepezil group showed a mix of normal mitochondria and broken cristae (bcMT). **J** The FD50 group exhibited vacuolated mitochondria (vMT). **K** The FD100 group showed fragmented cristae (fcMT) within the mitochondria (scale bar: 1 μm). **M–R** Hippocampal neuronal SER: **M, O, P** The control, donepezil, and FD50 groups revealed normal SER (nSER) structures, smooth, narrow, and continuous cisternae. **N** The AD-like rat model group showed abnormal SER (aSER) with scattered, fragmented appearances. **Q, R** The FD100 and FD200 groups exhibited abundant and nearly normal SER (nSER) and healthy associated mitochondria (scale bar: **M–O**: 1 μm ; **P–R**: 0.5 μm)

disorganization of neuronal layers, particularly within CA1 and DG, regions known to be highly vulnerable in AD [51, 52]. However, FD treatment significantly reduced neuronal degeneration and preserved pyramidal neuron diameter in a dose-dependent manner. These structural improvements parallel the behavioral recovery observed in the EPM and T-maze, indicating that preservation of hippocampal morphology may underlie the functional benefits of FD. The present findings are consistent with reports on other neuroprotective botanicals, such as *Ginkgo biloba* and *Centella asiatica*, which preserve hippocampal structure by mitigating OS and apoptotic signaling [53, 54]. Notably, this study, to the best of our knowledge, is among the first to demonstrate FD-mediated protection across all major hippocampal subfields in a chemically induced AD-like model.

Additionally, TEM further substantiated the protective role of FD at the subcellular level, whereby in this study, D-galactose and AlCl_3 -induced AD-like rat model caused pronounced ultrastructural abnormalities, including mitochondrial cristae disruption, myelin sheath degeneration, and SER fragmentation, pathological features closely associated with synaptic failure and neuronal loss in AD [55, 56]. Meanwhile, FD treatment markedly preserved mitochondrial ultrastructural morphology (as evidenced by reduced mitochondrial swelling and improved cristae organization), myelin integrity, and SER organization, particularly at the higher doses. These ultrastructural improvements provide

direct mechanistic evidence strengthening the behavioral and histological findings by underscoring that FD’s capacity not only preserves tissue architecture but also stabilizes subcellular integrity relevant to neuronal homeostasis. Besides, mitochondrial preservation is particularly relevant in the context of AD, as mitochondrial dysfunction is a central driver to disease pathogenesis through impaired adenosine triphosphate production, calcium dysregulation, and excessive ROS generation [57]. It should be noted, however, that the present study assessed mitochondrial protection based on ultrastructural morphology alone. Functional markers such as mitochondrial membrane potential, respiratory chain activity, and ATP production were not measured. Accordingly, the TEM findings are presented as evidence of preserved ultrastructural integrity rather than confirmed functional neuroprotection, and mechanistic conclusions regarding mitochondrial bioenergetics remain inferential. While FD largely mitigated morphological mitochondrial damage, residual abnormalities at lower doses highlight the dose-dependence of the protective response. Future studies should incorporate functional mitochondrial assays to confirm the bioenergetic significance of these morphological observations.

At the biochemical level, FD significantly attenuated oxidative stress, as evidenced by reduced MDA levels and restoration of antioxidant enzymes, including CAT, T-SOD, CuZn-SOD, and HO-1. Elevated MDA in the AD-like rat model group reflects enhanced lipid peroxidation and neuronal membrane damage, which are well-established features of AD pathology [58]. FD-mediated suppression of MDA aligns with its high flavonoid and phenolic contents, previously shown to inhibit lipid peroxidation and protein oxidation [59, 60]. In parallel, a concurrent upregulation of SOD and CAT activities indicates reinforcement of endogenous antioxidant defenses, a critical factor in sustaining neuronal resilience under chronic oxidative stress.

Besides, the observed modulation of HO-1 further supports the possibility that FD engages adaptive redox signaling pathways. HO-1 is a stress-inducible enzyme regulated by the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway and plays a pivotal role in cellular defense against oxidative and inflammatory insults [61]. Under physiological conditions, the protein Kelch-like ECH-associated protein 1 (Keap1) negatively regulates and keeps Nrf2 trapped in the cytoplasm, marking it for degradation [62]. During oxidative stress, Nrf2 breaks free from Keap1, translocates into the nucleus, and switches on the transcription of antioxidant genes like *HO-1*, *NQO1*, and *GSTs*, thereby restoring redox balance and reducing ROS burden [63]. In contrast, nuclear factor kappa B (NF- κB) is triggered by inflammatory signals such as TNF- α , IL-1 β , and lipopolysaccharide. These activate I κB kinase (IKK) complexes, which phosphorylate and degrade I $\kappa\text{B}\alpha$, freeing the p65/p50 complex to enter the

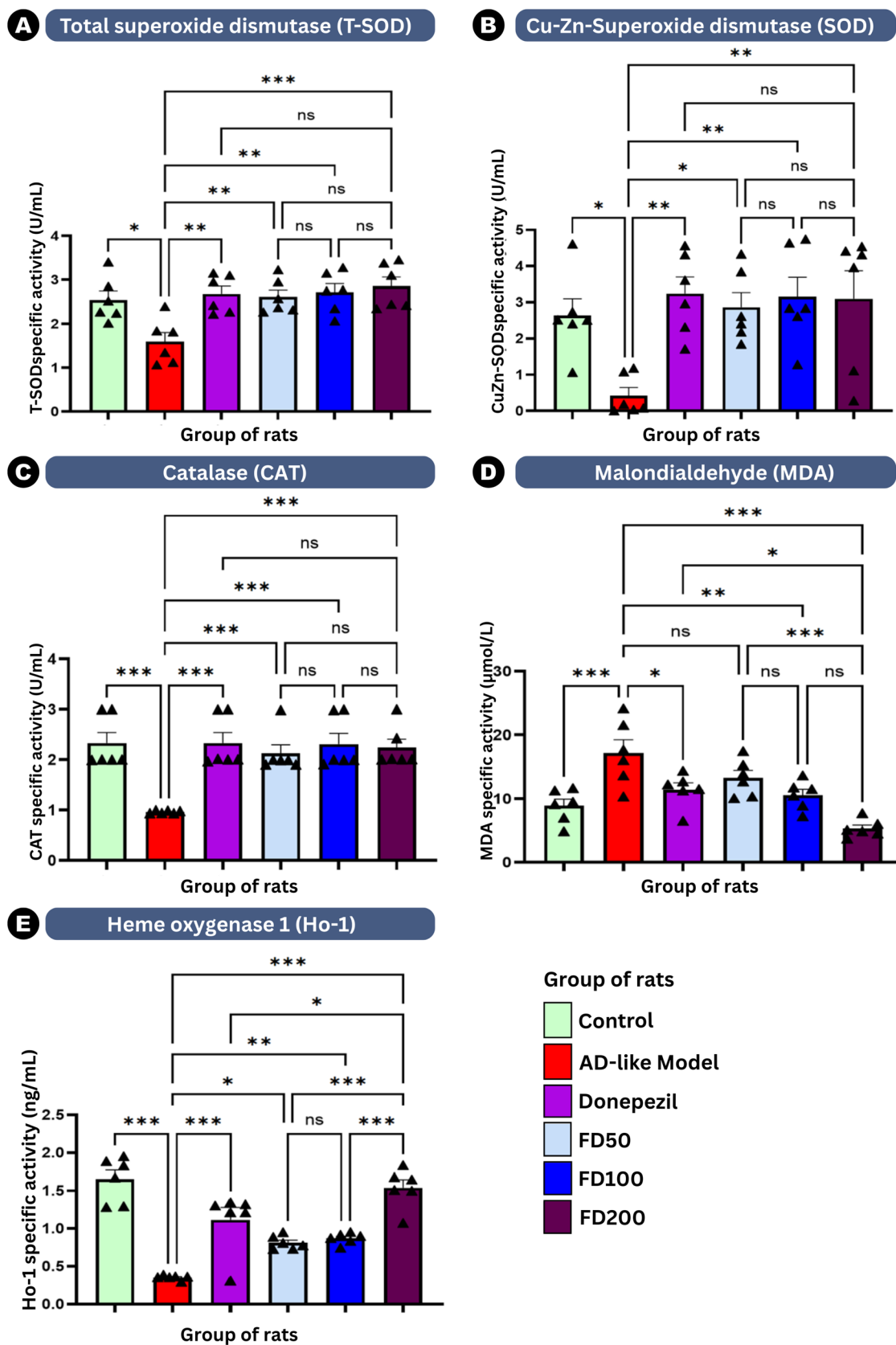


Fig. 9 Ameliorative effects of FD on oxidative stress markers in the hippocampus of D galactose and AlCl_3 -induced oxidative stress-driven AD-like rats. The panels illustrate the impact of varying doses of FD on antioxidant enzyme activities and lipid peroxidation: **A** T-SOD, **B** CuZn-SOD, **C** CAT, **D** MDA, and **E** HO-1. Antioxidant response: In the AD-like rat model group, a significant depletion of T-SOD, CuZn-SOD, CAT, and HO-1 was observed. Treatment with FD (50, 100, and 200 mg/kg) and donepezil significantly restored these antioxidant levels toward baseline. Lipid peroxidation: Elevated MDA levels in the AD-like rat model group were significantly reduced following administration of FD and donepezil, indicating a reduction in oxidative damage. Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n = 6$). Statistical significance is indicated by asterisks: * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$; “ns” denotes non-significance

nucleus and drive production of pro-inflammatory cytokines, chemokines, and adhesion molecules [64]. These two pathways work in functional opposition and are central to the regulation of oxidative stress and inflammation in neurodegenerative and chronic diseases [65].

In the present study, FD treatment enhanced HO-1 expression in a controlled manner, suggesting activation of protective antioxidant responses without exacerbating chronic stress signaling. This finding is consistent with emerging evidence suggesting that vitexin and related flavonoids may activate the Nrf2/Keap1 axis and thereby promote neuroprotection in AD-related contexts [66]. Although the observed restoration of HO-1, SOD, and CAT levels is compatible with activation of endogenous antioxidant defense systems, the present study did not directly assess upstream molecular regulators such as Nrf2, Keap1, NF- κ B, or MAPK signaling pathways. Consequently, the proposed mechanistic involvement of these pathways remains speculative and is based primarily on established literature describing flavonoid-mediated antioxidant responses. Future studies incorporating Western blotting, immunohistochemistry, quantitative PCR, and phosphoprotein analyses are required to determine whether FD directly modulates these signaling networks in the context of oxidative stress-driven neurodegeneration [67].

Taken together, the findings of this study indicate that FD exerts neuroprotective effects in a D-galactose and AlCl_3 -induced AD-like rat model that are associated with attenuation of oxidative stress, preservation of neuronal integrity, and improvement of behavioral outcomes. FD improved anxiety-like behavior and spatial working memory, preserved hippocampal neuronal architecture, reduced ultrastructural damage, and restored antioxidant defense mechanisms. These converging effects suggest that FD may protect against AD-like pathology, at least in part, by reducing oxidative stress and preserving neuronal integrity at both tissue and subcellular levels. Figure 10 schematically summarizes the proposed multitarget neuroprotective mechanisms of FD in a D-galactose and AlCl_3 -induced oxidative

stress-driven AD-like rat model, integrating behavioral, hippocampal structural, ultrastructural, and redox-related outcomes [68]. Although the present findings demonstrate significant neuroprotective effects of FD in a D-galactose and AlCl_3 -induced AD-like rat model, caution is warranted when considering their translational implications. The study was conducted in a single experimental model and did not evaluate critical translational parameters, including pharmacokinetics, bioavailability, tissue distribution, target engagement, chronic toxicity, or long-term efficacy. Furthermore, responses observed in rodent models may not fully predict therapeutic outcomes in humans because of species-specific differences in metabolism, disease progression, and treatment responsiveness. Consequently, the present findings should be regarded as preliminary preclinical evidence supporting further investigation rather than definitive proof of therapeutic utility in Alzheimer's disease.

Study Significance, Limitations, and Future Directions

The significance of this study lies in its integrated, region-specific, and multi-scale evaluation of FD neuroprotection in a D-galactose and AlCl_3 -induced oxidative stress-driven AD-like rat model. Unlike prior studies focusing primarily on behavioral or biochemical endpoints, the present work establishes a direct link between FD-mediated redox modulation with preservation of hippocampal subfields (including DG), maintenance of ultrastructural integrity, and recovery in functional cognitive. This comprehensive approach strengthens the translational relevance of FD as a multitarget botanical candidate for AD intervention.

Despite these strengths, several limitations warrant consideration. First, tau phosphorylation and amyloid- β burden were not directly assessed, which limits mechanistic insight into the effects of FD on the core AD pathological hallmarks. Second, although FD treatment significantly modulated HO-1 and other oxidative stress biomarkers, the upstream signaling pathways underlying the observed antioxidant effects were not directly investigated. In particular, key molecular regulators such as Nrf2/Keap1, NF- κ B, MAPK, and mitochondrial respiratory function, were not quantified. Therefore, the proposed involvement of redox-sensitive, neuroinflammatory, and mitochondrial pathways remains inferential and requires direct molecular confirmation in future studies through targeted molecular analyses such as Western blotting, immunohistochemistry, gene expression profiling, and mitochondrial functional assay.

Methodological limitations should also be acknowledged. The FD preparation used in this study was commercially standardized using vitexin and isovitexin as marker compounds; however, it was not subjected to independent in-house phytochemical analysis using validated analytical

Table 4 Effects of *Ficus deltoidea* (FD) on hippocampal oxidative stress and antioxidant markers in D-gal/ AlCl_3 -induced AD-like rats

Biomarker	Group	Mean $\pm$ SEM	<i>p</i> -value	Cohen's <i>d</i> †
T-SOD (U/mg protein)	Control	2.55 $\pm$ 0.206	0.016	1.60
	AD-like model	1.60 $\pm$ 0.209	—	—
	Donepezil	2.68 $\pm$ 0.175	0.005	2.40
	FD 50	2.62 $\pm$ 0.159	0.009	2.09
	FD 100	2.73 $\pm$ 0.189	0.003	2.42
	FD 200	2.86 $\pm$ 0.209	< 0.001	2.15
CuZn-SOD (U/mg protein)	Control	2.64 $\pm$ 0.464	0.044	3.41
	AD-like model	0.423 $\pm$ 0.224	—	—
	Donepezil	3.24 $\pm$ 0.459	0.005	4.42
	FD 50	2.87 $\pm$ 0.404	0.020	3.39
	FD 100	3.15 $\pm$ 0.540	0.007	4.00
	FD 200	3.10 $\pm$ 0.773	0.009	3.92
Catalase (CAT) (U/mg protein)	Control	2.00 $\pm$ 0.002	0.007	2.32
	AD-like	1.48 $\pm$ 0.222	—	—
	Donepezil model	2.00 $\pm$ 0.007	0.007	2.27
	FD 50	1.99 $\pm$ 0.004	0.007	2.25
	FD 100	2.00 $\pm$ 0.002	0.007	2.27
	FD 200	2.08 $\pm$ 0.070	0.001	1.19
MDA (nmol/mg protein)	Control	10.1 $\pm$ 1.62	0.014	−3.69
	AD-like model	17.2 $\pm$ 2.07	—	—
	Donepezil	11.4 $\pm$ 1.08	ns	−2.90
	FD 50	13.3 $\pm$ 1.17	ns	−1.86
	FD 100	10.1 $\pm$ 1.50	< 0.05	−2.69
	FD 200	4.50 $\pm$ 1.50	< 0.05	−1.74
HO-1 (relative units)	Control	1.65 $\pm$ 0.121	< 0.001	9.88
	AD-like model	0.351 $\pm$ 0.013	—	—
	Donepezil	1.12 $\pm$ 0.163	< 0.001	7.15
	FD 50	0.811 $\pm$ 0.037	0.022	4.08
	FD 100	0.869 $\pm$ 0.030	0.007	4.83
	FD 200	1.54 $\pm$ 0.106	< 0.001	12.1

Data are expressed as mean $\pm$ standard error of the mean (SEM) ($n = 9$ per group). *p* vs. Alzheimer's disease (AD)-like rats' model. †Cohen's *d* was calculated relative to the AD-like rats' model. Effect size interpretation: 0.2 (small), 0.5 (moderate), ≥ 0.8 (large). † Negative Cohen's *d* indicates therapeutic reduction in oxidative damage. *AlCl3*, aluminum chloride

methods such as HPLC, LC–MS, or GC–MS. Consequently, the relative abundance of individual bioactive constituents within the administered extract could not be independently verified, and direct correlations between specific phytochemicals and the observed dose-dependent neuroprotective effects cannot be established. Rigorous phytochemical profiling is important to identify major bioactive constituents, enable consistent dosing standardization, and support reproducibility across different laboratories and preparation batches. Future investigations should therefore incorporate comprehensive phytochemical characterization using validated analytical platforms to improve reproducibility, facilitate batch-to-batch comparisons, and strengthen mechanistic interpretation of bioactive constituent-specific effects.

The behavioral assessment was limited to the EPM and T-maze paradigms, which primarily evaluate anxiety-related

behavior and spatial working memory. Although these tests revealed significant functional improvements, they do not encompass the full spectrum of cognitive deficits associated with Alzheimer's disease. In the behavioral domain, although the EPM and T-maze tests indicated meaningful improvement, locomotor activity was not assessed. Because the EPM was performed prior to the T-maze, with a 48-h interval between tasks, the possibility of order-related influences on arousal state or cognitive strategy in subsequent testing cannot be fully excluded. Future investigations should incorporate a dedicated locomotor assay, such as the open field test, and counterbalance behavioral task order across cohorts to minimize potential confounding effects. Additional behavioral paradigms, including the Morris water maze, novel object recognition test, Y-maze spontaneous alternation task, and other measures of learning and

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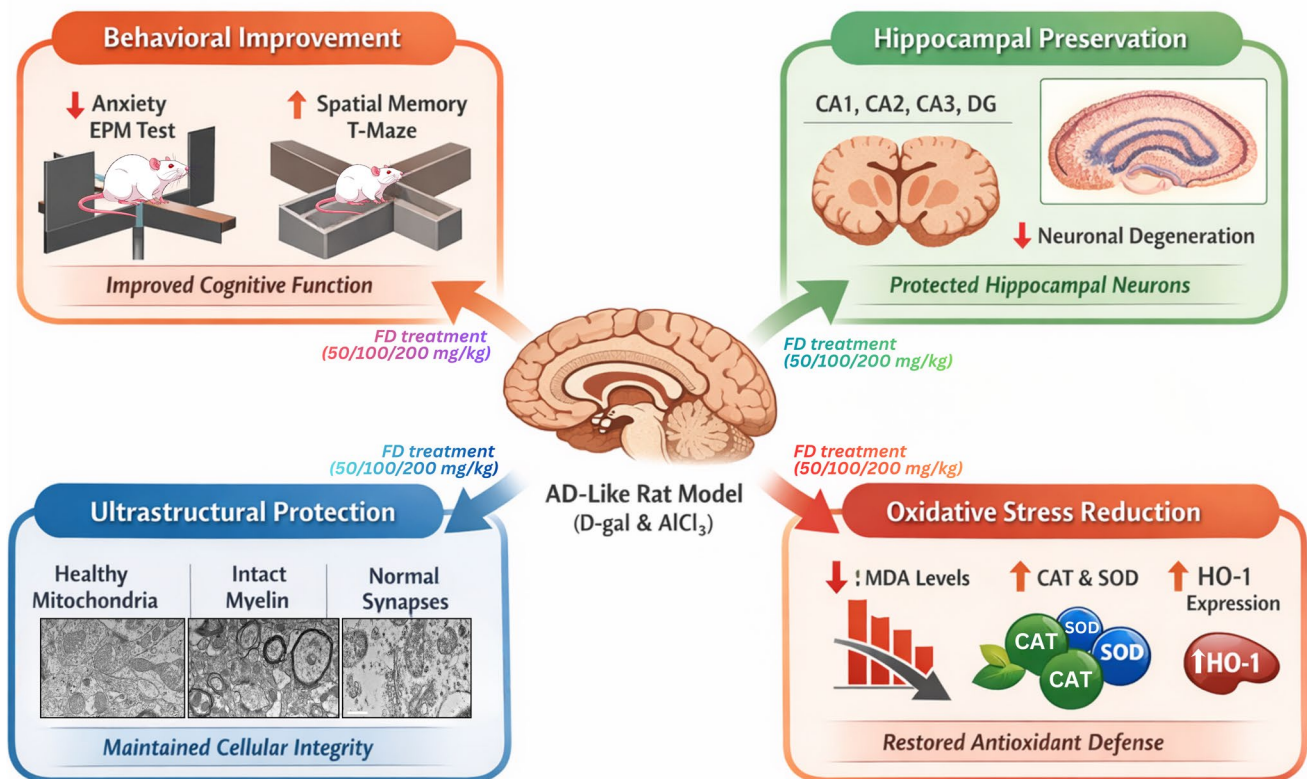


Fig. 10 Proposed neuroprotective actions of *Ficus deltoidea* (FD) in D-galactose and aluminum chloride (AlCl₃)-induced oxidative stress-driven AD-like rats. FD treatment ameliorates anxiety-like behavior (EPM) and restores spatial working memory (T-maze), reflecting improved hippocampal-dependent cognitive function. At the structural level, FD preserves hippocampal cytoarchitecture across cornu ammonis (CA1, CA2, and CA3), and dentate gyrus (DG) subfields by reducing neuronal degeneration and maintaining pyramidal neuron integrity. Ultrastructurally, FD protects neuronal homeostasis by

preserving mitochondrial morphology, myelin sheath integrity, and smooth endoplasmic reticulum (SER) organization. Biochemically, FD attenuates oxidative stress through suppression of lipid peroxidation ($\downarrow$ MDA) and reinforcement of endogenous antioxidant defenses ($\uparrow$ CAT, T-SOD, CuZn-SOD, and HO-1), likely involving redox-sensitive adaptive pathways. Collectively, these convergent effects highlight FD's multitarget mode of action linking redox modulation to hippocampal preservation and functional behavioral recovery in AD-like neurodegeneration

memory, would provide a more comprehensive evaluation of cognitive function and strengthen the translational relevance of the findings. The absence of Morris water maze data is recognized as a limitation, and inclusion of this paradigm in future work would enable a more robust assessment of spatial learning and reference memory. Overall, future studies should incorporate a broader behavioral test battery to validate and extend the cognitive benefits observed in the present work.

Although quantitative histomorphometric analyses of pyknotic neuron counts and neuronal diameter were performed, the ultrastructural assessment was primarily qualitative in nature. Quantitative stereological approaches, neuronal density measurements, mitochondrial morphometric analyses, organelle volume assessments, and validated ultrastructural scoring systems were not incorporated.

Consequently, the TEM findings should be interpreted as supportive morphological evidence rather than definitive quantitative measures of subcellular preservation. Future investigations should integrate unbiased stereological methods and quantitative ultrastructural analyses to further strengthen the structural characterization of FD-mediated neuroprotection. The mitochondrial findings should likewise be interpreted with caution. Although ultrastructural analyses demonstrated preservation of mitochondrial morphology, no corresponding functional validation measures, such as membrane potential, respiratory chain activity, or ATP production, were performed. The integration of functional assays is necessary to determine whether the observed structural preservation reflects genuine restoration of bioenergetics capacity and synaptic performance. Furthermore, the histological analysis was conducted using three rats per

group, with three sections analyzed per rat, yielding a total of nine sections per group in total. Although this approach is consistent with selected published protocols, expansion to larger cohorts, adoption of unbiased stereological methods, and inclusion of multilevel regional sampling would substantially enhance measurement precision and strengthen the external validity of structural findings.

The D-galactose and AlCl_3 -induced model is widely used as an experimental platform for investigating oxidative stress-related neurodegeneration and cognitive impairment, however, it does not fully recapitulate the complex pathobiology of human AD. The model reproduces several clinically relevant features, including hippocampal neuronal degeneration, mitochondrial dysfunction, oxidative stress, behavioral deficits, and age-related cognitive decline. Nevertheless, it lacks the progressive and multifactorial nature of human AD, including genetically driven susceptibility, sustained A β accumulation, neurofibrillary tau pathology, chronic neuroinflammatory progression, and the prolonged temporal evolution of disease. Therefore, the present findings should be interpreted as evidence supporting the neuroprotective effects of FD in an oxidative stress-driven AD-like model rather than definitive disease-modifying activity against Alzheimer's disease itself.

In addition, the present study did not investigate the long-term safety profile, tolerability, and potential off-target effects of chronic FD administration. Comprehensive dose-ranging toxicology studies, pharmacokinetic profiling, and drug interaction assessments are prerequisite steps before any consideration of clinical translation can be responsibly undertaken. Although a dose-dependent neuroprotective trend was observed, the present study was not designed to establish the optimal therapeutic dose or therapeutic window of FD. The highest tested dose (200 mg/kg) produced the greatest overall benefit among the evaluated groups; however, this should not be interpreted as evidence of dose optimality. Future investigations should incorporate detailed dose-escalation studies, pharmacokinetic analyses, bioavailability assessments, and long-term safety evaluations to define the minimum effective dose, maximum tolerated dose, and optimal therapeutic window.

Future studies should incorporate transgenic AD models, directly assess tau and amyloid pathology, conduct targeted analyses of mitochondrial bioenergetics and redox signaling pathways, and perform comprehensive pharmacokinetic profiling and long-term safety evaluations to define optimal dosing strategies and translational potential. Validation in transgenic and other mechanistically representative AD models will be essential to determine the broader translational relevance of these findings.

Finally, the present study exclusively used male Wistar rats to minimize hormonal variability that may confound oxidative stress and behavioral endpoints, consistent

with the established literature using this D-galactose and AlCl_3 -induced oxidative stress-driven AD-like rat model. However, this design limits the generalizability of the findings, as sex differences in AD vulnerability, hippocampal neurodegeneration, and antioxidant responses are increasingly recognized in both clinical and preclinical contexts. Future studies should incorporate both male and female animals to evaluate potential sex-dependent effects of FD neuroprotection.

Finally, the translational relevance of the present findings remains limited by the absence of pharmacokinetic, bioavailability, tissue distribution, target engagement, chronic toxicity, and long-term efficacy assessments. While no overt adverse effects were observed during the study period, comprehensive toxicological characterization was not performed. Therefore, the safety profile, optimal dosing strategy, and therapeutic applicability of FD cannot be fully established from the current data. Future studies should incorporate pharmacokinetic analyses, dose-escalation studies, long-term safety evaluations, and validation across multiple experimental models before clinical translation can be considered.

Conclusion

This study provides compelling multi-level evidence that FD exerts robust neuroprotective effects in a D-galactose and AlCl_3 -induced oxidative stress-driven AD-like rat model. FD treatment effectively mitigated anxiety-like behavior and spatial working memory deficits while preserving hippocampal neuronal architecture across the CA1, CA2, CA3, and DG subregions. These functional improvements were supported by marked attenuation of neuronal degeneration, preservation of the ultrastructural integrity of mitochondria, myelin sheaths, and smooth endoplasmic reticulum, and restoration of redox homeostasis within the hippocampus. Mechanistically, FD significantly reduced lipid peroxidation and enhanced endogenous antioxidant defenses, as reflected by normalization of MDA, CAT, T-SOD, CuZn-SOD, and HO-1 levels, indicating that modulation of oxidative stress pathways constitutes a central component of its neuroprotective action. The convergence of behavioral, structural, ultrastructural, and biochemical findings suggests that *Ficus deltoidea* exerts neuroprotective effects associated with attenuation of oxidative stress and preservation of hippocampal neuronal integrity. However, the specific molecular pathways responsible for these effects were not directly investigated and require further mechanistic validation. Collectively, these findings demonstrate that FD effectively attenuates oxidative stress-associated neuronal damage and cognitive dysfunction in this experimental model and support its potential as a promising phytotherapeutic

candidate for mitigating oxidative stress-driven neurodegeneration and cognitive decline associated with AD. However, because key AD hallmarks, including amyloid- β pathology, tau hyperphosphorylation, synaptic alterations, and neuroinflammatory signaling, were not directly evaluated, the present findings should not be interpreted as definitive evidence of disease-modifying effects on all aspects of Alzheimer's disease pathology. While further studies are required to delineate the effects of FD on amyloid and tau pathology and to validate its efficacy in transgenic models and clinical settings, this work establishes a strong preclinical foundation supporting the translational potential of FD as a complementary neuroprotective strategy for AD.

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Author Contributions All authors contributed to the study conception and design. The study design was proposed by Muhammad Zulfadli Mehat, with assistance from Hafizah Abdul Hamid. Material preparation, data collection, and analysis were performed by Bukar Alhaji Modu and Michael Ayuba. The first draft of the manuscript was written by Bukar Alhaji Modu. Sushmita Devi Manoharan, Abdulhamid Sani Usman and Umar Babagana Isa assisted with certain experiments. Statistical support was provided by Che Mohd Nasril Che Mohd Nassir and Zaw Myo Hein. Hafizah Abdul Hamid, Che Mohd Nasril Che Mohd Nassir, Zaw Myo Hein, Mohamad Aris Mohd Moklas and Muhammad Zulfadli Mehat contributed to the review and editing of previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Ethics Approval and Consent to Participate Ethical approval was obtained from the Institutional Animal Care and Use Committee of UPM (reference number: UPM/IACUC/AUP-R051/2022). All procedures were conducted in compliance with the guidelines of the Institutional Animal Care and Use Committee of UPM.

Competing interests The authors declare no competing interests.

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