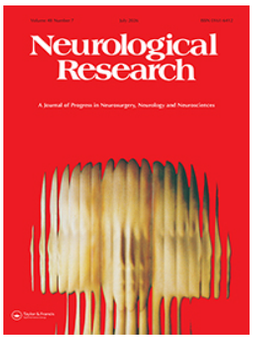

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Evaluation of neuroprotective effects of *Pyracantha crenulata* (D. Don) M. Roem against aluminium chloride induced memory impairment of rats

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ABSTRACT

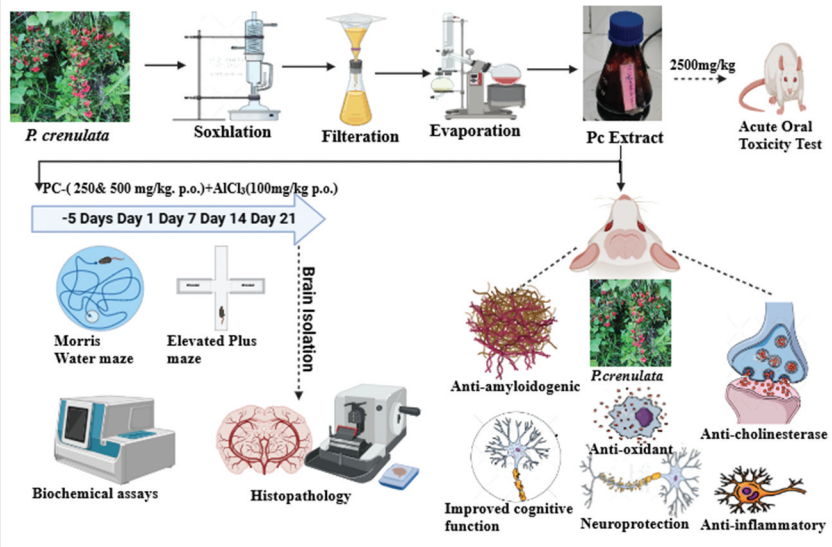
Ethnopharmacological relevance: *Pyracantha crenulata*, traditionally used in Himalayan folk medicine, valued for enhancing vitality, mental clarity, and combating age-related cognitive decline due to its antioxidant constituents.

Aim: This study evaluated the neuroprotective effect of hydroalcoholic extract of *P. crenulata* against aluminium chloride ($AlCl_3$)-induced Alzheimer's disease in rats.

Materials and Methods: Alzheimer's pathology was induced using $AlCl_3$, and rats were treated with *P. crenulata* extract (250 and 500 mg/kg) for 21 days. Cognitive and behavioural performance were assessed using the Morris Water Maze (MWM) and Elevated Plus Maze (EPM). Biochemical parameters, including oxidative stress markers (SOD), cholinesterase activity (AChE), and myeloperoxidase levels (MPO), were measured. Histopathological examination of the hippocampus and cerebral cortex was conducted.

Results: Aluminium intoxication led to marked deficits in learning and memory, as evidenced by performance in the Morris Water Maze and Elevated Plus Maze tests. Treatment with *P. crenulata* extract significantly enhanced spatial and long-term memory in a dose-dependent manner, with the higher dose producing the most pronounced improvement.

Conclusion: The findings of this study highlight the notable neuroprotective effects of *P. crenulata*, demonstrated through modulation of biochemical parameters and suppression of amyloid precursor protein and Tau, key pathological hallmarks of Alzheimer's disease, further validated by histopathological evidence.



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Introduction

Alzheimer's disease (AD), is most prevalent & devastating neurodegenerative disorder, manifesting as a gradual decline in cognitive functions, ultimately leading to significant memory loss, impaired judgment, and alterations in personality and behaviour [1,2]. The rising incidence of AD worldwide emphasises how critical it is to understand the complex molecular processes underlying its pathophysiology in order to create efficient treatment approaches [3]. The insidious nature of Alzheimer's disease is characterised by a prolonged preclinical phase, during which subtle pathological changes accumulate in the brain decades before the emergence of overt clinical symptoms [4]. In AD, age-related changes set the stage during the initiation and progression of the disease [5]. Up to 107 million people are predicted to be impacted globally by 2050 [6]. The accumulation of amyloid-beta plaques (A-β) and neurofibrillary tangles (NFT) are the two hallmarks of Alzheimer's disease, disrupting neuronal function and triggering a cascade of neuroinflammatory events, contributing to altered synaptic dysfunction and neuronal loss [7]. The precise molecular mechanisms underlying neuronal degeneration and cognitive decline in Alzheimer's disease remain under active investigation [8]. Extracellular plaques consist of A-β peptide, and NFTs are the polymers of abnormally hyperphosphorylated tau protein [9]. Aβ plaques can develop and accumulate in both the cerebral vasculature and the brain parenchyma [10]. These pathological changes, initially identified over a century ago, remain central to our understanding of the disease, although their precise roles in Alzheimer's disease are still in progress [11]. Furthermore, in AD oxidative stress, mitochondrial dysfunction, inflammation, metal ion dyshomeostasis, decreased neurotrophic support, and genetic mutations are the prevalent factors [12]. Amyloid precursor protein (APP) undergoes sequential cleavage by β-secretase and γ-secretase, producing amyloid-β peptides that aggregate into plaques characteristic of Alzheimer's disease [13]. Aluminium chloride is the metal that is found to be affecting CNS due to its water exposure and use of metal foil for wrapping food. The aluminium chloride is an inducing compound that causes increased ROS, which leads to Aβ and Tau protein degradation and neuroinflammation [14]. Current USFDA-approved treatments for Alzheimer's disease (AD) consist of two drug classes: cholinesterase (ChE) inhibitors and NMDA antagonists [15]. Although these medications may offer some improvement in memory and cognitive function, their benefits are limited, and they do not prevent the progression of the disease. These medications have a number of adverse effects, including bronchospasm, decreased intraocular tension, increased respiratory secretion, and hypotension [16].

India is particularly rich in these plants, which are known for their fewer side effects and ability to target multiple biological pathways. Given their effectiveness, safety, and accessibility. Traditional medicinal plants are key focus in treating various diseases, especially neurodegenerative diseases. In facts, the World Health Organisation (WHO) even recommended using traditional herbal remedies during the COVID-19 pandemic due to these benefits [17].

Pyracantha crenulata (D. Don) M. Roem, a member of the 'Rosaceae' family and 'Pyracantha' genus. *Pyracantha crenulata* enriched with flavonoids like quercetin, tannins, saponins, alkaloids, glycosides, protein, phenols etc. bioactive molecule [18]. The hydroalcoholic extract, abundant in flavonoids, demonstrates strong antioxidant activity [19] and shows promising free radical scavenging as well as anti-inflammatory properties [20]. Further, its reported to have anti-hypertensive effect, anti-urolithogenic and provide analgesic properties [21,22].

Pyracantha crenulata contains flavonoids and phenolic compounds with potent antioxidant activity [19, which may protect neuronal cells from oxidative damage induced by aluminium chloride. Oxidative stress plays a critical role in neurodegeneration associated with Alzheimer's disease. *Pyracantha crenulata* is rich in flavonoids and phenolic compounds known for their anti-oxidant potential. However, its neuroprotective effects against aluminium-induced cognitive impairment have not been extensively explored. Therefore, the present study aimed to evaluate the neuroprotective potential of the hydroalcoholic extract of *Pyracantha crenulata* in an aluminium chloride -induced neurotoxicity model.

Moreover, this hydroalcoholic extract of *P. crenulata* has been employed to find the neuroprotective properties of novel bioactive flavonoids via using AlCl3 model of experimental rats via investigation of behavioural, biochemical AChE & histopathological evaluations.

Materials and methods

Chemicals used

Methanol (Rankem), Distilled water (Thermo Fisher Scientific), Aluminium chloride ($AlCl_3$), Rivastigmine & other analytical graded chemicals.

Plant material

Whole plant parts of *Pyracantha crenulata* were procured and identified by the local people of the village of Kanda, Champawat, Uttarakhand, at an altitude of 1000–2600 m and authenticated by the plant botanist Mr S.K. Singh, Botanical Survey of India (BSI), Dehradun. The account number: 1466. For documentation purposes, the collected plant specimen was preserved as a herbarium sample. Fresh material was rinsed thoroughly with tap water to eliminate surface impurities, then cut into smaller sections and dried in the shade at ambient temperature. Once dried, the plant material was ground into a coarse powder using a grinder, after which it was processed for extraction.

Extraction

One kilogram of coarse powder prepared from the whole plant was extracted using a Soxhlet apparatus with a hydroalcoholic solvent mixture (water: methanol, 1:1). Methanol was selected because it is highly effective in extracting polar phytochemicals such as phenolic compounds and flavonoids due to its polarity and efficient penetration into plant tissues. Previous studies have reported that methanol often yields higher phenolic content and antioxidant activity compared to ethanol-based extract [23]. The resulting extract was concentrated under reduced pressure at 45–55°C with a rotary evaporator. The semi-solid residue was air-dried, stored in an amber glass container, and kept refrigerated. Phytochemical screening was carried out following established protocols [24].

Total flavonoid content

Flavonoids were quantified using aluminium chloride colorimetric technique. 1 mg/ml of hydroalcoholic extract sample of *Pyracantha crenulata* was dissolved in methanol, 1 ml of $AlCl_3$ (2%) in methanol was added, and after incubation for 10 min, the absorbance was measured at 430 nm. The analyses were replicated ($n = 3$), and the contents given as mean values, plus or minus the standard deviation. Flavonoids were measured as quercetin equivalents and expressed as milligrams of each compound per 100 g of dry weight of hydroalcoholic extract [25,26].

Total phenolic content

The Folin-Ciocalteu phenol assay was employed for the determination of total phenolic content. The hydroalcoholic extract sample of *Pyracantha crenulata* was prepared at a concentration of 5 mg/ml in 30% DMSO. A sample (20 µl) of the plant extract and a standard solution of gallic acid was mixed with Folin-Ciocalteu phenol reagent (100 µl) and sodium carbonate (80 µl). After a 30-min incubation, the absorbance was measured at 765 nm using a microplate reader (Synergy LX, Bio-Tek, Instrument, Inc., U.S.A.). The total phenolic content was expressed as milligrams of gallic acid equivalent per gram of plant extract [27–29].

Experimental animals

To assess the memory-enhancing potential of *Pyracantha crenulata*, male Wistar rats (150–180 g, 3–4 months old) were obtained from the Central Animal House, ISF College of Pharmacy, Moga, Punjab. The animals were housed in clean, translucent polypropylene cages under controlled conditions. They were randomly divided into five groups ($N = 5$, $n = 6$; where N denotes the number of groups and n the number of animals per group). All rats were maintained on a 12/12 h natural light – dark cycle at 22 ± 3°C with 60% relative humidity. The experimental

protocol was approved (ISFCP/IEAC/CPCSEA/Meeting No. 08/2025/105) and conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA) [29].

Experimental study design

Male Wistar rats (150–180 g) with age of 3–4 months were allocated into five groups for the study design. The LD_{50} of *Pyracantha crenulata* was previously reported as 2500 mg/kg[21] over 28 days with no mortality; therefore, two intermediate doses (250 mg/kg and 500 mg/kg, equivalent to 1/10th of the LD_{50}) were selected for experimentation. The normal control group received carboxymethyl cellulose (0.1%) throughout the study. The disease control group was administered $AlCl_3$ suspended with 0.5% carboxymethylcellulose (100 mg/kg/day, p.o.) [30–32] for 21 days, while the positive control (PC) group received $AlCl_3$ (100 mg/kg/day, p.o.) along with rivastigmine (5 mg/kg/day, p.o.) for 21 days. The PC250 and PC500 groups were treated with $AlCl_3$ (100 mg/kg/day, p.o.) in combination with the hydroalcoholic extract of *P. crenulata* (250 mg/kg/day and 500 mg/kg/day, respectively) for 21 days. Prior to the intervention, acquisition trials were conducted for all animals using the Morris Water Maze (MWM) and Elevated Plus Maze (EPM) tests. Following treatment, latency time was recorded on days 0 (first dose), 7, 14, and 21. At the end of the *in -vivo* experiments, rats were euthanized by 80 mg/kg ketamine by intraperitoneal route, and brains were isolated for analysis of acetylcholinesterase (AChE) levels in the cerebral cortex and hippocampus, as well as whole-brain myeloperoxidase (MPO) and superoxide dismutase (SOD) activity. Histopathological examination of brain tissues was also performed [33–38].

Study design

An *in-vivo* experimental plan was employed in Table 1, with $N = 5$ (number of groups), and $n = 6$ (number of animals per group) and total number of animals = 30

In-vivo screening model for memory

Morris water maze (MWM) test

Morris water maze (MWM) was used in accordance with Morris's methodology to assess learning and spatial memory. It makes use of a circular water tank with a diameter of 180 cm and a height of 60 cm that is filled to a depth of 40 cm with tap water that is 25 ± 1°C. Fresh, non-fat milk was added to the water to make it opaque. The tank was split into four equal quadrants: north-east (NE), north-west (NW), south-west (SW), and south-east (SE). In the pool, an escape platform with a diameter of 10 cm was positioned 2 cm below the water's surface. One of the four quadrants had a concealed platform (10 cm) buried 2 cm below the water's surface. For the duration of the experiment, this platform's location remained constant. Rats were free to swim in the pool for 120 s without a platform during the training phase. For training, four trial sessions were performed each day (once from each starting position) for 4 days, with a cut-off time of 120 s and a 30-s interval between each trial. Initial latency was defined as the time it took to find the hidden platform (latency in seconds). The animal must carefully pick and place on a secret platform for 30 s if it cannot find one within 120 s. Thus, each animal's initial delay was noted in order to evaluate their capacity for learning [39,40].

Index of memory improvement – Escape Latency Time (ELT)

Table 1. Shows that an *in-vivo* experimental plan.

Groups (N)	Treatment (n)
Normal Control	Received Carboxy methyl cellulose(0.1%)-21 days
Disease Control	Received $Alcl_3$(100 mg/kg *p.o.)* – 21 days
Positive control (PC)	Received $Alcl_3$ (100 mg/kg/day *p.o.)* + Rivastigmine (5 mg/kg *p.o.)* –21 days
PC 250 group	Hydroalcoholic extract (250 mg/kg *p.o.)* + $Alcl_3$ (100 mg/kg *p.o.)* – 21 days
PC 500 group	Hydroalcoholic extract (500 mg/kg p.o.) + $Alcl_3$ (100 mg/kg *p.o.)* – 21 days

Probe trial

In order to evaluate the animal learning, a probe trial was carried out by removing the hidden platform, following a 24-h navigation trial. It contains a measurement of the amount of time spent in the quadrant where the platform was removed. Time spent in the target quadrant (SW) (TSTQ) is calculated as a measure of spatial memory.

Elevated plus maze

On days 1,7,14, and 21, the EPM was carried out by placing each rat at the end of the track, which records rat's crossing time from the open arm to either of the enclosed arms while keeping its arm out and facing away from the central platform. After the measurement of transfer latency in the plus maze, the rat was given 20 s to freely explore the entire apparatus, regardless of whether it entered open or closed arms. A shorter transfer latency, meaning the rat quickly moved from the open arm to the closed arm, was interpreted as reflecting enhanced memory ability [41–43].

Index of memory improvement: Transfer Latency Time.

In-vivo biochemical estimation

Determination of SOD

Superoxide dismutase activity was assessed following the method of Sun and Zigman [43,44]. Each 1.5 ml reaction mixture included 100 mM TRIS/HCl (pH 7.8), 75 mM NBT, 6 mM EDTA and 200 µL supernatant. Following the catalytic activity finally the observed blue formazan production observed. One unit of SOD was defined as the amount needed to inhibit NBT reduction by half. Enzyme activity was expressed as units per mg protein and the absorbance of solutions were taken at 560 nm [44–46].

Determination of acetylcholinesterase activity

Brain acetylcholinesterase (AChE) activity was quantified using the Ellman method, with minor adaptations. This measurement relied on the development of yellow coloration from the reaction between thiocholine and dithiobisnitrobenzoate ions. The rate of thiocholine production from acetylthiocholine iodide, catalyzed by brain cholinesterase, was quantified spectrophotometrically. Into a 25 ml volumetric flask, 0.5 ml of supernatant from the brain homogenate was pipetted, followed by dilution with freshly prepared DTNB solution. From the volumetric flask, 4 ml was transferred into two test tubes; 2 drops of serine solution were added to one tube. 1 ml of substrate solution (75 mg acetylcholine iodide dissolved in 50 ml distilled water) was added to both test tubes, followed by incubation at 30°C for 30 min. The test tube with serine solution served as the blank, and the sample's absorbance change per minute was recorded spectrophotometrically at 590 nm [47,48]

Estimation of myeloperoxidase (MPO) assay

Supernatant of brain tissues suspended in hexadecyltrimethylammonium bromide (HTAB) buffer (0.5% HTAB in 50 mM phosphate buffer, pH 6.0). Hexadecyltrimethylammonium bromide, a detergent that liberates MPO from neutrophil primary granules, was used for resuspension. Cell suspensions were sonicated on ice for 10 s, then centrifuged at 20, 000 g for 15 min. The supernatant was analysed for MPO activity. Myeloperoxidase activity was quantified spectrophotometrically by mixing 0.1 ml supernatant with 2.9 ml of 50 mM phosphate buffer (pH 6.0) containing 0.167 mg/ml o-dianisidine hydrochloride and 0.005% hydrogen peroxide. Absorbance change at 460 nm was recorded using a spectrophotometer. One unit of MPO activity was defined as the amount degrading 1 pmol peroxide per minute at 25°C. For tissue MPO activity, was minced in a beaker with 1 ml Hexadecyltrimethylammonium Bromide buffer on ice, transferred to a test tube, and homogenized using a homogenizer on ice cube. The combined homogenate and rinses were sonicated for 10 s, subjected to three freeze- cycles, and analyzed for MPO activity at 460 nm [42,49].

Histopathology examination

After euthanasia, the brains were excised and fixed in 10% formalin. The tissue was sectioned into 3 mm blocks, which were subsequently embedded in paraffin. Standard haematoxylin and eosin staining was performed, and brain sections of 20 µm thickness and magnification 40X were prepared for histological evaluation [50].

Statistical analysis

Data were presented as mean± S.D. Behavioural parameters recorded across multiple time points were evaluated using two-way ANOVA with Bonferroni post hoc test. Biochemical and molecular data were analysed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was considered at $p < 0.05$ [51].

Results

Plant extraction and phytochemical screening

The hydroalcoholic extract obtained from the whole plant of *Pyracantha crenulata* weighed 75 g, corresponding to a percentage yield of 7.4% (w/w). Preliminary phytochemical screening of the extract revealed the presence of alkaloids, carbohydrates, saponins, glycosides, proteins, phenols, tannins, flavonoids, and amino acids.

Total flavonoid and phenolic content

The hydroalcoholic extract of *Pyracantha crenulata* was found to contain a total flavonoid content of 44.94 mg quercetin equivalents per gram of dry extract. Similarly, the total phenolic content was determined to be 268.95 mg gallic acid equivalents per gram of dry extract.

In-vivo studies

Morris water maze: effect of pyracantha crenulata on escape latency time (ELT). During the acquisition trials, escape latency time (ELT) and time spent in the target quadrant (TSTQ) were recorded. On day 1, no significant differences were observed among the groups. By day 7, however, the disease control group showed a marked increase in ELT $^{@}(p < 0.001)$ compared to the normal control group, whereas treatment with PC250 significantly reduced ELT $^{\$}(p < 0.01)$ relative to disease control group. On days 14 and 21, the disease control group continued to exhibit a pronounced rise in ELT, while both PC250 and PC500 groups demonstrated a significant decline $^{\lambda}$ $(p < 0.05)$ compared to disease control. The effects of PC250 and PC500 on ELT and TSTQ are illustrated in Figures 1(a) and 1(b).

Elevated plus maze(EPM): effect of Pyracantha crenulata on transfer latency (TL). Memory retrieval was assessed using transfer latency (TL) in the elevated plus maze, where a reduction in TL reflects improved recall. On day 0, TL values were comparable to acquisition trial readings. By day 7, the disease control group exhibited a significant increase in TL $^{@}(p < 0.001)$ compared to the normal control group, while treatment with rivastigmine (PC), PC250, and PC500 produced a notable reduction in TL relative to disease control. On days 14 and 21, TL remained significantly elevated in the Disease control group $^{\$}(p < 0.01)$, whereas both PC250 and PC500 groups showed a marked decline in TL compared to disease control $^{\lambda}$ $(p < 0.05)$. The effects of PC250 and PC500 on TL are illustrated in Figure 2.

In-vivo biochemical estimation

Crenulata effect on superoxide dismutase(SOD). In the disease control group treated with $AlCl_3$, the superoxide dismutase (SOD) level in brain homogenate tissue decreased significantly (3.88 ± 0.37) compared to the Normal control group (9.55 ± 0.87). Treatment with *P. crenulata* extract at doses of 250 mg/kg (4.96 ± 0.14) and 500 mg/kg (6.54 ± 0.35) produced a significant increase in SOD levels relative to disease control. The effects of *P. crenulata* on SOD activity in brain homogenate tissue are presented in Figure 3(a) and Table 2.

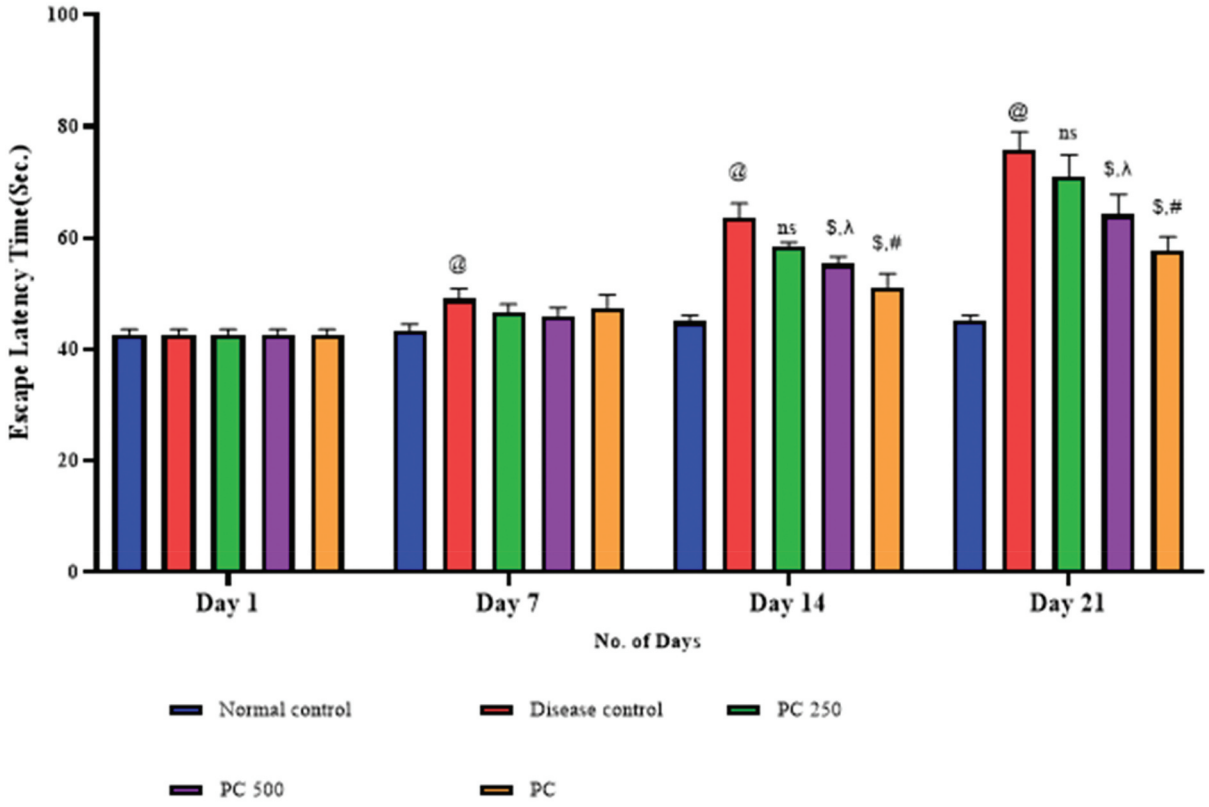

Figure 1a. Effect of *Pyracantha crenulata* on escape latency in Morris water maze. Data was statistically confirmed by two-way ANOVA followed by Bonferroni's multiple comparison tests. Values are expressed as mean ± standard deviation, with $p < 0.05$ was considered statistically significant: $^{@}p < 0.001$ vs normal control, $^{\$}p < 0.01$ vs disease control, $^{\lambda}p < 0.05$ vs pc 250, $^{\#}p < 0.05$ vs pc 500.

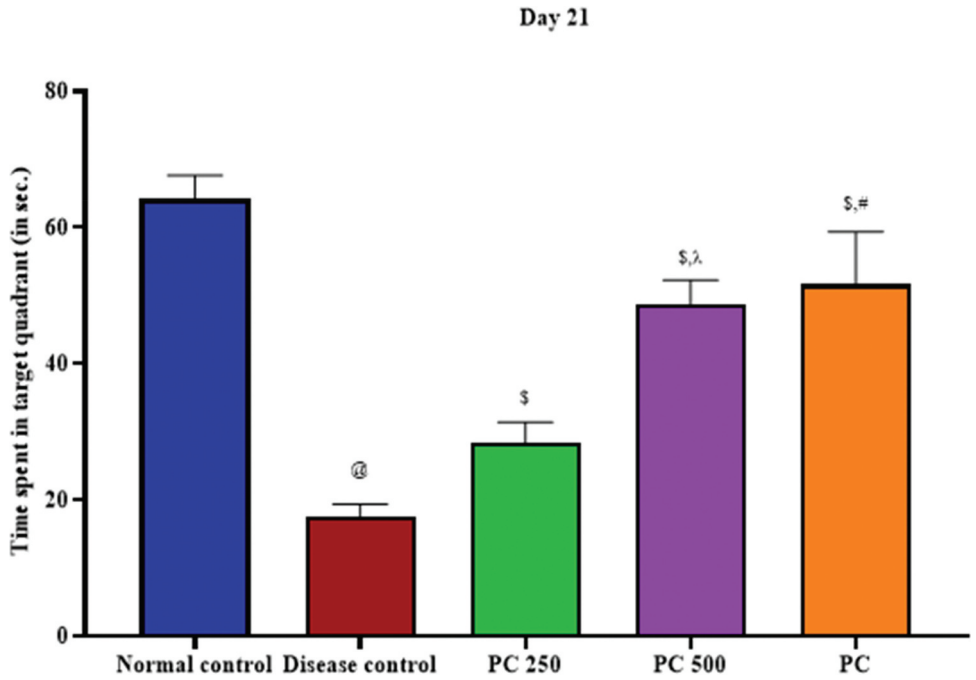

Figure 1b. Effect of *Pyracantha crenulata* on time spent in target quadrant (SW) in morris water maze. Data was statistically confirmed by two-way ANOVA followed by Bonferroni's multiple comparison tests. Values are expressed as mean ± standard deviation with $p < 0.05$ was considered statistically significant: $^{@}p < 0.001$ vs normal control, $^{\$}p < 0.001$ vs disease control, $^{\lambda}p < 0.05$ vs PC 250, $^{\#}p < 0.05$ vs PC 500.

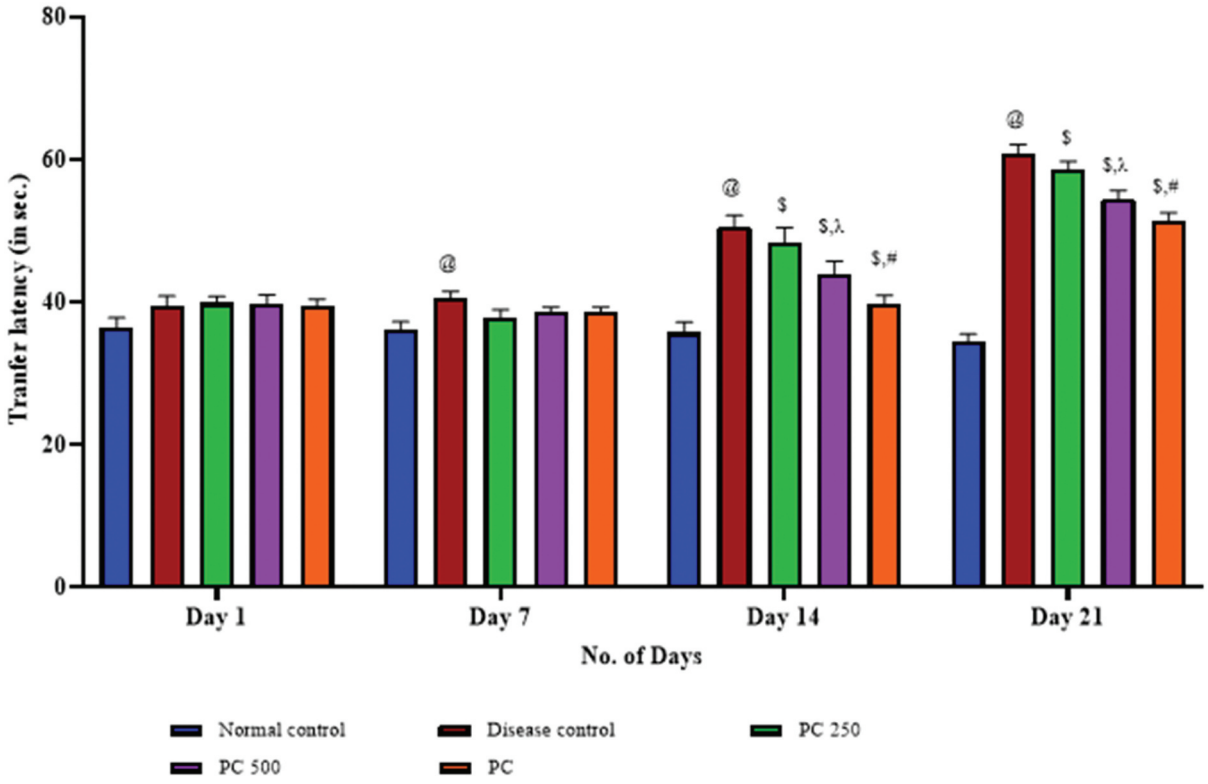

Figure 2. Effect of *Pyracantha crenulata* on the transfer latency in elevated plus maze test. Data was statistically confirmed by two-way ANOVA followed by Bonferroni's multiple comparison tests. Values are expressed as mean ± standard deviation with $p < 0.05$ was considered statistically significant: $^{@}p < 0.001$ vs normal control, $^{\$}p < 0.01$ vs disease control, $^{\lambda}p < 0.05$ vs pc 250, $^{\#}p < 0.05$ vs pc 500.

Crenulata effect on AChE level. Administration of $AlCl_3$ in the Disease control group (0.395 ± 0.01) led to a marked elevation of acetylcholinesterase (AChE) levels in the cerebral cortex and hippocampus compared to the normal control group (0.091 ± 0.007). In contrast, treatment with rivastigmine (PC), PC250, and PC500 significantly reduced AChE activity in these regions $^{@}(p < 0.001)$ relative to disease control. Both *P. crenulata* treatment groups demonstrated a strong inhibitory effect on AChE. The impact of *P. crenulata* on AChE levels in brain homogenate tissue is presented in Table 2 and Figure 3(b).

Crenulata effect on MPO level. In the disease control group treated with $AlCl_3$, myeloperoxidase (MPO) levels in brain tissue increased significantly (196.38 ± 5.25) compared to the normal control group (78.40 ± 4.37). In contrast, treatment with *P. crenulata* extract at doses of 250 mg/kg (164.82 ± 7.36) and 500 mg/kg (125.61 ± 9.24) produced a marked reduction in MPO levels relative to disease control. The effects of *P. crenulata* on MPO activity in brain homogenate tissue are presented in Table 2 and Figure 3(c).

Histopathological analysis

Histopathological changes in the brain hippocampus section of rats. Histopathological assessment of the brain sections of rats in the, normal control group, disease control group, PC group, PC 250 and PC500. The yellow arrow represents inflammatory cell infiltration and black arrow shows a degeneration of hippocampus neurons in disease control group. In this administration of PC250 and PC500 prevented the neuronal loss and restored the neurons (represents by green arrow) as compared to disease control group. Furthermore, the positive group shown near to comparative effect as compared to PC 500 group. The effect of *P. crenulata* on histopathological changes in the brain hippocampus section of rats is shown in the Figure 4

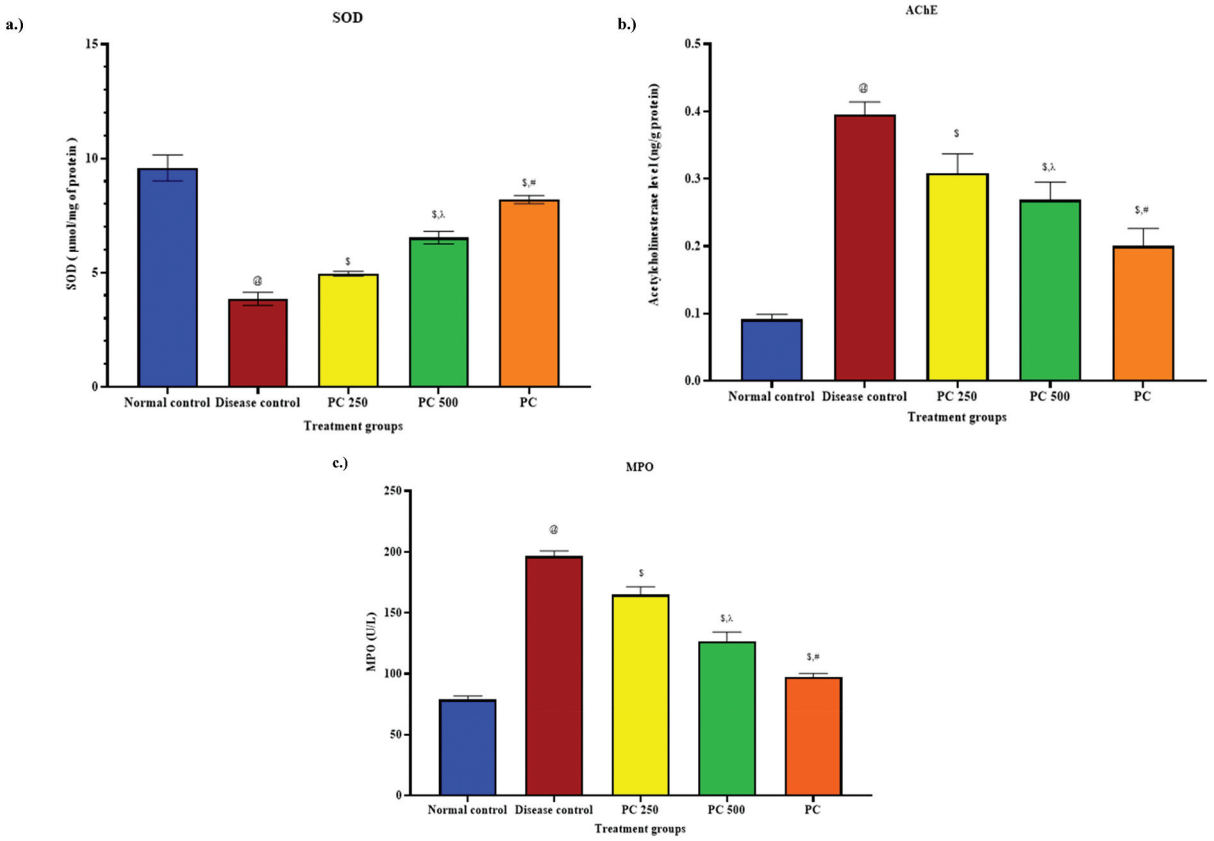

Figure 3. Effect of *P. crenulata* on the level of SOD,MPO and AChE in brain homogenate tissue. a. sod b. AChE c. MPO. Data was statistically confirmed by one way ANOVA followed by Tukey's multiple comparison tests. Values are expressed as mean ± standard deviation with $p < 0.05$ was considered statistically significant: $^{@}p < 0.0001$ vs normal control, $^{\$}p < 0.001$ vs disease control, $^{\lambda}p < 0.01$ vs pc 250, $^{\#}p < 0.05$ vs pc 500.

Table 2. Protective effect of *P. crenulata* on level of MPO,AChE and sod in rat brain homogenate.

Experimental Groups	MPO (U/L)	AChE (ng/g protein)	SOD (µmol/mg protein)
Normal control	78.40 ± 4.37	0.09 ± 0.007	9.55 ± 0.87
Disease control	196.38 ± 5.25$^{@}$	0.39 ± 0.018$^{@}$	3.88 ± 0.37 $^{@}$
PC 250	164.82 ± 7.36$^{\$}$	0.30 ± 0.028$^{\$}$	4.96 ± 0.14$^{\$}$
PC 500	125.61 ± 9.24$^{\$,\lambda}$	0.26 ± 0.026$^{\$,\lambda}$	6.54 ± 0.35$^{\$,\lambda}$
PC	97.38 ± 3.76$^{\$,\#}$	0.20 ± 0.026$^{\$,\#}$	8.16 ± 0.22$^{\$,\#}$

Discussion

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive decline, cholinergic dysfunction, oxidative stress and neuroinflammation. Although currently available cholinesterase inhibitors provide symptomatic relief, their therapeutic benefits remain limited and primarily offer short-term improvement without preventing disease progression [52]. Therefore, there is growing interest in exploring multitarget therapeutic strategies capable of modulating multiple pathological pathways involved in AD. Herbal medicines have gained attention in this context because they contain diverse bioactive compounds with anti-oxidant, anti-inflammatory, and neuroprotective properties [53]. Previous studies have suggested that plant-derived phytochemicals can mitigate neurodegeneration by improving neuronal survival, enhancing cerebral circulation, promoting neurogenesis, and reducing oxidative and inflammatory damage associated with AD pathology [54].

In the present study, the neuroprotective potential of *Pyracantha crenulata* was evaluated in an aluminium chloride (AlCl3)-induced cognitive impairment model. Chronic exposure to AlCl3 is known to

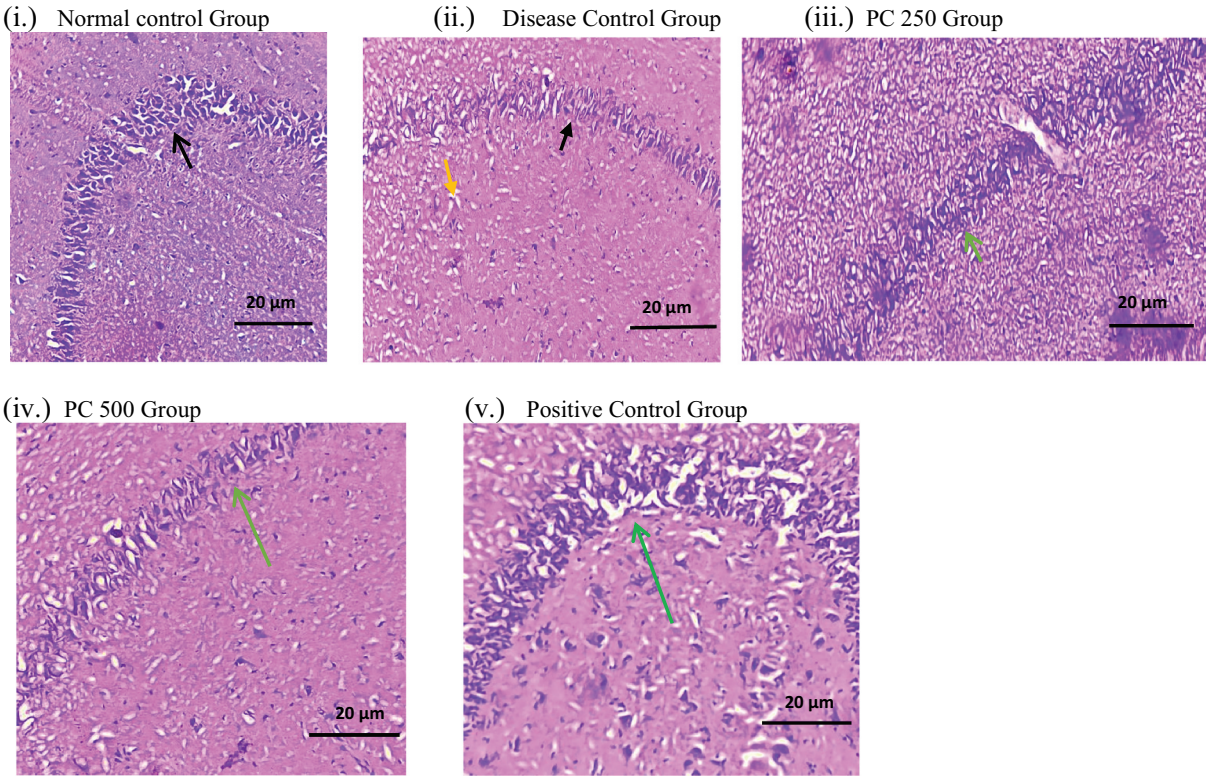

Figure 4. Effect of *P. crenulata* on histopathological changes in the brain hippocampus section of rats.

produce several neuropathological features resembling Alzheimer's disease, including oxidative stress, cholinergic dysfunction, neuronal degeneration, and memory impairment [55–57]. Consistent with previous reports, AlCl3 administration significantly impaired spatial learning and memory in rats, as demonstrated by increased escape latency and reduced time spent in the target quadrant in the Morris water maze test. Similar findings have been reported in earlier studies where aluminium exposure disrupted hippocampal neuronal function and impaired spatial memory performance [58]. In the present investigation, treatment with *P. crenulata*, particularly at the higher dose (PC500), significantly improved cognitive performance, as evidenced by reduced latency time and enhanced quadrant preference, inducing improved spatial memory retention.

The elevated plus maze test further supported these findings, where AlCl3- treated animals exhibited increased transfer latency, reflecting impaired learning and memory. However, chronic administration of *P. crenulata* significantly reduced transfer latency, suggesting improvement in memory consolidation. The behavioural improvements observed in this study were comparable to those produced by rivastigmine, a clinically approved cholinesterase inhibitor used for the symptomatic treatment of AD [59]. Similar neuroprotective effects have been reported for several plant extracts rich in polyphenols and flavonoids, which improve cognitive deficits in experimental Alzheimer's models through antioxidant and cholinergic mechanisms [60].

Cholinergic dysfunction represents a central feature of AD pathology, characterised by reduced acetylcholine levels due to increased activity of acetylcholinesterase (AChE) [61]. Inhibition of AChE remains one of the primary therapeutic strategies for improving cognitive function in AD patients. In the present study, AlCl3 administration significantly increased AChE activity in the brain, whereas treatment with *P. crenulata* produced a marked reduction in AChE activity in both the cerebral cortex and hippocampus. The observed inhibitory effect may be attributed to phytoconstituents such as Flavonoids, phenolic compounds, saponins, and sterols reported in *P. crenulata*. Previous studies have demonstrated that flavonoids can enhance cholinergic neurotransmission by inhibiting AChE activity and protecting cholinergic neurons from oxidative damage [62].

Oxidative stress also plays a crucial role in the pathogenesis of Alzheimer's disease. Excessive production of reactive oxygen species (ROS) disrupts cellular redox homeostasis, leading to lipid peroxidation, protein oxidation, and neuronal damage [63]. Superoxide dismutase (SOD) is an important antioxidant enzyme that protects neuronal cells by scavenging superoxide radicals. In the present study, AlCl3 administration significantly reduced SOD levels in the brain, indicating oxidative stress-induced neuronal injury. However, treatment with *P. crenulata* significantly restored SOD activity, suggesting enhancement of endogenous antioxidant defences. These findings are consistent with previous studies reporting that plant extracts rich in phenolic and flavonoid compounds possess strong free-radical scavenging activity, as demonstrated through in vitro anti-oxidant assays such as DPPH, FRAP, and ABTS [64].

Neuroinflammation is another key pathological hallmark of AD and contributes to progressive neuronal damage. Myeloperoxidase (MPO), an enzyme released by activated microglia and inflammatory cells, catalyses the formation of cytotoxic oxidants that exacerbate neuronal injury [65]. Increased MPO expression has been observed in Alzheimer's disease brain tissues and is associated with amyloid plaque formation and neuroinflammatory responses [66]. In the present study, AlCl3 exposure significantly elevated MPO activity, indicating inflammatory responses in brain tissue. Treatment with *P. crenulata* resulted in a dose-dependent reduction in MPO activity, suggesting potential anti-inflammatory effects. These findings indicate that suppression of MPO-mediated oxidative damage may represent an additional mechanism contributing to the neuroprotective effects of *P. crenulata*.

Histopathological evaluation further supported the biochemical and behavioural findings. Brain sections from $AlCl_3$-treated animals showed pronounced neuronal degeneration and inflammatory cell infiltration in the hippocampus, a region critically involved in memory processing. In contrast, animals treated with *P. crenulata* or rivastigmine exhibited improved neuronal architecture with reduced inflammatory infiltration, suggesting protection against aluminium-induced neurotoxicity.

Overall, the present findings suggest that *Pyracantha crenulata* exerts neuroprotective effects through multiple mechanisms, including improvement of cholinergic neurotransmission, enhancement of antioxidant defence systems, and attenuation of inflammatory responses. These multitarget actions may contribute to the observed improvement in cognitive function in the $AlCl_3$-induced model of Alzheimer-like neurodegeneration.

Limitations

Although the findings are encouraging, several limitations should be acknowledged. First, the study relied solely on a chemically induced Alzheimer's disease (AD) model. While this approach is useful for exploring oxidative stress, cholinergic dysfunction, and certain amyloid and tau-related mechanisms, it does not fully capture the progressive pathology or the genetic and temporal complexity of sporadic and familial human AD. Future validation in transgenic AD models would enhance translational relevance. Second, individual bioactive compounds were not isolated or tested to confirm their specific roles. Third, the work was constrained by limited institutional funding. Fourth, although we proposed Western blot analyses of BDNF, BACE-1, phosphorylated tau, cleaved Casp-3, Nrf2 nuclear translocation, and GSK-3β activity as follow-up targets, these protein-level changes remain to be validated. Moreover, the involvement of signaling pathways such as PI3K/Akt, MAPK, or NFκB was not examined and warrants further study to deepen mechanistic insights. Finally, future research should also address pharmacokinetics, safety, and the effects of long-term administration.

Conclusion

The present study investigated the memory-enhancing potential of *Pyracantha crenulata* using an $AlCl_3$-induced cognitive impairment model in rats. By boosting cholinergic system activity, controlling antioxidant pathways, and lowering myeloperoxidase levels, long-term *Pyracantha crenulata* therapy ameliorates memory impairment brought on by $AlCl_3$. The study used Morris water maze (MWM) and Elevated plus maze (EPM) to show how *Pyracantha crenulata* and $AlCl_3$ both affected memory function. The study's findings can be compared to rivastigmine, a medication that has received clinical approval. To further evaluate the therapeutic potential of *Pyracantha crenulata* in Alzheimer's disease, additional validation using alternative AD models and gene expression studies is necessary, as the current findings are limited to preclinical investigations.

Author contributions

CRediT: **Payal Saxena:** Conceptualization, Investigation, Methodology, Writing – original draft, Writing – review & editing; **Preeti Kothiyal:** Supervision; **Parminder Ratan:** Conceptualization.

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No potential conflict of interest was reported by the author(s).

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Data availability statement

The data will be made available on request.

Ethics statement

Ethical approval was obtained from the institutional animal care and use committee under the protocol number ISFCP/IEAC/CPCSEA/Meeting No. 08/2025/105, 17 October 2025 of the department of pharmacology, central animal house ISF college of pharmacy, Moga, Punjab.

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