Ind. J. Pharm. Edu. Res., 2026; 60(1s):s194-s204.
https://www.ijper.org

Original Article

Protective Effects of *Petroselinum crispum* Ethanolic Extract against D-Galactose and Aluminum Chloride-Induced Alzheimer's Disease in Rats: A Behavioral and Biochemical Approach

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ABSTRACT

Background: Alzheimer's disease is one of the progressive neurodegenerative disorders affecting the elderly population without a clear etiology, accounting for more than 80% of dementia worldwide. We found the D-Gal and $AlCl_3$-induced models to be the most economical, promising, and convenient among the other models for AD induction. *Petroselinum crispum* (parsley), with its established ethnomedicinal value mainly due to its antioxidant and neuroprotective activity, made us select it as a relevant choice for anti-AD activity. **Materials and Methods:** D-gal 60 mg/kg/day, i.p and $AlCl_3$ 200 mg/kg/day, p.o. were exposed to all groups except the control; additionally, donepezil 1 mg/kg/day, i.p. and ethanolic leaf extract of *Petroselinum crispum* 100 mg/day, p.o. as well as 200 mg/day, p.o. were administered to the standard, test-low dose, and test-high dose groups, respectively, for 70 days. The behavioral parameters of the animals were assessed by modified EPM, MWM, and OFT. Rat brain homogenate was used to assess biochemical parameters such as AchE inhibitory activity, Antioxidant activity (SOD, GPx, and CAT), and MDA for lipid peroxidation. In addition to that, histopathology of the hippocampus and cortex was done on all groups of rat brains. **Results:** Potent anti-AD activity emerged for behavioral activity, AchE inhibitory activity, lipid peroxidation inhibitory effects, as well as moderate free radical scavenging activity, for the EPC group. The histopathology of the EPC group demonstrates an almost complete reversal of AD pathology. **Conclusion:** The results emphasize that EPC might be a good alternative for the treatment of AD.

Keywords: Alzheimer's disease, Ethanolic leaf extracts *Petroselinum crispum*, Elevated Plus Maze, Morris Water Maze, and Open Field Test.

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Received: 17-04-2025;
Revised: 06-06-2025;
Accepted: 26-08-2025.

INTRODUCTION

Alzheimer's disease is one of the progressive neurodegenerative disorders affecting the elderly population without a clear etiology, accounting for more than 80% of dementia worldwide.[1] Evidence suggests there is a directly proportional relationship between dementia[2,3] and the concentration of A peptide-soluble aggregates that appear as senile plaque deposits.[4,5] The progress of AD is closely linked with the accumulation of oxygen-free radicals.[6-8] Histological observation suggests that chronic administration of D-galactose is responsible for cognitive impairment by accelerating neuronal damage.[9-13] Hence, the cognizant impairment associated with memory deficit by administration of D-galactose would be the ideal model for screening potential pharmacotherapeutic agents for AD.

Aluminum (Al), one of the toxic heavy metals that cause neurodegeneration, affects the brain as well as bone, liver, and spleen; besides, it increases the level of Acetylcholinesterase (AchE) and Malondialdehydes (MDA) through oxidative stress.[14-17] Therefore, co-administration of D-galactose and Aluminum Chloride ($AlCl_3$) would be ideal to accelerate the pathogenesis, and it is one of the established models for anti-AD screening methods.[18]

Petroselinum crispum, popularly known as parsley, is one of the most important medicinal plants. It has the following list of ethnomedicinal values: antioxidant, neuroprotective, anti-diabetic, hepatoprotective, analgesic, anti-ulcer, laxative, estrogenic, diuretic, hypotensive spasmolytic, immunosuppressant, anti-coagulant, anti-bacterial, and antifungal activities.[19-25]

DOI: 10.5530/ijper.20263885

The phytochemical constituents of leaf and seed extracts include flavonoids [Luteolin, Chrysoeriol, QuercetinIsorhamnetin, Apiose, and Petroside. osmosiin, Oxypeucedanin hydrate, Apiin, 6"-Acetylapiin, Cnidilin, Diosmetin, 7-O--D-glucopyranoside Kaempferol, 3-O--D-glucopyranoside Kaempferol), Essential oil [(Myristicin, Apiol, α-Pinene Sabinene, β-Pinene ρ-Cymene Limonene, β-Phellandrene, γ-Terpinene Elemicin, 1-Allyl-2,3,4,5-tetra methoxy-benzene Carotol, Eugenol, -Elemene, -Caryophyllene, Phenylacetaldehyde, γ-Elemene, α-Terpineol α-Thujene, Toluene Camphene Hexanal, 3-Carenem- and/or -Xylene Myrcene, -Phellandrene, -Terpinene, 2-Pentylfuran cis--Ocimene, trans--Ocimene, -Terpinolene-1,3,8-Menthatriene, cis-Hex-3-en-l-ol, 4-isopropenyl-1-Methylbenzene, -Cubebene Benzaldehyde, -Copaene Cryptone, -Bisabolene, -Elemene, 2-(-Tolyl) propan-2-ol, -Cadinol Nonanal Decanal)] Furanocoumarins [(Oxypeucedanin, Psoralen, 8-Methoxypsoralen, 5-Methoxypsoralen, Imperatorin, Isoimperatorin)], Carotinoid [(-Carotene, Lutein, Violaxanthin, Neoxanthin), Terpenes (Crispane, Crispanone, l-methyl-4-(methylethenyl)-2,3-dio [2.2.2] Oct-5-ene)] vitamin [(ascorbic acid)], Essential oil[26-33] components (mainly Myristicin and apiol), coumarins, and furocoumarins might be responsible for the various pharmacological activities, making it more appropriate to select *Petroselinum crispum*as a test drug against AD.

MATERIALS AND METHODS

Extraction of ethanoic extract of *Petroselium crispum*

Dried leaves were crushed with a mixer to a fine powder, and the powdered leaves of *Petroselinum crispum* were defatted with 1 L of petroleum ether for 72 hr. The obtained marc was further extracted with 1 L of 70% ethanol (700 mL of ethanol to 300 mL of water) in a conical flask. The mixture was stirred thoroughly with a glass rod. The conical flask was kept with intermittent shaking for 72 hr, and the mixture was filtered using a muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated using a heating mantle at 40°C, and the resultant residue was kept in a refrigerator (20°C) till further use.

Animals

Thirty male Albino Wister rats (100-200 g), aged 6-8 weeks old (offered by KMCH College of Pharmacy, Coimbatore), were used for the study. The rats were kept in cages with 5-6 animals per cage in a climate-controlled environment with 12 hr light and dark cycles and free access to food and water. $AlCl_3$, D-gal, EPC, and donepezil were administered in the morning between 8:00 a.m. and 10:00 a.m., while the behavioral tests were performed starting at 1:00 p.m. The study protocol was approved by the Institutional Animal Ethics Committee and was guided by the Committee for Control and Supervision of Experiments on Animals (CPCSEA) guidelines of the Government of India.

Grouping and experimental design

The rats were divided randomly into 5 groups, with each group comprising 6 rats, and the following vehicle and drugs were administered for 70 days as presented in Table 1. D-gal was dissolved in distilled water and administered by i.p., while $AlCl_3$, donepezil, and PC extract were dissolved in distilled water for oral administration. The doses of D-gal, $AlCl_3$, and EPC leaf extract administered were selected based on published research literature.[34] After 70 days of treatments, the rats were evaluated by OFT for their locomotor activities, modified EPM, and MWT for memory after completion of all *in vivo* experiments, animals were killed by decapitation to perform histopathological studies.[35] The decapitation procedure was chosen to avoid contamination of brain tissues by the chemicals used, like anesthetics.

Behavioral Study on Rats-Modified Elevated Plus Maze

The wooden maze is elevated to 40 cm and divided into 4 quadrants, consisting of 2 opposing open and closed arms (50x-10x-40 cm; length, width, and height) at the center of a square space (10x10 cm) in such a way to connect the 4 quadrants. The experiment started with an acquisition session. Each animal was kept at the center of the arm and directed towards the open arm, and the time elapsed to reach the closed arm was recorded as Initial Transfer Latency (ITL). Further, the animals were allowed to explore for 20 sec. If the animal failed to enter the closed arm within 90 sec, it was guided towards the closed arm and allowed to explore for 20 sec. Only if the rat both arms were inside the imaginary line drawn from the center square, was it considered that the rat was inside the closed arm. To avoid the influence of the olfactory effect, the apparatus is cleaned with 70% alcohol between each trial.

Before induction of AD, the retention session was performed (after 24 hr of training to ensure the animals are intact for the study for all the groups), and after Induction of AD, the retention session was performed 24 hr, and 7 days after the acquisition session in a similar way, and the Initial Transfer Latency (ITL) and Second Transfer Latency (STL) were noted if any animal did not enter the closed arm within the 90s, and the value was recorded as 90s. This experiment is one of the most reliable for the demonstration of the effect of various chemicals on memory; the shortened ITL and STL denote the memory-enhancing property of the drug.[36]

Morris Water Maze Test

The apparatus comprises a metal water pool of about 170 cm in diameter and 58 cm in height, divided into 4 quadrants as NE, NW, SE, and SW by an imaginary boundary line that marks the center of the pool. It is filled with water about 40 cm deep, and the temperature is maintained at 25°C. An invisible platform was kept at the center of the first quadrant below the water level to facilitate the identification of the invisible platform; milk powder

was kept below the platform. The experiment commences with the acquisition of rats to reach the escape platform by swimming and memorizing the platform location.[37] It is done in the trial session by placing the rat on any one of the quadrants facing toward the wall of the pool, and the animal is allowed to swim until it attains the escape platform. If it fails to reach the escape platform even after 60 sec, then the animal is guided towards the platform until it memorizes. After the acquisition, each animal was placed in any one of the quadrants facing the wall of the water pool and allowed to swim. The time elapsed to climb the hidden platform was noted as the latency period. At the end of the trial, the animals were dried with a clean cotton cloth. After induction, AD Latency was recorded after 24 hr and 1 week of the trial period.

Open Field Test

The device consists of an open top with a 75-x-75 cm base and a 40 cm height made up of square-based Plexiglas; the entire base was subdivided into 25 equal squares (15-x-15 cm). The test is meant for CNS activity based on the exploration of a novel environment as well as the motor activity of rodents. The experimental protocol[38] starts with exposing each rat on the 1st day to the middle of the device and allowing it to explore a novel environment for 5 min for acclimatization. On the 2nd day, the same procedure was repeated to assess the number of lines crossed and the speed of the rat moment with the help of video tracks. The crossing of the square would be considered only if all limbs of the rodent were outside the line. The device was cleaned between the tests with 70% alcohol. The test was performed at the end of AD induction for all groups.

Estimation of Biochemical Parameters of Rat Brain Homogenate

Preparation of brain homogenates

After the completion of the probe trial (the end of 77 days), the animals were sacrificed by decapitation. An incision was made on the dorsal side of the skull, and the brains were collected. The brain (hippocampus and cortex areas severely affected in AD) were dissected and separated as previously described. A 10% w/v of tissue homogenate was prepared in ice-cold phosphate buffer pH 7.4. The brain homogenates were used to determine acetylcholinesterase, catalase, glutathione peroxidase, and catalytic activities as well as lipid peroxidation levels using standard protocols.

Acetylcholinesterase activity assay

The brain homogenate level of the AChE enzyme was determined by Ellmanet *et al.*[39] It involves the hydrolysis of Ach into acetic acid and thiocholine by adding 0.01 mL of hippocampus homogenate into a mixture of 1.5 mL of phosphate buffer (100 mmol/L, pH 8.0), 0.01 mL of acetylthiocholine solution (75 nmol/L), and 0.05 mL of DTNB, increasing the formation of the yellow anion, 5-thio-2-nitrobenzoate, measured at 410 nm by UV spectrophotometry.

Estimation of Glutathione Peroxidase (GPx)

The brain Glutathione peroxidase levels were estimated by the spectrophotometric principle[40] by mixing 100 µL of tissue homogenate with 900 µL of Ellman's reagent prepared in tris-HCl buffer (0.1 M, pH 6.5). The mixture was then incubated at room temperature for 30 min, and the absorbance was measured at 412 by a UV spectrophotometer against the blank.

Estimation of Superoxide Dismutase (SOD)

The assay was performed based on the Oberley method,[41] using the brain tissue supernatant of 20 µL with a mixture of 960 µL of 100 mM sodium carbonate buffer (pH 7.8) containing 0.1 mM xanthine, 0.025 mM nitroblue-tetrazolium (NBT), and 0.1 mM EDTA, and 20 µL of xanthine oxidase. The absorbance difference was measured after the formation of blue formazan spectrophotometrically at 560 nm. One unit of SOD is equivalent to the quantity required to inhibit the rate of NBT reduction by 50%.

Estimation of Catalase (CAT)

The catalase assay was done under the Sinha method.[42] The mixture was prepared by the addition of 50 µL of homogenate of brains, 750 µL of phosphate buffer (0.01 M; pH 7.0), and 200 µL of hydrogen peroxide (200 mM). The reaction was stopped after 60 s by adding 2 mL of dichromate to acetic acid (1:3 v/v of 5% potassium dichromate with concentrated acetic acid). After heating at 100°C for 10 min, tubes were cooled in an ice bath, and the optical densities were recorded at 570 nm against the blank (50 µL of 0.9% NaCl).

Table 1: Grouping of Animals.

Groups	Sample size	Group specification
Group 1	6	The Control group received normal saline i.p and distilled water p.o.
Group 2	6	The Disease control group received D-gal 60 (mg/kg/day i.p) and $AlCl_3$ 200 (mg/kg/day, p.o).
Group 3	6	Donepezil (1 mg/kg/day, p.o) was received and D-gal 60 (mg/kg/day i.p) and $AlCl_3$ 200 (mg/kg/day, p.o) received.
Group 4	6	PC leaf extract (100 mg, p.o) was received and D-gal 60 (mg/kg/day, i.p) and $AlCl_3$ 200 (mg/kg/day, p.o) were received.
Group 5	6	PC leaf extract (200 mg, p.o) was received and D-gal 60 (mg/kg/day, i.p) and $AlCl_3$ 200 (mg/kg/day, p.o) were received.

Estimation of Malondialdehyde (MDA)

Brain tissue homogenate MDA estimated as per the Yagi method,[43] by adding a mixture of, 500 µL of 1% Thiobarbituric Acid (TBA reagent), and 500 µL of 1% phosphoric acid into that 100 µL of brain homogenate was introduced, heated in a water bath at 100°C for 15 min and then cooled for 30 min by a water bath. The resultant mixture was centrifuged at 3000 g for 10 min, and the absorbance of the supernatant was read at 532 nm against the blank.

Histological analyses of the hippocampus and cortex

After 77 days of the experimental period, the animals were sacrificed by decapitation. The brains were dissected out and immersed in the same fixative for post-fixation. Sections were cut in a coronal plane of 8 m thick using a microtome. The sections were stained with crystal violet and mounted. The stained sections of the rat hippocampus and cortex were observed under a light microscope and photographed using a digital camera to study the morphological changes of pyramidal neurons in the brain regions.

Statistics

Data were analyzed by one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison tests using Prism 8.0. Data were expressed as the mean±standard error of the mean and values of $p<0.05$ were considered statistically significant.

RESULTS

Behavioral Study on Rats-Modified Elevated Plus Maze

The whole purpose of the test is to find out the improvement in spatial learning and enhancement of memory of the test and standard drug against D-gal and $AlCl_3$-induced AD. The latency time among all the animals was almost the same before the induction of AD, which implies all the groups of animals were fit for the study. However, the percentage decreased latency time of the standard, low-dose, high-dose group was found to be 22.2, 46.7, and 57.7 after 24 hr of AD induction, and at the end of the 77th day, it was about 12.2, 36.6, and 48.8 indicates the memory enhancement was persisted for EPC and the memory enhancement was almost 3 times more than the standard drug (Table 2).

The Water Maze Experiment

The results demonstrate (Table 3) that the escape latency and time to find the north quadrant for the vehicle-treated group were substantially less (2-fold reduction) as compared to the negative control group, which indicates the development of AD; however, the standard group and low and high-test doses showed significant percentage reductions (50, 57.1, and 66.7), and the standard group and low and high test doses showed significant percentage decreases (35.5, 45.2, and 58.1) to find the NW quadrant. All 3 behavior parameters prove that the EPC has significant and dose-dependent neuro-protective activity in AD-induced rats.

Table 2: Effect of EPC on modified Elevated Plus Maze.

Sl. No.	Group	Initial Transfer-Latency-Before AD-induction (sec)	Retention Trial- 24 hr after-AD induction (sec)	Retention Trial-1 week after-AD induction (sec)
1	Control	43±7.81	13±3.69	15±4.75
2	Negative Control	43±7.50	45±10.44	44±9.76
3	Standard	40±4.22	35±9.09	35±7.69
4	Test-1 (Low dose)	45±10.21	24±6.83	27±2.86
5	Test-2 (High dose)	41±8.26	19±3.35	21±1.84

Values are expressed as the Mean±SEM Statistical significance (p) calculated by One-way ANOVA followed by Dunnett's multiple comparison test using with Prism 8.0. ns-not significant $^{**}p<0.05$, $^{***}p<0.001$ calculated by comparing treated group with control group.

Table 3: Effect of EPC on Morris Water Maze.

Sl. No.	Group	Escape latency (sec)	Time latency to find to NW Quadrant (sec)
1	Control	7±7.27	7±3.61
2	Negative Control	70±31.70	31±8.26
3	Standard	47±20.42	20±11.94
4	Test-1 (Low dose)	26±15.70	17±7.54
5	Test-2 (High dose)	20±7.17	13±3.38

Values are expressed as the Mean±SEM Statistical significance (p) calculated by One-way ANOVA followed by Dunnett's multiple comparison test using with Prism 8.0. ns-not significant $^{**}p<0.05$, $^{***}p<0.001$ calculated by comparing treated group with control group.

Table 4: Effect of EPC on Biochemical parameters on rat brain.

Sl. No.	Group	Activity of AchE (UAChE/ mg/min/protein)	Estimation of MDA (µmol/g)	GPx (UGPx/mg/ min/ protein)	SOD (USOD/mg/ min/ protein)	CAT (UCAT/mg/ min/ protein)
1	Control	7.71±0.99	15.46±1.23	17.01±1.02	5.44±1.39	3.27±0.52
2	Negative Control	14.45±1.41	27.23±1.05	9.64±1.40	2.86±1.19	1.40±0.30
3	Standard	12.40±0.71	19.25±1.08	14.09±1.29	5.84±0.97	2.69±0.31
4	Test-1 (Low dose)	11.88±1.03	21.58±1.54	12.43±0.84	4.29±0.61	2.05±0.71
5	Test-2 (High dose)	10.21±1.07	18.57±1.36	13.17±0.91	5.02±0.46	2.24±0.71

Values are expressed as the Mean±SEM Statistical significance (p) calculated by One-way ANOVA followed by Dunnett's multiple comparison test using with Prism 8.0. ns-not significant $^{**}p<0.05$, $^{***}p<0.001$ calculated by comparing treated group with control group.

Open Field Test

This study has proven there is no difference in motor activity among all groups (Figure 1), reflecting that the negative group treated with D-gal and $AlCl_3$ does not affect motor activity to rule out that D-gal and $AlCl_3$ have no motor impairment.

A Biochemical Study on the Rat Brain-Activity of Acetylcholinesterase

The significant and dose-dependent percentage inhibition of AchE level (low-dose of 17.8 and high-dose of 29.3), and more importantly, the inhibitory effect of High-dose is almost twice as strong as that of donepezil (the standard drug), confirms that phytochemicals in EPC might have an inhibitory level of AchE and that may be one of the possible mechanisms of neuro-protective effect (Table 4).

Radical scavenging activity

EPC has moderate free radical scavenging activity in the brain homogenate, formed during various phases of the pathology of AD. The percentage increase in activity for SOD (for Standard, test low-dose, and high-dose of EPC was found to be 51, 33.3, and 43, respectively), as in the case of GPx (for standard, test-low dose, and high-dose of EPC was found to be 31.6, 22.45, and 426.8, respectively), and similarly for CAT (for standard, test-low dose, and high-dose of EPC was found to be 29.6, 20.7, and 31.8, respectively) reinforces the anti-AD effect (Table 4).

Malondialdehyde reduction activity (MDA)

The significant and dose-dependent reduction of MDA was observed in brain homogenate for EPC; the percentage reductions for standard, low-dose, and high-dose EPC were found to be 29.3, 20.7, and 31.8, respectively (Table 4).

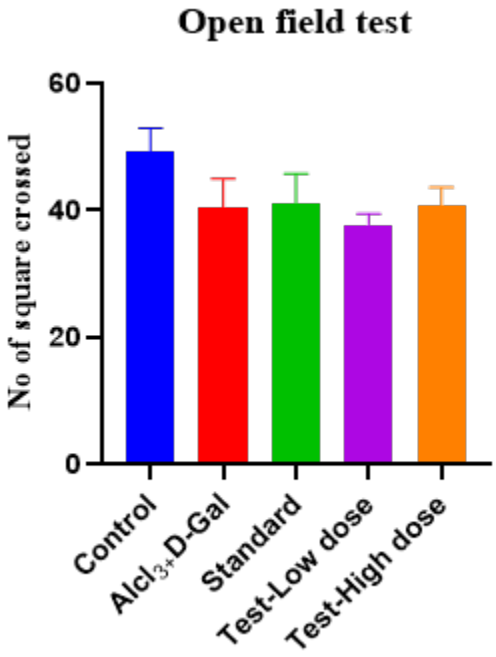


Figure 1: Effect of EPC on Open Filed Test.

Histopathological study of the rat brain

The control group of rat hippocampus shows normal neurons and normal dentate gyrus, and the cerebral cortex shows normal morphology, in contrast with scattered inflammatory infiltrates, neurovascular degeneration of the cortex, and neuronal loss in the hippocampus, which indicates the induction of AD in groups treated with D-gal and $AlCl_3$. Donepezil-treated animals showed gliosis of the cortex and neuronal degeneration in the cortex and hippocampus areas of rat brains. However, the EPC low-dose group animal cortex shows (Figure 2) mild edema, gliosis, and focal pyknotic changes, and similarly, there was mild edema in the hippocampus region. The case of the EPC high-dose group animal

demonstrates almost normal morphology of the hippocampus with no evidence of hippocampus sclerosis, implying there is no neuronal degeneration and the histopathology is almost the same as the control group (Figure 3).

DISCUSSION

It is well established that co-administration of D-gal and $AlCl_3$ in rats caused cognitive dysfunction and degeneration of pyramidal cells of the hippocampus that mimicked natural aging processes in rats. The present study proves to be a good choice for the study of the effect of test drugs on AD. The modified EPM experiment was one of the most important tools for the evaluation of spatial learning and memory processes in rats, based on their ability to learn and remember to find a safe location. The data shows a severe decline of spatial learning and memory in groups that received D-gal and $AlCl_3$, but significant dose-dependent spatial and memory retention was observed with the EPC group as compared to the AD-control group, and the effect is much higher as compared to the standard group treated by donepezil.

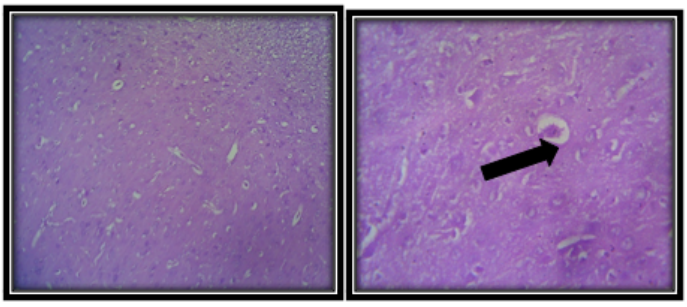
Similar results were observed in the case of the MWM experiment, which is also one of the most time-tested tools for spatial memory and accuracy. The results showed a clear-cut distinction for the EPC and standard groups with significant retention of spatial memory and accuracy as compared to the D-gal and $AlCl_3$ groups with severe loss of cognizant effect. It proves the AD induction by D-gal and $AlCl_3$ as well as its effectiveness against AD for both the EPC and donepezil groups. The OFT was performed to confirm that D-gal and $AlCl_3$ have impairments of cognition but not motor impairment. The results were obvious, as there was no difference in the number of crossings among all the groups.

Donepezil is one of the proven drugs for the treatment of AD, with a specific mechanism of inhibition of the AchE enzyme in the cholinergic neurons. Our current research has narrowed down the possibility that the AchE enzyme might be the main mechanism for the neuro-protective effect. Free radical generation and compromised scavenging activity were observed during the development and progression of AD through many literature reviews, where the scavenging effect of free radicals was found

Values are expressed as the mean±SEM Statistical significance (p) calculated by One-way ANOVA followed by Dunnett's multiple comparison test using with Prism 8.0. ns-not significant **$p<0.05$, ***$p<0.001$ calculated by comparing treated group with control group.

Group-I: Control.

Gross Appearance: Specimen of brain measuring 3.0x1.8x1.2 cms.

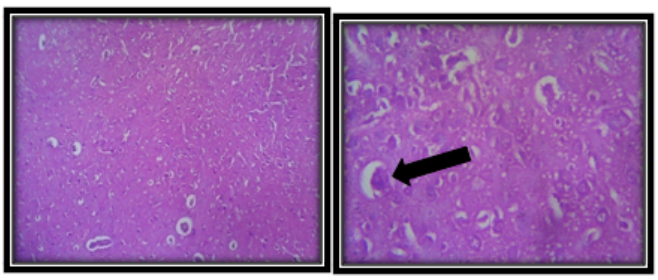


10X **40X**

2A: Histopathology of group 1 animals in the cortex, section studied from brain shows, cerebral cortex showing normal morphology.

Group-II: $AlCl_3$+Galactose.

Gross Appearance: Specimen of brain measuring 2.6x1.4x1.3 cms.



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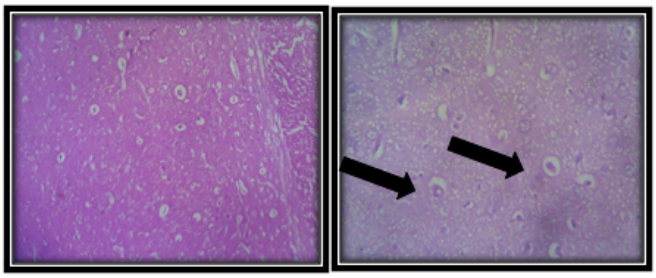
2B: Histopathology of group 2 animals in the cortex, showing scattered inflammatory infiltrates, neurovacuolar degeneration and neuronal loss.

Group-III: $AlCl_3$+D-Galactose+Donepezil.

Gross Appearance: Received a specimen of brain measuring 2.8x1.6x1.5 cms.



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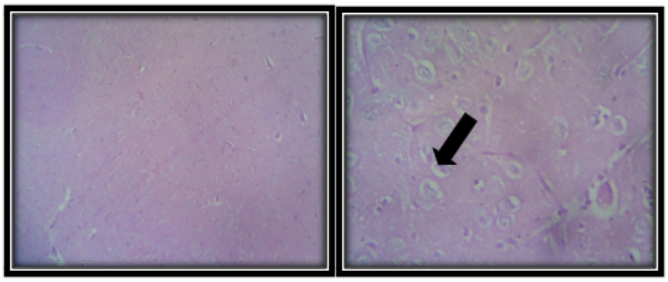
2C: Histopathology of group 3 animals in the cortex section showing gliosis, neuronal degeneration.

Group-IV: $AlCl_3$+Galactose+PC-LD.

Gross appearance: Received a specimen of brain measuring 3.1x1.7x1.4 cms.



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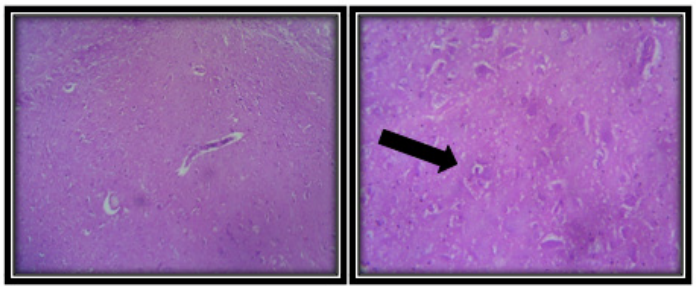
2D: Histopathology of group 4 animals in the cortex, showing mild edema, gliosis and focal pyknotic changes noted.

Group- V: $AlCl_3$+D-Galactose+PC High dose 200 mg.

Gross Appearance: Received a specimen of brain measuring 3.0x1.5x1.2 cms.



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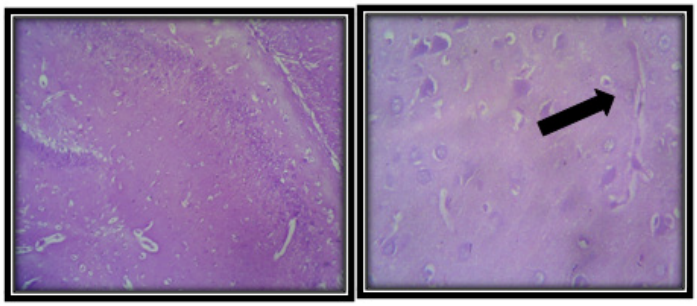
2E: Histopathology of group 5 animals in the cortex, showing normal morphology.



10X **40X**

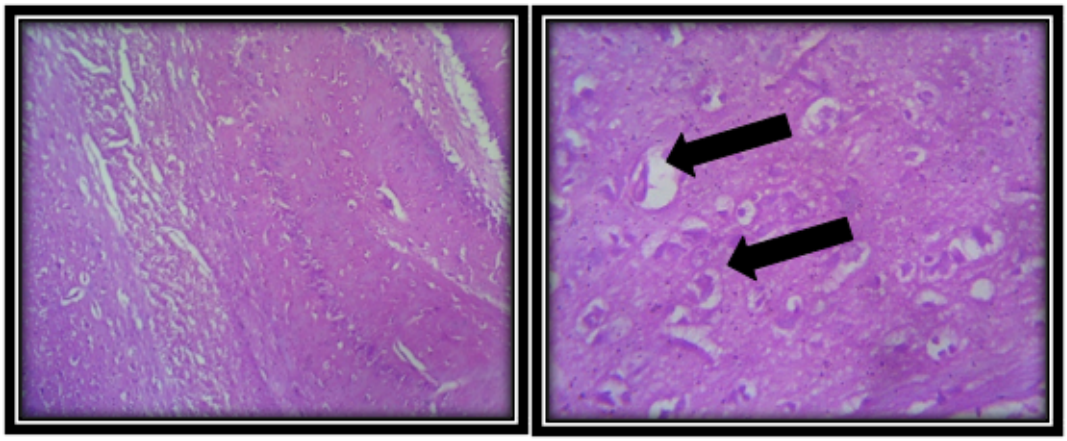
Figure 2: Histopathology of rat cortex (A-E).

to be moderate for EPC. This effect might be due to the direct increased activity of SOD, GPx, and CAT in the hippocampal region of the rat brain, which may enhance the neuro-protective effect.

MDA was one of the important indicators of lipid peroxidation; the levels of MDA were found to be increased in AD due to the up regulation of phospholipase-A_2 in brain tissue homogenate. Our current research showed the high-dose EPC has an almost similar effect on reducing the MDA level as the standard drug donepezil, suggesting possible reinforcement of the neuro-protective effect, which might be mediated by some of the specific phytochemicals present in the EPC.

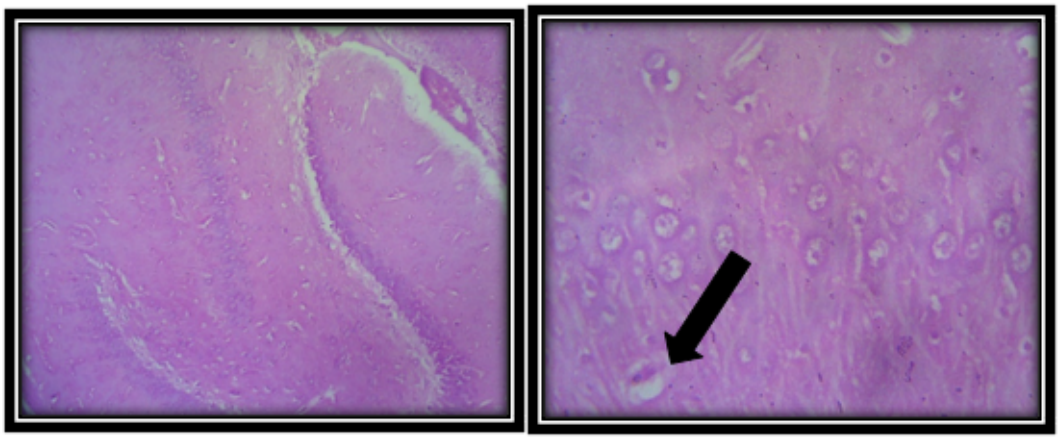
The histopathological study illustrates that there was mild edema, gliosis, and focal pyknotic changes in the hippocampus region for the low-dose EPC treated group, as well as almost normal morphology of the hippocampus and no hippocampus sclerosis in the high-dose EPC treated group. It implies the results were more significant even as compared with donepezil (standard) treated animals, which showed gliosis of the cortex and neuronal degeneration in the cortex and hippocampus areas of the rat brain.

3A: Histopathology of group 1 animals in the hippocampus shows normal neurons and normal dentate gyrus.



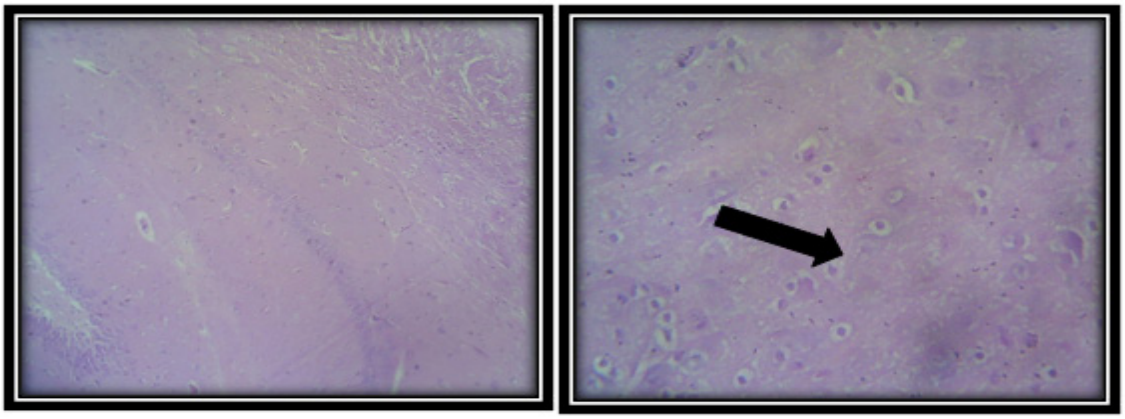
10X **40X**

3B: Histopathology of group 2 animals in the hippocampus shows neuronal degeneration and neuronal loss.



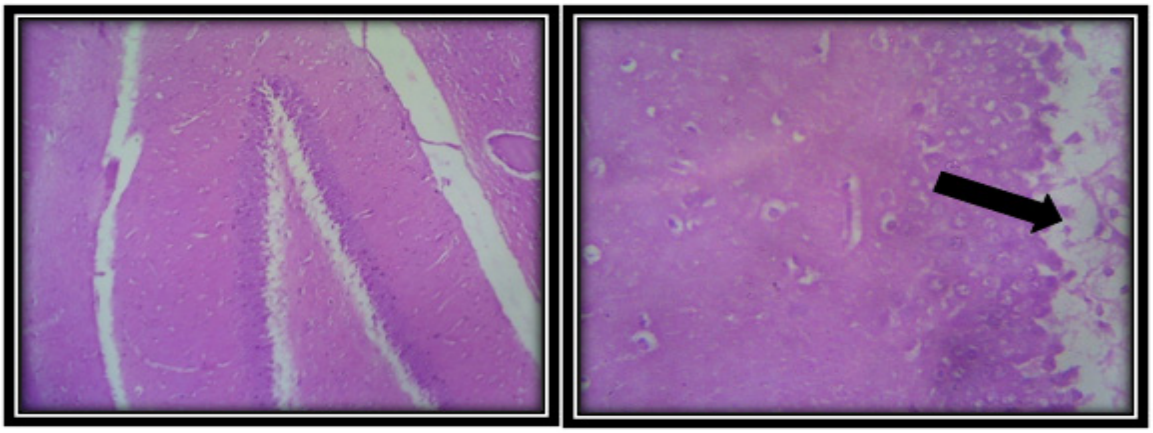
10X **40X**

3C: Histopathology of group 3 animals in the hippocampus shows neuronal degeneration and neuronal loss.



10X 40X

3D: Histopathology of group 4 animals in the hippocampus shows mild edema.



10X 40X

3E: Histopathology of group 5 animals in the hippocampus there is no evidence of hippocampus sclerosis/neuronal degeneration.

Figure 3: Histopathology of rat hippocampus (A-E).

CONCLUSION

Our research work emphasizes that EPC has highly potent anti-AD activity against D-gal and $AlCl_3$-induced AD models in rats. Although the description of the precise mechanism of the EPC was beyond the scope, the significant AchE inhibition, moderate free radical scavenging effect, and potent lipid peroxidation inhibitory effect of some specific phytochemicals might contribute to their significant anti-AD activity. Therefore, EPC might be a good alternative in the prevention and treatment of AD as compared to donepezil. However, isolation and characterization of individual phytochemicals might provide drugability against AD.

ACKNOWLEDGEMENT

I am thankful to KMCH College of Pharmacy management for providing valuable infrastructure support for the successful completion of our research work.

ABBREVIATIONS

AD: Alzheimer's Disease; **CAT:** Estimation of Catalase; **EPC:** Ethanolic Extract of Leaf Extract *Petroselinum crispum*; **EPM:** Elevated Plus Maze; **GPx:** Estimation of Glutathione Peroxidase; **ITL:** Initial Transfer Latency; **MDA:** Malondialdehyde; **MWM:** Morris Water Maze Test; **OFT:** Open Field Test; **STL:** Second Transfer Latency; **SOD:** Superoxide Dismutase.

CONFLICT OF INTEREST

The authors declare that that there is no conflict of interest.

ETHICAL STATEMENT

The entire research work on animals was done in strict adherence to CPCSEA norms (KMCRET/ReRc/Mpharm/10/2021).

SUMMARY

The crux of our research work was to investigate the neuro-protective effect of *Petroselinum crispum* (Parsley) ethanolic extract on rats based on literature backup. The AD was induced by the combination of D-Gal and $AlCl_3$, making the model very promising and convenient. All groups except the control were exposed to D-gal 60 mg/kg/day, i.p. and $AlCl_3$ 200 mg/kg/day, p.o. Additionally, donepezil 1 mg/kg/day, p.o. and ethanolic extract of *Petroselinum crispum* 100 mg/day, p.o. as well as 200 mg/day, p.o. were administered to rats for 70 days, respectively. The behavioral study was performed using modified EPM, MWM, and OFT, and the biochemical parameters such as AchE inhibitory activity, antioxidant activity (SOD, GPx, and CAT), and MDA for lipid peroxidation were measured using rat brain homogenate. In addition to that, histopathology of the hippocampus and cortex was done on all groups of the rat brain. EPC establishes a potent anti-AD activity by its significant behavioral activity, AchE inhibitory activity, and lipid peroxidation inhibitory effect, as well as moderate free radical scavenging activity and, above all, by its almost complete reversal of AD pathology as compared to the disease control group. The results conclude that EPC might be a good alternative for the treatment of AD.

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Cite this article: Aswin P, Sivakumar G. Protective Effects of *Petroselinum crispum* Ethanolic Extract against D-Galactose and Aluminum Chloride-Induced Alzheimer's Disease in Rats: A Behavioral and Biochemical Approach. Indian J of Pharmaceutical Education and Research. 2025;60(1s):s194-s204.