Pharmacogn. Res., 2026; 18(4):1318-1329.
https://www.phcogres.com

Original Article

Neuroprotective Effect of Chloroform Fraction of *Pterocarpus marsupium* Roxb Bark and its Isolated Compound (Afzelechin)

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ABSTRACT

Background: Neuroprotection is crucial in the development of novel therapy for the treatment of neurodegenerative disorders. The present study was aimed to evaluate the neuroprotective effects of Chloroform Fraction of *Pterocarpus marsupium* Roxb Bark (CFPMB) and its isolated compound (Afzelechin) on Aluminium Chloride ($AlCl_3$) induced neurodegeneration in *Sprague Dawley* rats. **Materials and Methods:** Acute toxicity was assessed for CFPMB and its isolated compound (Afzelechin). All the animals except the vehicle-treated group received $AlCl_3$ (17 mg/kg/p.o) for 21 days for the induction of neurodegeneration. Group III, IV, V and VI were administered Donepezil Hydrochloride (3 mg/kg/p.o), CFPMB (200 mg/kg/p.o), CFPMB (400 mg/kg/p.o) and isolated compound (Afzelechin) (30 mg/kg/p.o) respectively. Body weight changes were measured on day 0, 7, 14 and 21st day. At the end of 3rd week study, behavioural changes were measured by Water maze test, elevated plus maze test, Pole climbing test and Open field test. Biochemical parameters such as Acetylcholine, Acetylcholinesterase, Dopamine, Glutamate and antioxidant level such as Superoxide dismutase, Catalase, Nitric oxide, Total protein and TBARS level were measured. After that one brain from each group were examined for histopathological changes. **Results:** CFPMB and its isolated compound (Afzelechin) significantly attenuated the $AlCl_3$ induced alterations in body weight, and behavioural parameters and increased neurotransmitters such as Acetylcholine and dopamine level. CFPMB and its isolated compound (Afzelechin) also reduced oxidative stress by increasing SOD, Catalase, Total protein level and decreasing Nitric oxide, TBARS levels and exhibited best regenerative and healing property in histopathological studies. **Conclusion:** The findings propose that CFPMB and Afzelechin isolated from CFPMB may be a possible therapeutic option for neurodegenerative disorders.

Keywords: Afzelechin, Antioxidant enzyme, Neuroprotective effect, Oxidative stress, *Pterocarpus marsupium*.

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Received: 23-02-2026;
Revised: 14-04-2026;
Accepted: 08-06-2026.

INTRODUCTION

In most countries, the numerous debilitating Neurodegenerative Disorders (NDDs) such as Alzheimer's Diseases (AD), Huntington's Disease (HD), Parkinson's Disease (PD) are believed to be the main problem and impose significant economic pressure on the health care system (Amarjot *et al*., 2021). Neuroprotection refers to the strategies and relative mechanisms able to struggle down the Central Nervous System (CNS) against neuronal damage caused by various neuropsychiatric and neurodegenerative disorders such as Alzheimer's disease, anxiety, cerebrovascular impairment, seizures, Parkinson's disease, etc. (Phani *et al*., 2015). NDDs is a hereditary or sporadic condition that results in slow and irreversible loss of neurons and their processes (axons, dendrites, synapses) with a corresponding progressive impairment in neuronal function (Ponmalai *et al*., 2020). NDDs such as Alzheimer's disease resulted in very serious problem to patients themselves and also made their society pay huge expenses (Jung *et al*., 2020). Parkinson's disease is defined by the degeneration of dopaminergic neurons in the *substantia nigra*, which ultimately leads to the death of these neurons. The depletion of neurons leads to motor dysfunctions characterized by tremors, rigidity, and bradykinesia (Mateusz *et al*., 2024).

Pterocarpus marsupium Roxb. (*P. marsupium* Roxb) belonging to the family *fabaceae* is popularly known as Indian Kino tree or Bijasar or Vijaysar in Hindi (Anshul *et al*., 2013). The phytochemicals isolated from *P. marsupium* Roxb exhibit a diverse range of pharmacological activities such as antidiabetic, anti-inflammatory, antioxidant, hepatoprotective, cardioprotective, neuroprotective and wound-healing effects

DOI: 10.5530/pres.20260269

(Vrushali *et al*., 2025). Traditionally stem of *P. marsupium* Roxb have been used for the treatment of neurological problems (Bhupendra *et al*., 2012). *P. marsupium* Roxb has shown neuroprotective, analgesic, and memory enhancing activities in animal models (Sakshiparsutkar *et al*., 2025).

Oxidative stress, necrosis, cytotoxicity, ions imbalance, mitochondria dysfunction, cellular inflammation, apoptosis, increased blood-brain permeability, and morphological changes are pathological alterations in response to injuries, which aggravate medical conditions and give hints to screen alternative neuroprotection approaches (Muneeb *et al*., 2019).

Oxidative stress is mainly related to secondary cell death in many central nervous system disorders (Aditi *et al*., 2020). Living cells of man, animals and plants when exposed to external environment leads to the formation of free radicals and tissue damage resulting in diseases such as atherosclerosis, heart failure, neurodegenerative disorders, aging, cancer, diabetes mellitus, hypertension and several other diseases and are becoming increasingly recognized (Deepa *et al*., 2014).

Flavonoids are capable of crossing the Blood-Brain Barrier (BBB), which makes them potential agents in preventing neurodegenerative disorders (Haroon *et al*., 2020). Flavan-3-ols have been reported to exhibit several health beneficial effects by acting as antioxidant, anticarcinogen, cardio-preventive, antimicrobial, anti-viral, and neuro-protective agents (Patricia *et al*., 2008).

The aim of this study was to investigate possible neuroprotective effect of the Chloroform Fraction of *Pterocarpus marsupium* Roxb Bark (CFPMB) and its isolated compound (afzelechin) in Aluminium Chloride ($AlCl_3$) induced neurodegeneration in *Sprague dawley* rats in order to point out the role of this plant and its isolated compound (afzelechin) as potential sources for the development of therapeutic agents for neurodegenerative disorders.

MATERIALS AND METHODS

Plant material

P. marsupium Roxb plant parts were collected from Vedhagireeswar hills, Bhavani, Erode district, Tamil Nadu state, India, during the month of November-December. It was dried under shade at temperature not exceeding 40°C. It was authenticated by Botanical Survey of India, Coimbatore, Tamil Nadu. The authentication certificate number is BSI/SRC/5/23/2023/Tech-597 dated 17-08-2023.

Preparation of CFPMB (Harborne 1998)

About 1 kg o*f P. marsupium* Roxb bark was dried under shade. The dried material was ground into powder by using mechanical grinder. Then this powder was passed through sieve no. 40 to get uniform powder. This bark powder was successively extracted by soxhlet apparatus with solvents of increasing polarity, i.e. n-hexane, chloroform, ethyl acetate and ethanol until the change of colour of solvent. The content of round bottom flask was evaporated in the dry air in boiling water bath to get the extract. The percentage yield, consistency and colour of the extracts were noted. Then the extracts were transferred to sterile container and labelled.

Experimental animals

Sprague Dawley rats (150-200 g) were used for the experiments. Animals were purchased from Mass Biotech (Regn No.2084/PO/RcBt/S/19/CPCSEA), Chengalpet, Tamil Nadu, India and maintained at standard housing conditions. A standard commercially available diet was provided with water *ad libitum* during the experiment. The animals were kept in clean and dry polycarbonate cages and maintained in a well-ventilated animal house with 12 hr light/dark cycle. All the experimental procedures were carried out in accordance with the CPCSEA guidelines. The experimental protocol was approved by Institutional Animal Ethics Committee (Approval no. JKKMMRAFCP/IAEC/2024/012, dated 20.07.2024) for conducting this study.

Acute Oral Toxicity Study (OECD 2001)

Acute toxicity study of the CFPMB and compound isolated from CFPMB (Afzelechin) were performed according to OECD-guidelines 423. Three animals of same sex were used in each group. The CFPMB and isolated compound were administered to each group at 5, 50, 300 and 2000 mg/kg (p.o) respectively. The animals were fasted over-night before the administration of extract. Animals were observed regularly for 14 days for any signs and symptoms of toxicity.

Neuroprotective effect of CFPMB and Isolated compound (Afzelechin)

In this study, CFPMB and Isolated compound (Afzelechin) were tested for their reversibility of neurodegeneration caused by $AlCl_3$ administration at 17 mg/kg/p.o (Prachi *et al*., 2023) for 21 days. 6 groups of 8 rats weighing between 150-200 g in each group were divided randomly.

Group - I (Vehicle control) rats were administered with Distilled water. Group- II rats were administered with $AlCl_3$ (17 mg/kg/p.o) only. Group - III rats were administered with $AlCl_3$ (17 mg/kg/p.o) + Donepezil hydrochloride (3 mg/kg, p.o.) (Laxmi *et al*., 2017). Group IV and V were administered with $AlCl_3$ (17 mg/kg/p.o) and CFPMB (200 mg/kg/p.o and 400 mg/kg/p.o) respectively and Group - VI rats were administered with $AlCl_3$ (17 mg/kg/p.o) and isolated compound (30 mg/kg, p.o). After 24 hr of the administration of the last dose, animals were subjected to behavioural tests and finally sacrificed and the brain was extracted for histopathological studies and biochemical analysis.

Examination of general behaviour

Change in Body weight

The change in body weight of individual animals was recorded once in a week (Hemlata *et al*., 2021).

Behavioural pharmacology studies

Morris water maze test

Morris water maze test is a method to assess spatial or place learning (Ponmalai *et al*., 2021). It consists of an open circular pool with having a diameter of 100 cm and 50 cm in height with a featureless interior surface. A circular platform was hidden 2 cm below the water level. The circular pool was filled with water maintained at a constant temperature of 23±1°C and camouflaged with 500 mL of milk. All the animals were trained with 3 trials per day with the interval of 5-10 min for 5 days a week. The animals were allowed to stay in the water maze for maximum period of 2 min. Each trial started from one of four assigned polar positions with a different sequence each day. The first step to calculate learning is the escape latency. It is the time taken by the animal to reach the platform.

Pole climbing test

Cook's pole climbing test apparatus was used to study the effect of test compounds on Aluminium Chloride induced memory impaired animals. The apparatus consists of a 25 cm × 25 cm × 40 cm chamber along with dim light and sound box. The electric shock was given to animals in grid floor of the chamber. The animals avoided electric stimulus by jumping onto the pole. This jumping was noted as an escape time of the animals to avoid foot shock. However, jumping on to the pole before shock due to buzzer sound was considered as avoidance. The experiment was terminated after 5 trials with intervals of 30 sec. A significant reduction in escape latency time was considered as successful retention of avoidance memory (Shukla *et al*., 2021).

Elevated Plus Maze Test (EPM)

EPM is a conflict paradigm that consists of two closed arms (length 30 cm × width 5 cm × height 15 cm), two open arms (length 30 cm × width 5 cm), and a central platform (5 cm × 5 cm). The maze was elevated 15 cm above in a dimly illuminated room. Rats were placed individually into the center of the maze, facing open arms. The time spent and number of entries in open arms was recorded for 5 min. Increased activity in the open arms was interpreted as an index of potential anxiolytic activity (Kumar *et al*., 2021).

Open Field Test (OFT)

The OFT provides a useful method to simultaneously measure of the exploratory behaviour, total locomotor activity levels and anxiety-like behaviour in rodents. The test is based on the tendency of the rodents to explore the novel area and avoid bright light. The open field is an enclosed space, with surrounding walls that prevent the animal from escaping. The field is marked with a grid and square crossings. The center of the field is marked with a different colour to differentiate from the other squares. On the 22nd day of the experiment, the animals were subjected to OFT. The animals were placed in the open field and observed for 5 min. The behaviour assessments included line crossings referring the rate with which the animals cross a grid line with all four paws. These behaviours indicated change in exploratory behaviour (Kada *et al*., 2022).

Estimation of Biochemical parameters

Brain Tissue Homogenate Preparation

Rats were sacrificed with mild ether anaesthesia and brain dissected out, washes it thoroughly with saline solution, and divided into two halves. One-half of the brain of each rat was homogenized instantaneously in a solution containing Tris-HCl (50 mM, pH 7.4) and sucrose (300 mM). The tissue homogenate was centrifuged at 10000 RPM for 10 min at 4°C, and the supernatant was separated for the biochemical estimation (Ramachandran *et al*., 2020).

Estimation of Acetylcholine (Ach) content

1 mL of rat brain homogenate was placed in a boiling water bath for 5 min to terminate the Acetylcholinesterase enzyme activity and also to release the bound ACh. To the homogenate, 1 mL of alkaline hydroxylamine hydrochloride was added followed by 1 mL of 50% hydrochloric acid solution. The contents were mixed thoroughly and centrifuged. To the supernatant, 0.5 mL of0.37 M ferric chloride solution was added and the brown colour developed was read at 540 nm against a reagent blank (1 mL of alkaline hydroxylamine hydrochloride +1 mL of 50% hydrochloride + 1 mL of distilled water + 0.5 mL of 0.37 M ferric chloride solution) in a spectrophotometer. The Acetylcholine content was expressed as µ moles of ACh/gm wet weight of tissue (Santhi *et al*., 2020).

Determination of AChE Activity

The brain concentration of acetylcholinesterase was estimated by the method of Ellman *et al* with slight modification. Brain homogenate (0.1 mL) was mixed with 6 mL of 0.1M sodium phosphate buffer (pH 8), 0.2 mL of 0.075M acetylthiocholine iodide, and 0.01 mL of 0.01M5,5'-dithio-bis- (2-nitrobenzoic acid (DTNB, the Ellman reagent). The changes in the absorbance of the mixture were measured at 412 nm. The results were expressed as µM of acetylthiocholine hydrolysis per milligram of protein (Ponmalai *et al*., 2021).

Estimation of Dopamine level

The dopamine level in rat brain was estimated by admixing of homogenised supernatant liquid (1 ml) with 1 mL of ferric chloride (1.5 × 10-2 M) and 1 mL of potassium ferricyanide (1.5

× 10-2 M) in 25 mL distilled water. It was kept aside for 30 min and the developed colour was estimated using the UV-visible double beam spectrophotometer at 735 nm. The results were expressed as mole of dopamine per mL of supernatant liquid (Chandravadivelu *et al*., 2019).

Estimation of Glutamte content

The supernatant from brain homogenate was evaporated to dryness at 70ºC in an oven and the residue was reconstituted in 100 mL double distilled water. The 2 mM glutamate standard solution with the sample was spotted on Whatman no.1 chromatography paper using a micropipette. It will be placed on a chamber containing butanol: acetic acid: water (12: 3: 5 v/v) as solvent. When the solvent front reached the top of the paper, it was removed and dried and sprayed with ninhydrin reagent and placed in an oven at 100ºC for 4 min. The portions which carry glutamate corresponding with the standard was cut and eluted with 0.005% CuSo4 in 75% ethanol. Their absorbance was read against blank at 515 nm in spectrophotometer. The concentration of glutamate was expressed as µmol/gram wet weight tissue (Phani *et al*., 2017).

Evaluation of antioxidant activity

Estimation of Superoxide Dismutase (SOD)

The supernatant (500 µL) was added to 0.8 mL of carbonate buffer (100 mM, pH 10.2) and 100 µL of epinephrine (3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 sec. Parallel blank was run for determination of SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto-oxidation. The reaction mixtures are diluted 1/10 just before taking the readings in the spectrophotometer (Prakash *et al*., 2017).

Estimation of Catalase (CAT)

The activity of CAT was measured as the amount of hydrogen peroxide consumed per minute per milligram of the protein assayed by the method of Takahara *et al*. (1960). To 1.2 mL of 50 mM phosphate buffer pH 7.0, 0.2 mL of the tissue homogenate was added and reaction was started by the addition of 1.0 mL of 30 mM H2O2 solution. The decrease in absorbance was measured at 240 nm at 30 sec intervals for 3 min. The enzyme blank was run simultaneously with 1.0 mL of distilled water instead of hydrogen peroxide. The enzyme activity was expressed as nanomoles of H2O2 decomposed per minute per milligram protein (Manju *et al*., 2010).

Determination of Nitric oxide (NO)

The production of NO in the brain may occur due to oxidative stress and it can be determined by estimation of nitrite level. The nitrite level was determined spectrophotometrically with Griess reagent (0.1% N-1-naphthyl ethylene amine dihydrochloride, 1% sulphanilamide and 2.5% phosphoric acid). Brain homogenate and Griess reagent was mixed equally and this mixture was incubated for 10 min and the absorbance was measured at 546 nm. The standard curve of sodium nitrite was prepared and the concentration of nitrite in the supernatant was determined from standard curve (Souravh *et al*., 2015).

Estimation of Protein

Protein concentrations of the tissue homogenates were determined by the standard method of (Lowry *et al*., 1951) using bovine serum albumin as the standard. As per this method, colour change of the sample solution was in proportion to protein concentration which was measured using colorimetric techniques. 1 mL of brain homogenate was taken and mixed with 10 mL buffer (N/10 Acetic acid and N/10 Sodium acetate). Then it was centrifuged at 2500 rpm and supernatant was collected. About 0.5 mL supernatant was taken and 0.5 mL distilled water was added to it, and to this about 5 mL of alkaline solution (NaOH+ Sodium potassium tartarate) and 0.5 mL of folin reagent was added. The optical density was measured at 600 nm (Charushila *et al*., 2024).

Estimation of brain Lipid Peroxidation

The quantitative measurement of lipid peroxidation in the brain was performed according to the method of Wills (1966). In this, 0.1 mL of supernatant was incubated with 0.5 mL Tris-HCl (0.1 M, pH 7.4) for 2 hr. To this, 1 mL of trichloroacetic acid (10%, w/v) was added and centrifuged at 1,000×g for 10 min. To 1 mL supernatant, 1 mL (0.67%, w/v) Thiobarbituric Acid (TBA) was added and kept in the boiling water bath for 10 min, cooled, and added 1 mL distilled water. The amount of lipid peroxidation products was measured by reaction with TBA at 532 nm using the spectrophotometer (UV-1700, Shimadzu, Japan). The values were calculated using molar extinction coefficient of chromophore (1.56×105 M−1 cm−1) and expressed as micromoles per milligram protein (Dinesh *et al*., 2012).

Histopathological Studies

After behavioural studies, one rat from each group was sacrificed under mild ether anaesthesia and brain were immediately removed and preserved in 10% formalin and send for histopathological examination (Srinivasa *et al*., 2015).

Statistical Analysis

The experimental results were reported as means with SEM. Statistical analysis was performed using GraphPad Prism software. Analysis of variance (ANOVA) and Tukey's Multiple Comparison test were used to compare the experimental groups with the controls. $p < 0.05$ were considered as statistically significant.

RESULTS

Acute toxicity of CFPMB and its isolated compound (Afzelechin)

There was no mortality and the animals displayed normal behaviour and did not reveal any abnormality or pathological significance. We found that the CFPMB and its isolated compound (afzelechin) were safe when given orally and no drug-related toxicity was observed even at the highest dose 2000 mg/kg and 300 mg/kg respectively.

Effect of CFPMB and Afzelecin on body weight

In comparison to the Normal control group animals, the $AlCl_3$ only treated animals were found to be decreased in body weight throughout the 21-day treatment period. Treatment with CFPMB (200 mg/kg, 400 mg/kg) and isolated compound (30 mg/kg) prevented the decrease in body weight due to $AlCl_3$ treatment (p<0.001) as shown in Table 1.

Effect of CFPMB and its isolated compound (Afzelechin) on Behavioural parameters

Administration of $AlCl_3$ (17 mg/kg/p.o) produced significant impairment in learning and memory in $AlCl_3$ only treated group when comparing to the normal control group (p<0.001) (Table 2). This impairment was indicated by increase in escape latencies in water maze test to reach the hidden platform and increase in Escape latency (sec) in Pole climbing test to jump to the pole to avoid electric shock and decrease of time spent in open arm and increase of time spent in closed arm in Elevated plus maze test and decreased number of lines crossed in open field test.

Effect of CFPMB and its isolated compound (Afzelechin) on Biochemical parameters

Administration of $AlCl_3$ (17 mg/kg/p.o) produced significant decrease in acetylcholine and dopamine level and increase in acetylcholinesterase and glutamate level in $AlCl_3$ only treated group when comparing to the normal control group (p<0.001) (Table 3). The rats that treated with CFPMB (400mg/kg /p.o) and Afzelechin (30 mg/kg/p.o) exhibited increase in acetylcholine and dopamine level and decrease in acetylcholinesterase and glutamate level when compared to $AlCl_3$ only treated group (p<0.001) (Table 3).

Effect of CFPMB and its isolated compound Afzelechin on antioxidant system

$AlCl_3$ (17 mg/kg/p.o) only treated group showed significant decrease in Super oxide dismutase, Catalase and Total protein level and increase in Nitric oxide and TBARS level when comparing to the normal control group (p<0.001) (Table 4). The rats that treated with CFPMB (400 mg/kg/p.o) and Afzelechin (30 mg/kg/p.o) exhibited increase in Super oxide dismutase, Catalase and Total protein level and decrease in Nitric oxide and TBARS level when compared to $AlCl_3$ only treated group (p<0.001) (Table 4).

Table 1: Effect of CFPMB and Afzelecin on body weight.

Group (n=6)	Treatment	Day 0 (g)	7th Day (g)	14th Day (g)	21th Day (g)
I	Distilled water (p.o)	184.67±1.33	187.66±1.892	188.67±2.17	190.67±2.17
II	$AlCl_3$ (17 mg/kg/p.o)	182±1.16	175.67±1.59*	165.66±1.89***	160.66±1.89***
III	$AlCl_3$ (17 mg/kg/p.o)+ Donepezil hydrochloride (3 mg/kg, p.o.)	185.67±1.59	187.66±1.892ns	190.67±1.52***	192.67±1.52***
IV	$AlCl_3$ (17 mg/kg/p.o)+ CFPMB (200 mg/kg, p.o)	184±1.71	184.67±1.33ns	185.67±2.17***	186.67±2.17***
V	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (400 mg/kg, p.o)	185.67±1.59	188.67±2.17*	190.67±1.52***	192.67±1.52***
VI	$AlCl_3$ (17 mg/kg/p.o)+ Afzelechin (30 mg/kg, p.o)	185.67±1.59	187.67±1.59***	189.67±1.59***	191.67±1.59***

Values are expressed as Mean ± SEM, (n=6). One-way ANOVA followed by Tukey's Multiple Comparison test was performed. Group II was compared with Group I. Group III to VI was compared with Group II. ns - not significant, *p<0.05; **p<0.01; ***p<0.001.

Histopathological Studies

Effect of CFPMB and its isolated compound Afzelechin on Cerebral cortex and Hippocampus

The rats treated with Afzelechin (30 mg/kg/p.o) exhibited best regenerative and healing property and CFPMB (400 mg/kg /p.o) treated rats exhibited good regenerative and healing property in par with standard drug donepezil treated group. CFPMB (200 mg/kg /p.o) treated rats exhibited least regenerative and healing property.

DISCUSSION

Many age-related problems and neurodegenerative diseases are driven by enhanced oxidative stress, therefore the search for natural medicinal agents that boost cognitive function and enable neuroprotective effects via antioxidant action is of significant interest (Danni *et al*., 2023). In the current study, CFPMB and its isolated compound (Afzelechin) alleviated the behavioural, biochemical, and neurochemical problems and have potential as a neuroprotective drug.

Administration of $AlCl_3$ (17 mg/kg/p.o) produced significant impairment in learning and memory in $AlCl_3$ only treated group when comparing to the normal control group (p<0.001) (Table 1). This indicates that there is a severe degeneration in the brain as a result of exposure to $AlCl_3$, which contributes to deficits in episodic memory and recognition capacity. Aluminium is a pro-oxidant and indirectly results in the production of free radicals leading to oxidative damage and reduced levels of ROS, which indirectly affect acetyl cholinesterase enzyme activity (Suvarchala *et al*., 2020). The possible mechanism of $AlCl_3$ induced neurotoxicity may involve severe oxidative stress followed by inflammatory changes leading to neurodegeneration. $AlCl_3$ can cause degeneration of cholinergic nerve terminals in cortical and hippocampus areas leading to cellular depletion and severe learning disability (Mundugaru *et al*., 2017).

The Morris maze was employed to assess spatial memory (Venkataramaiah *et al*., 2018) and animals administered with $AlCl_3$ alone had taken longer time to reach the position of the hidden platform. Treatment with CFPMB and Afzelechin decreased the escape latency in morris water maze test. Cooks pole climbing apparatus is widely used to assess the ability of an

Table 2: Effect of CFPMB and isolated compound (Afzelechin) on Behavioural pharmacology studies.

Group (n=6)	Treatment	Escape latency (sec) in water maze test	Escape latency time (sec) in Pole climbing test	Time spent in Arms (sec) in Elevated plus maze test		Number of line crossed in Open Field Test
				Open Arm	Closed Arm	
I	Distilled water (p.o)	15.00 ± 1.18	8.83±0.65	49.00±7.47	249.17±7.84	70.00±10.36
II	$AlCl_3$ (17 mg/kg/i.p)	100.00± 6.41**	83.33±3.89***	13.83±2.12***	286.17±2.12***	56.00±8.08ns
III	$AlCl_3$ (17 mg/kg/p.o) + Donepezil hydrochloride (3 mg/kg, p.o)	16.83±1.64**	25.17±0.95***	40.67±4.00**	259.33±4.00**	74.33±10.92 ns
IV	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (200 mg/kg p.o)	45.67± 1.94**	84.50±1.65ns	19.17±6.18ns	279.17±6.36 ns	45.50±11.74 ns
V	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (400 mg/kg p.o)	34.33±2.06**	37.50±0.76***	30.17±1.54 ns	269.00±1.73 ns	70.67±5.63 ns
VI	$AlCl_3$ (17 mg/kg/p.o) + Afzelechin (30 mg/kg, p.o)	17.17±1.99**	32.33±4.22***	38.00±2.02**	261.33±2.16**	72.83±7.68 ns

Values are expressed as mean ± SEM, (n=6). One-way ANOVA followed by Tukey's Multiple Comparison test was performed. Group II was compared with Group I. Group III to VI was compared with Group II. ns - not significant, *p<0.05; **p<0.01; ***p<0.001.

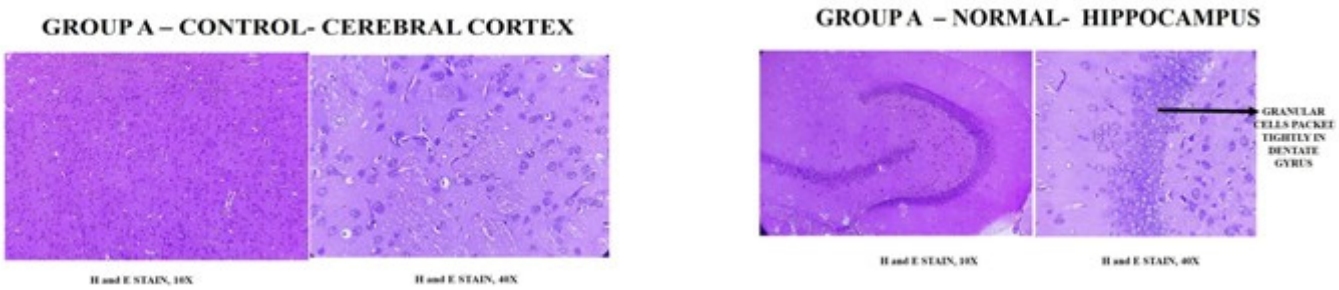

Figure 1: Group I - Distilledwater (p.o) treated group. H and E stained section shows brain with Hippocampus and ce rebral cortex. The hippocampus proper show layers of pyramidal cells and dentate gyrus show densely packed granular cells. The cerebral cortex show granular cells, pyramidal cells, blood vessels.

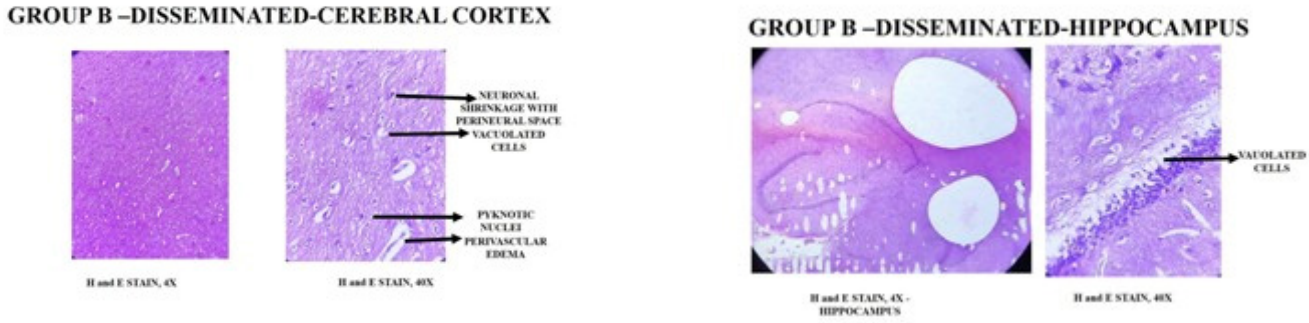

Figure 2: Group II - $AlCl_3$ (17 mg/kg/p.o). H and E stained section shows brain. The hippocampus region show decrease in thickness of pyramidal cells in CA1, CA2, CA3, CA4 region of hippocampus proper. Pyramidal cells are separated from each other showing irregular outline. More vacuolated cells are observed in the dentate gyrus region below the granular layer. The lateral ventricles are dilated. The cerebral cortex show dilated and congested blood vessels, with perivascular edema. Neuronal shrinkage with perineuralvacuolation, vacolated cells, gliosis and pyknotic nuclei are noted in higher magnification.

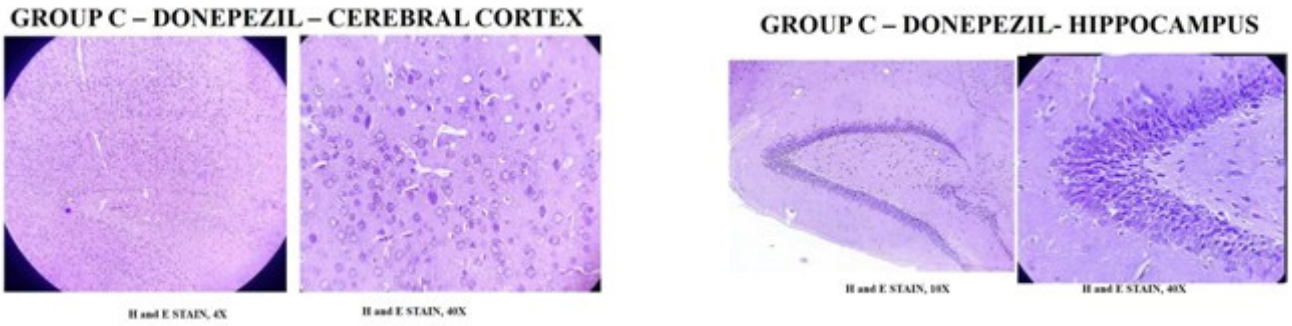

Figure 3: Group III - $AlCl_3$ (17 mg/kg/p.o) + Donepezil hydrochloride (3 mg/kg, p.o.). H and E stained section shows densely packed granular cells in dentate gyrus,vacuolation below granular layer is not seen. The thickness of hippocampus proper is almost normal. Granular cells, pyramidal cells, blood vessels are noted with perivascular edema at very few places.

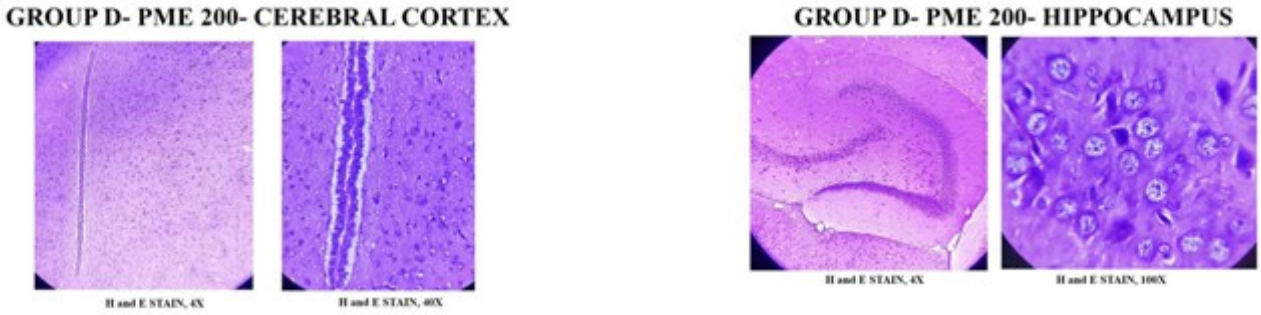

Figure 4: Group IV - $AlCl_3$ (17 mg/kg/p.o) + CFPMB (200 mg/kg, p.o). H and E stained section shows brain with hippocampus proper and dentate gyrus. No vacuolated cells below the granular layer are seen. Perivascular edema with congested blood vessels, vacuolated cells, neuronal shrinkage are observed at few places.

Table 3: Effect of CFPMB and Afzelechin on Biochemical parameters.

Group (n=6)	Treatment	Acetylcholine level (µ moles of Ach/gm wt of wet tissue)	Acetylcholinestrase (AChE) activity (µM of acetylthiocholine hydrolysis per milligram of protein)	Dopamine level (Mole/mL)×10^{-5}	Glutamate level (µ moles/gram wet weight tissue)
I	Distilled water (p.o)	0.959 ± 0.039	87.75±2.48	2.27±0.09	1.41±0.04
II	$AlCl_3$ (17 mg/kg/i.p)	0.237±0.006***	223.42 ± 3.53***	0.46 ± 0.03***	3.68±0.19***
III	$AlCl_3$ (17 mg/kg/p.o) + Donepezil hydrochloride (3 mg/kg, p.o.)	0.828 ± 0.026***	101.46 ± 2.13***	2.16 ± 0.02***	1.65±0.06***
IV	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (200 mg/kg, p.o)	0.465 ± 0.025**	180.78 ± 3.28***	1.04 ± 0.02***	3.03±0.15*
V	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (400 mg/kg, p.o)	0.617 ± 0.031***	141.24 ± 2.17***	1.66 ± 0.09***	2.72±0.06***
VI	$AlCl_3$ (17 mg/kg/p.o) + Afzelechin (30 mg/kg, p.o)	0.776 ± 0.046***	121.19 ± 1.66***	2.00 ± 0.03***	2.07±0.07***

Values are expressed as Mean ± SEM, (n=6). One-way ANOVA followed by Tukey's Multiple Comparison test was performed. Group II was compared with Group I. Group III to VI was compared with Group II. ns - not significant, *p<0.05; **p<0.01; ***p<0.001.

animal to acquire, retention and retrieve the memory process and which was indicated by increasing number of avoidance response (Reddy *et al*., 2020). Treatment with CFPMB and Afzelechin decreased the escape latency time in Cooks pole climbing test. The Elevated Plus Maze task is widely used to predict the anxiety responses of drugs in rodents (Sahba *et al*., 2019). Animals which were received only $AlCl_3$ decreased in the number of entries in open arm, and increased in the number of entries in closed arm. The treatment with CFPMB and Afzelechin reverse effects on $AlCl_3$ induced neurodegeneration in rats. The open field test is a criterion for detecting the level of neuronal excitability and analysing the influence of drug therapy on general behavior (Gagarani *et al*., 2022). The memory deficit caused by $AlCl_3$ was reversed in animals treated with CFPMB and Afzelechin demonstrates the elevated memorizing capacity of CFPMB and Afzelechin. Improvement in behavioural abnormalities may due to the presence of flavonoids in CFPMB as flavonoids have been reported to have neuroprotective effects (Paolo *et al*., 2023).

The cholinergic function is vital and is actively involved in the process of learning and memory, and its alteration directly leads to the development of cognitive impairment (Amandeep *et al*., 2021). The dopaminergic system plays important roles in neuromodulation, such as motor control, motivation, reward, cognitive function, maternal, and reproductive behaviours (Marianne *et al*., 2018). $AlCl_3$ only Administered rats showed decrease in acetylcholine and dopamine level (p<0.001) (Table 3) and Treatment with CFPMB and Afzelechin reversed acetylcholine and dopamine levels.

Acetylcholinesterase (AchE) is a broadly distributed particularly potent enzyme in the brain that has several roles in cholinergic and neuromuscular synapses and is linked with building and preserving learning memory in the brain (Danni *et al*., 2023). The elevations in AChE are either a direct result of the neurotoxic effect of metals or due to increased lipid peroxidation. AchE is a biologically important enzyme that hydrolyses acetylcholine thereby terminating the cholinergic neurotransmission (Pandy *et al*., 2022). AChE inhibition is an important target for the management of Alzheimer disease (Natalie *et al*., 2015). Glutamate is a neurotransmitter that can cause excitatory neurotoxicity when its extracellular concentration is too high, leading to disrupted calcium balance and increased production of reactive oxygen species (Huizhen *et al*., 2024). $AlCl_3$ only Administered rats showed increase in acetylcholinesterase and glutamate level (p<0.001) and Treatment with CFPMB and Afzelechin reversed acetylcholinesterase and glutamate level (p<0.001). The active constituents like phenolics, flavonoids, had shown improvement in cognitive function by inhibiting AChE (Suvarchala *et al*., 2020).

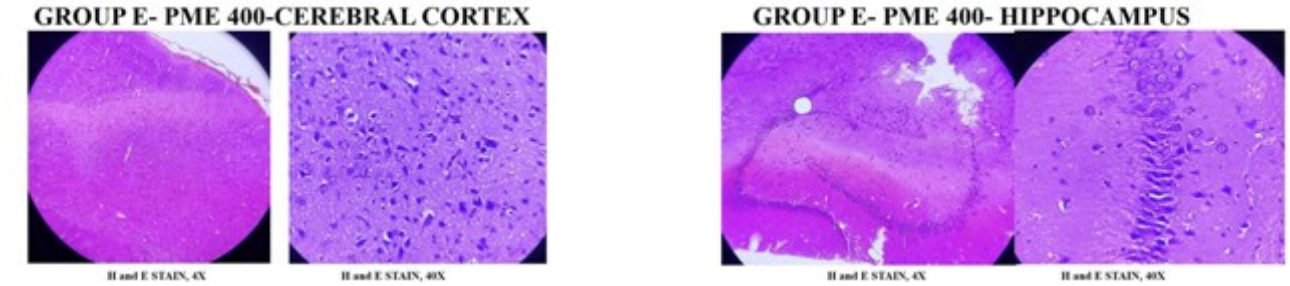

Figure 5: Group V- $AlCl_3$ (17 mg/kg/p.o) + CFPMB (400 mg/kg, p.o). H and E stained section shows brain with slightly reduced thickness in hippocampus region. Lateral ventricles are normal in size. No vacuolation in dentate gyrus region. Cerebral cortex is normal with pyramidal cells, granular cells and blood vessels.

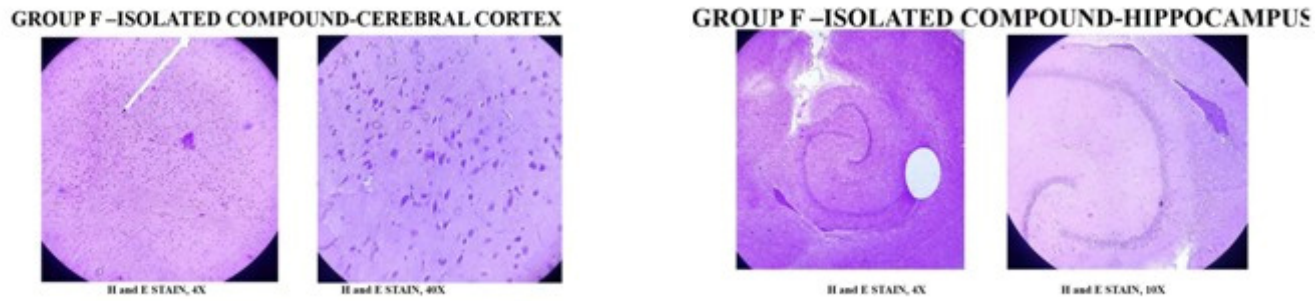

Figure 6: Group VI- $AlCl_3$ (17 mg/kg/p.o) + Afzelechin (30 mg/kg, p.o). H and E stained section shows normal hippocampus and cerebral cortex. Lateral ventricle size is normal.

Table 4: Effect of CFPMB and Afzelechin on antioxidant system.

Group (n=6)	Treatment	Superoxide Dismutase (SOD) level (units/min/ mg protein)	Catalase level µmoles of catalase/mg protein	Nitric Oxide (NO) level (µmole/ mL of tissue)	Total protein (mg/mL)	TBARS (µmol/mg protein)
I	Distilled water (p.o)	1.87±0.03	377.17±16.00	91.17 ± 2.79	1.74±0.07	3.73±0.22
II	$AlCl_3$ (17 mg/kg/i.p)	0.35±0.14 ns	43.19±5.16***	452.33±18.15***	0.40 ± 0.06***	8.41 ± 0.20***
III	$AlCl_3$ (17 mg/ kg/p.o) + Donepezil hydrochloride (3 mg/kg/p.o)	1.81±0.03 ns	364.42±21.50***	115.33 ± 6.55***	1.61 ± 0.05***	4.02 ± 0.05***
IV	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (200 mg/kg/p.o)	0.93±0.02ns	169.47±22.71***	310.33 ± 1.38***	0.81 ± 0.06**	6.66 ± 0.12***
V	$AlCl_3$ (17 mg/kg/p.o) + CFPMB (400 mg/kg/p.o)	1.39±0.04 ns	285.42±8.06***	240.50 ± 1.43***	1.02 ± 0.05***	5.50 ± 0.15***
VI	$AlCl_3$ (17 mg/kg/p.o) + Afzelechin (30 mg/ kg/p.o)	1.74±0.04 ns	326.33±16.25***	159.50 ± 12.68***	1.33 ± 0.04***	4.63 ± 0.05***

Values are expressed as Mean ± SEM, (n=6). One-way ANOVA followed by Tukey's Multiple Comparison test was performed. Group II was compared with Group I. Group III to VI was compared with Group II. ns - not significant, *p<0.05; **p<0.01; ***p<0.001).

$AlCl_3$ only treated rats shown elevated oxidative stress, as evidenced by a significant rise in lipid peroxidation as well as nitrite concentrations, along with a reduction in superoxide dismutase, catalase, and total protein levels in our study. The rats treated with CFPMB (400mg/kg /p.o) and Afzelechin (30 mg/kg/p.o) exhibited increase in Catalase and Total protein level and decrease in Nitric oxide and TBARS level (p<0.001) (Table 4). Neuroprotective activity of CFPMB and its isolated compound Afzelechin is related to its antioxidant properties, due to which the susceptible neurons are subjected to less oxidative stress leading to reduced neuronal harm and enhanced neuronal function.

The hippocampus and Dentate Gyrus of brain are mainly responsible for memory formation (Bindhu *et al*., 2019). Hippocampus and cortex neuronal are normal and healthy in normal control group rats (Figure 1) whereas hippocampus and cortex neuronal cell shrinkage was observed in $AlCl_3$ (17 mg/kg/p.o) only treated rats (Figure 2). Treatment with Donepezil hydrochloride (3 mg/kg, p.o.) protected hippo¬campus and Dentate Gyrus neurons from $AlCl_3$-induced neurotoxicity (Figure 3). Treatment with CFPMB (200 mg/kg p.o) had some effect on hippocampus and dentate gyrus neuronal damage caused by $AlCl_3$ (17 mg/kg p.o.) but the results were not satisfactory (Figure 4). Treatment with CFPMB (400 mg/kg /p.o) (Figure 5) and Afzelechin (30 mg/kg/p.o) (Figure 6) protected hippocampus and Dentate Gyrus neurons from $AlCl_3$ induced neurotoxicity. Treatment with CFPMB and Afzelechin provided a good intracellular antioxidant potent agent against oxidative stress variables. The good neuroprotective action of CFPMB and Afzelechin was exhibited in best regenerative and healing property in histopathological studies.

CONCLUSION

In conclusion, the present study reveals that CFPMB (400 mg/kg /p.o) and Afzelechin (30 mg/kg/p.o) effectively ameliorates $AlCl_3$ induced motor deficits, cognitive impairment, and oxidative stress through its various neuroprotective mechanisms. The current study indicates that the underlying mechanism of CFPMB and Afzelechin may involve modulations of the cholinergic system, dopamine and glutamate pathway and the reduction of oxidative stress. The active principles such as flavonoids and poly phenolic compounds present in the CFPMB may be active against neurodegenerative disorders such as Alzheimer and parkinsonism. The findings of the current study propose that CFPMB and Afzelechin (flavon-3-ol) isolated from CFPMB may be a possible therapeutic option for neurodegenerative disorders.

ACKNOWLEDGEMENT

We thank Management of JKKMMRF's - Annai JKK Sampooraniammal College of Pharmacy, B. Komarapalayam - 638183, Namakkal District, Tamil Nadu, India for providing necessary facilities to carry out this work.

We thank Prof. Dr. A. Yamuna Devi, MDS., NDCH - Department of Oral Pathology and Microbiology, Nandha Dental college and Hospital, Erode-638052, Tamil Nadu, India for histopathological studies.

ABBREVIATIONS

$AlCl_3$: Aluminium chloride; **CFPMB:** Chloroform fraction of *Pterocarpus marsupium* Roxb bark; **NDDs:** Neurodegenerative disorders; **AD:** Alzheimer's disease; **HD:** Huntington's disease; **PD:** Parkinson's disease; **CNS:** Central nervous system; **SOD:** Superoxide dismutase; **TBARS:** Thiobarbituric acid reactive substances; **BBB:** Blood-brain barrier; **OECD:** Organisation for Economic Co-operation and Development; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **IAEC:** Institutional Animal Ethics Committee; **EPM:** Elevated Plus Maze; **OFT:** Open Field Test; **SEM:** Standard error of the mean; **ANOVA:** Analysis of variance; **CAT:** Catalase; **NO:** Nitric oxide; **ACh:** Acetylcholine; **AChE:** Acetylcholinesterase.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTION

Conceptualization, Data collection, Data analysis, and interpretation, drafting the article-Muthukumaran Gurupackiyam, Ph D Scholar (Part-time), Department of Pharmaceutics. JKKMMRF's - Annai JKK Sampooraniammal College of Pharmacy, B. Komarapalayam, Namakkal, Tamil Nadu, India.

Supervision - Senthilkumar Natesan, Principal and Professor, Department of Pharmaceutical Chemistry, JKKMMRF's - Annai JKK Sampooraniammal College of Pharmacy, B. Komarapalayam, Namakkal, Tamil Nadu, India.

SUMMARY

CFPMB and Afzelechin exihibited neuroprotective against $AlCl_3$ (17 mg/kg/p.o) induced neurodegeneration in Sprague Dawley rat model. Treatment with $AlCl_3$ (17 mg/kg/p.o) decreased body weight, Ach, Dopamine, SOD, Catalase level and increased AchE, Glutamate and NO, TBARS level. CFPMB (400 mg/kg, p.o) and Afzelechin (30 mg/kg, p.o) treatment suppressed AchE, Glutamate and NO, TBARS level and increased body weight, Ach,

Dopamine, SOD, Catalase level. CFPMB and Afzelechin appear to have neuroprotective effect.

REFERENCES

Anshul Chawla, J. (2013). Systemic Review: Pharmacognosy, Phytochemistry, Pharmacology and Clinical Applications of *Pterocarpus marsupium* Roxb. Kaur, Anil Kumar Sharma. International Journal of Pharmaceutical and Phytopharmacological Research, 2(5), 319–327.

Aron, P. M., & Kennedy, J. A. (2008). Flavan-3-ols: Nature, occurrence and biological activity. Molecular Nutrition and Food Research, 52(1), 79–104. https://doi.org/10.1002/mnfr.200700137

Phani Kumar. G, Anilakumar. K.R and Naveen. S.(2015). Phytochemicals Having Neuroprotective Properties from Dietary Sources and Medicinal Herbs. Pharmacognosy Journal, 7(1),1-17.

Bellavite, P. (2023). Neuroprotective potentials of flavonoids: Experimental studies and mechanisms of action. Antioxidants, 12(2), Article 280. https://doi.org/10.3390/antiox12020280

Bhintade, C. P., Dr. (2024). Pharmacological evaluation of anti depressant Potential of kaempferol in alloxan induced diabetic Rat. Hemant Kamble, Ghodke S.R, Gaikwad Ashlesha S. World Journal of Pharmaceutical Research, 13(19), 705–725.

Bhupendra Chauhan, A. K. C. (2012). Memory enhancing activity of methanolic extract of *Pterocarpus marsupium* Roxb. Phytopharmacology, 2(1), 72–80.

Bindhu, K. H., & Vijayalakshmi, A. (2019). Neuroprotective effect of *Carica papaya* Leaf extract against aluminium toxicity: An experimental study on cognitive dysfunction and biochemical alterations in rats. Indian Journal of Pharmaceutical Education and Research, 53(3s)(3 Suppl. 2), s392–s398. https://doi.org/10.5530/ijper.53.3s.111

Chandravadivelu Gopi, V.G. (2019). Effect of novel phenothiazine derivatives on brain dopamine in Wistarr ats. Sastry and Magharla Dasaratha Dhanaraju. Beni-Suef University Journal of Basic and Applied Sciences, 8, 7.

Cortes, N., Alvarez, R., Osorio, E. H., Alzate, F., Berkov, S., & Osorio, E. (2015). Alkaloid metabolite profiles by GC/MS and acetylcholinesterase inhibitory activities with binding-mode predictions of five Amaryllidaceae plants. Journal of Pharmaceutical and Biomedical Analysis, 102, 222–228. https://doi.org/10.1016/j.jpba.2014.09.022

Deepa, R., Manjunatha, H., Krishna, V., & Kumara Swamy, B. E. (2014). Evaluation of antimicrobial activity and antioxidant activity by electrochemical method of ethanolic extract of *Pterocarpus marsupium* Roxb Bark. Journal of Biotechnology and Biomaterials, 4, 166.

Dhull, D. K., Jindal, A., Dhull, R. K., Aggarwal, S., Bhateja, D., & Padi, S. S. V. (2012). Neuroprotective effect of cyclooxygenase inhibitors in ICV-STZ induced sporadic Alzheimer's disease in rats. Journal of Molecular Neuroscience, 46(1), 223–235. https://doi.org/10.1007/s12031-011-9583-6

Dongare, V., & Kolhe, S. (2025). Pharmacological screening of heartwood of *Pterocarpus marsupium* Roxb in stress induced neurobehavioral changes in albino Wistar rat. Brain Disorders, 17, Article 100194. https://doi.org/10.1016/j.dscb.2025.100194

Gagarani, M. B., & Patil, P. H. (2022). Antidepressant activity of different fractions of *Cassine albens* in an Olfactory bulbectomized Mouse Model. Indian Journal of Pharmaceutical Education and Research, 56(3s), s503–s507.

Grewal, A. K., Singh, T. G., Sharma, D., Sharma, V., Singh, M., Rahman, M. H., Najda, A., Walasek-Janusz, M., Kamel, M., Albadrani, G. M., Akhtar, M. F., Saleem, A., & Abdel-Daim, M. M. (2021). Mechanistic insights and perspectives involved in neuroprotective action of quercetin. Biomedicine and Pharmacotherapy, 140, Article 111729. https://doi.org/10.1016/j.biopha.2021.111729

Harborne, J. B. (1998). Phytochemical methods. In A Guide to modern techniques of plant Analysis Article 3rd. New Delhi, Springer (India), Pvt Ltd., 5-16.

Hemlata Dewangan, R. K. T., Sharma, V., Shukla, S. S., & Pandey, R. K. (2021). Assessment of toxicity and drug–drug interaction of combination therapy prescribed by physicians/clinicians for treatment of diabetes using experimental animals. Indian Journal of Pharmaceutical Education and Research, 55(2), S605–S615.

Jung, Y. S., Lee, H. W., & Ma, C. J. (2020). Neuroprotective effect of compounds isolated from Euonymus A latus on glutamate-induced oxidative stress in HT22 hippocampal cells. Pharmacognosy Magazine, 16, S308–S314.

Kada, A. S., Nantia, E. A., Chin, F. J., Manfo, F. P. T., Vijayakumar, N., Nchinda, J. T., & Atsamo, A. D. (2022). *Momordica foetida* (*Cucurbitaceae*) prevents behavioral impairment, motor incoordination and brain oxidative stress induced by subchronic exposure to Parastar pesticide formulation. Journal of Drug Delivery and Therapeutics, 12(3–S), 44–50. https://doi.org/10.22270/jddt.v12i3-S.5368

Kaur, A., Jaiswal, G., Brar, J., & Kumar, P. (2021). Neuroprotective effect of nerolidol in traumatic brain injury associated behavioural comorbidities in rats. Toxicology Research, 10(1), 40–50. https://doi.org/10.1093/toxres/tfaa100

Kciuk, M., Garg, N., Dhankhar, S., Saini, M., Mujwar, S., Devi, S., Chauhan, S., Singh, T. G., Singh, R., Marciniak, B., Gielecińska, A., & Kontek, R. (2024). Exploring the comprehensive neuroprotective and anticancer potential of afzelin. Pharmaceuticals, 17(6), Article 701. https://doi.org/10.3390/ph17060701

Khan, H., & Hammad Ullah, M. (2020). Neuroprotective Effects of Quercetin in Alzheimer's Disease. Aschner, Wai San Cheang and Esra Küpeli Akkol. Biomolecules, 10(59), 1–20.

Klein, M. O., Battagello, D. S., Cardoso, A. R., Hauser, D. N., Bittencourt, J. C., & Correa, R. G. (2019). Dopamine: Functions, signaling, and association with neurological diseases. Cellular and Molecular Neurobiology, 39(1), 31–59. https://doi.org/10.1007/s10571-018-0632-3

Kola, P. K., Akula, A., NissankaraRao, L. S., & Danduga, R. C. S. R. (2017). Protective effect of naringin on pentylenetetrazole (PTZ)-induced kindling; possible mechanisms of antikindling, memory improvement, and neuroprotection. Epilepsy and Behavior: E&B, 75, 114–126. https://doi.org/10.1016/j.yebeh.2017.07.011

Kumar, M., & Thamotharan, G. (2021). Comparative study of anti-anxiety and anti-depressant potentials of leaves and root of methanolic extract from *achyranthes bidentata* blume on mice. Internal Journal of Pharmaceutical Sciences and Research, 12(10), 5378–5387.

Li, D., Man, X., Ma, X., & Sun, J. (2023). Neuroprotective role of periplocin against aluminium chloride-stimulated Alzheimer's disease in a rat model by modulation of oxidative stress and inflammation. Indian Journal of Pharmaceutical Education and Research, 57(1), 147–154. https://doi.org/10.5530/001954642194

Manju versus Subramanian and TJ James. (2010). Age-related protective effect of deprenyl on changes in the levels of diagnostic marker enzymes and antioxidant defense enzymes activities in cerebellar tissue in Wistar rats. Cell Stress and Chaperones,15, 743-751.

Mukherjee, A., Madhulika Yadav, A. S., Afsar Alam, S. B., & Supradip Mandal, A. B. (2020). Review on neuroprotective activity of herbal drugs. European Journal of Pharmaceutical and Medical Research,7. Dhrubo Jyoti Sen and Beduin Mahanti, 7, 975–983.

Mundugaru, R., Sivanesan, S., Udaykumar, P., Rao, N., & Chandra, N. (2017). Protective effect of *Pluchea lanceolata* against aluminum chloride induced neurotoxicity in Swiss albino mice. Pharmacognosy Magazine, 13(Suppl. 3), S567–S572. https://doi.org/10.4103/pm.pm_124_17

OECD. (2001). Test No. 423: OECD guideline for testing of chemicals. Acute oral toxicity—Acute toxic class [Method].1–14.

Ojha, P. S., Biradar, P. R., Tubachi, S., & Patil, V. S. (2023). Evaluation of neuroprotective effects of *Canna indica* L. against aluminium chloride induced memory impairment in rats. Advances in Traditional Medicine, 23(2), 539–556. https://doi.org/10.1007/s13596-021-00627-x

Pandy, V., Shaik, B. R., Chunduru, D., & Munnangi, D. (2022). *In vitro* acetylcholinesterase inhibitory activity of different capsicum varieties by using chicken brain extract prepared by employing a home mixer-jar as an alternative to expensive tissue homogenizer. Indian Journal of Pharmaceutical Education and Research, 56(3), 810–815. https://doi.org/10.5530/ijper.56.3.132

Pattanashetti, L. A., Taranalli, A. D., Parvatrao, V., Malabade, R. H., & Kumar, D. (2017). Evaluation of neuroprotective effect of quercetin with donepezil in scopolamine-induced amnesia in rats. Indian Journal of Pharmacology, 49(1), 60–64. https://doi.org/10.4103/0253-7613.201016

Prakash, R., Sandhya, E., Ramya, N., Dhivya, R., Priyadarshini, M., & Sakthi Priya, B. (2017). Neuroprotective effect of ethanolic extract of *Tinospora cordifolia* on LPS induced neuroinflammation. Translational Biomedicine, 8(4), 1–17.

Ramachandran, V., Umakanth, S., & Ahamed, H. N. (2020). Neuroprotective effect of Leucasaspera in streptozotocin induced alzheimer rat model. Internal Journal of Pharmaceutical Sciences and Research, 11(8), 3702–3708.

Reddy, K. S., Likithasree, P., Peraman, R., Jyothi, M. V., Babu, C. N., Pradeepkumar, B., & Sudheer, A. (2020). Spatial Long-Term Memory Retention by Banana and Papaya Peel Extract: *In silico* and *in vivo* Evaluation. International Journal of Pharmaceutical Investigation, 10(2), 202–207. https://doi.org/10.5530/ijpi.2020.2.37

Rehman, M. U., Wali, A. F., Ahmad, A., Shakeel, S., Rasool, S., Ali, R., Rashid, S. M., Madkhali, H., Ganaie, M. A., & Khan, R. (2019). Neuroprotective Strategies for Neurological Disorders by Natural Products: An update. Current Neuropharmacology, 17(3), 247–267. https://doi.org/10.2174/1570159X16666180911124605

Sahba Jafarian, K.-H. L., Hassan, Z., & LuaPerimal-Lewis, M. R. (2019). Effect of zerumbone on scopolamine-induced memory impairment and anxiety-like behaviours in rats. Sulaiman, Enoch Kumar Perimal. *Alzheimer's and Dementia*: Translational Research and Clinical Interventions, 5, 637–643.

Sakshiparsutkar, & Anjaliwankhade. (2025). Therapeutic Potential of Pterocarpus marsupium: From Traditional Medicine to Modern Pharmacological Applications with Its Phytochemistry. International Journal of Pharmarmaceutical Sciences. Vivek Paithankar, 3(4), 2561–2572.

SanthiKrupa, D., & ChLochana, P. K. (2020). Antialzheimer's Potential of *Abrus pectoris* hydro alcoholic root extract. Journal of Pharmaceutical Sciences and Research, 12(7), 914–919.

Shukla, D., Srivastava, S., & Jawaid, T. (2021). Learning and memory enhancing activity of polyherbal formulation on streptozotocin induced memory impairment in rats via reducing mitochondria-targeted cytochrome. Pharmacognosy Journal, 13(1), 230–240. https://doi.org/10.5530/pj.2021.13.33

Souravh Bais, N. S. (2015). Neuroprotective Effect of *Juniperus communis* on Chlorpromazine Induced Parkinson Disease in Animal Model. Gill, and Nitan Kumar. Chinese Journal of Biology. Hindawi Publishing Corporation, Article ID 542542.

Srinivasan, P., Arul, B., & Kothai, R. (2021). Neuroprotective effect of ethanolic leaf extract of Commiphora caudata (Wight & Arn.) against lipopolysaccharide-induced neurotoxicity in Wistar rats. Journal of Herb Med Pharmacology, 10(4), 500–507. https://doi.org/10.34172/jhp.2021.57

Srinivasan, P., R, K., & Arul, B. (2020). Neurodegenerative diseases: An overview. International Research Journal of Pharmacy, 11(2), 20–24. https://doi.org/10.7897/2230-8407.110214

SrinivasaRao, T., Kavimani, S., & VeereshBabu, S. S. P. (2015). Nootropic effect of hypocholesterolemic drugs in scopolamine induced amnesic rats. International Journal of Biological and Pharmaceutical Research, 6(12), 991–1000.

Sun, H., Wei, S., Gong, Y., Ding, K., Tang, S., Sun, W., Yuan, C., Huang, L., Liu, Z., Chen, C., & Yao, L. (2024). Neuroprotective effects of cordycepin inhibit glutamate-induced apoptosis in hippocampal neurons. Cell Stress and Chaperones, 29(1), 10–20. https://doi.org/10.1016/j.cstres.2024.01.001

Suvarchala Reddy, N. V. L., Ganga Raju, M., Rahul Goud, M., & Shabnamkumari, T. (2020). Neuroprotective activity of Methanolic extract of *Terminala bellerica* Fruit against aluminium chloride and haloperidol induced amnesia in mice. Journal of Young Pharmacists. Asian Journal of Pharmaceutical and Clinical Research,11(5), 25-29, 12(2)(Suppl.), s87–s90.

Venkataramaiah.CH, G. Swathi, W. Rajendra. (2018). Morris water maze - A bench mark test for learning and memory disorders in animal models: a review.

Cite this article: Gurupackiyam M, Natesan S. Neuroprotective Effect of Chloroform Fraction of *Pterocarpus marsupium* Roxb Bark and its Isolated Compound (Afzelechin). Pharmacog Res. 2026;18(4):1318-29.