

Comparative Neuroprotective and Antioxidant Effects of *Prunus armeniaca* (L.) and *Murraya paniculata* (L.) in AlCl₃-Induced Neurodegeneration in Mice

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Abstract: The present study was designed to assess the neuroprotective efficacy of crude extract of the kernels and leaves of *Prunus armeniaca* L. (PA) and *Murraya paniculata* L. (MP), respectively, against the ALCL₃-induced neurodegeneration model in experimental mice. Extract of PA (200mg/kg p.o.) showed the strongest neuroprotective activity in the ALCL₃ induced neurodegeneration model. Parameters such as behavioral and functional defects, including time spent on the rota rod, fall-off time, and locomotor activity in the actophotometer, were evaluated. The antioxidant potential of the crude extracts from the leaves and kernels of the plants was determined using lipid peroxides (LPO) and superoxide dismutase (SOD) assays. The result of the experiment suggests that showed that PA has better antioxidant and neuroprotective potential. On account of this finding, the extracts demonstrating memory enhancement efficacy could result in the discovery of novel memory enhancement agents. Conclusion: These findings suggest that *P. armeniaca* may exert neuroprotective actions potentially through antioxidative or cholinergic modulation pathways. This positions it as a promising candidate for the development of plant-based therapeutics targeting cognitive decline and neurodegenerative disorders such as Alzheimer's disease. The comparative potency of these two plants also underscores the importance of species-specific phytochemical profiling in ethnopharmacological research.

Keywords: Leaves and kernels, Extract, Memory enhancement activity, phytochemical analysis, MWM.

1. Introduction:

One of the human body's most intricate structures is the brain. Neuroglia and neurons make up its composition. [1] A variety of abnormalities in the structure or function of neurons or the nervous system are referred to as neurodegenerative disorders (NDs). Such long-term, gradual damage may result in cognitive, memory, mobility, and thinking impairments. The most common NDs include Huntington's disease (HD), multiple sclerosis (MS), Parkinson's disease (PD) and illnesses associated with it, Alzheimer's disease (AD) and other forms of dementia, and amyotrophic lateral sclerosis (ALS). Genetic predisposition, age, way of life, diet, chemicals, some viruses, and exposure to certain environmental pollutants. [2] The pathophysiology of chronic neurodegenerative diseases, including Alzheimer's, Down syndrome, multiple sclerosis, etc., is mostly characterized by inflammation and oxidative stress. [3]

It is commonly recognized that acetylcholine (ACh), a neurotransmitter that is essential to the neuronal system, can be released by cholinergic nerve terminals. It is a neurotransmitter that was initially identified and controls respiration, digestion, heart rate, and movement in addition to other autonomic processes between neurons and non-neural tissues. The human brain contains a large number of cholinergic neurons, which are involved in cognition. ACh is necessary for cholinergic signal transduction linked to memory and learning. [4] The central nervous system's cholinergic system plays a critical role in controlling synaptic plasticity, neurogenesis, neuronal differentiation, and neuroprotection. [5] However, because there are many acetylcholine esterase (AChE) and/or butyrylcholine esterase (BChE) in interstitial spaces, acetylcholine is quickly broken down into choline and acetic acid. [6] Nevertheless, central cholinergic neuron degeneration could happen, impairing memory,

learning, attention, and sleep control. [7] Since ACh is involved in several physiological processes (such as memory, attention, learning, sensory information, and other critical functions), the degeneration of cholinergic neurons in the brain leads to reduced ACh levels, affecting cholinergic transmission, generating cognitive deficits. [8, 9] The main factor for the degeneration of neurons is due to the increased activity of cholinesterases (ChEs), which leads to a decrease in acetylcholine (ACh) levels, which in turn stops the neuronal transmission signals. [10]

Therefore, the acetylcholinesterase enzyme (AChE), which hydrolyzes the neurotransmitter acetylcholine, has become an important therapeutic target for AD. [11] Different AChE inhibitors (AChEIs) were developed based on the cholinergic hypothesis. [9] Herbal medicines, as the fundamental part of traditional medicine (such as in China and India), have been gradually accepted for use in the treatment of various diseases worldwide due to their multilevel function characteristics and remarkable efficacy (in some cases) with fewer adverse effects. [12]

Many natural compounds are known to have neuroprotective effects during AD. In modern pharmacological research, the ingredients or extracts of herbal medicines (such as *Acanthopanax*, *Alpinia*, and *Astragalus*) indeed have been demonstrated to exhibit continuous and considerable effects on the models of PD. [13] A large family of plant isolates has proven to be a modality for treatment by their inhibitory effect on the cholinesterase enzyme. Potent inhibition of the cholinesterase enzyme by plant products or phytoconstituents isolated from plant extracts also substantiates the neuroprotective effect. Natural substances were the first molecules to be employed as medicinal agents, and they are still used today. Natural chemicals are currently being studied for their potential neuroprotective properties, piquing the interest of both science and industry. A wide range of natural compounds derived from various sources is effective in the prevention and treatment of a few diseases, including neurological disorders like AD. Natural compounds have been shown to have therapeutic potential in various *in vitro* and *in vivo* experiments. [14]

Neuroprotection encompasses strategies and mechanisms that safeguard the central nervous system (CNS) from neuronal injury resulting from both acute events, such as stroke or trauma, and chronic neurodegenerative conditions, including Alzheimer's disease (AD) and Parkinson's disease (PD). Herbal medicine, also known as phytotherapy, encompasses the medical application of plant organs—such as leaves, stems, roots, flowers, fruits, and seeds—due to their therapeutic properties. Herbal products typically comprise complex mixtures of active components, such as phenylpropanoids, isoprenoids, and alkaloids.

Consequently, identifying the specific component(s) responsible for biological activity can be challenging. [15,16]

1.1 About plants: Apricot is botanically known as *Prunus armeniaca* Linn. It is also called Khubani and belongs to the family Rosaceae. [17] The cultivation of apricots is near about 600 hectares in Kashmir, 375 hectares in Himachal Pradesh, and 1600 hectares in the Kumaun region (Uttarakhand). [18] Apricot kernels contain various micronutrients and macronutrients such as fatty acids, sterol derivatives, polyphenols, carotenoids, volatile compounds, and cyanogenic glycosides (CNGs). They are divided into two types of sweet and bitter kernels, depending on the amount of CNGs, mainly amygdalin (D-mandelonitrile- β -D-gentiobioside) which causes a bitter taste.

Murraya paniculata (Linn.) Jack, synonym *M. exotica* Linn, known as Orange Jasmine, mock orange, Chinese box, or Kamini, family Rutaceae, is commonly grown in gardens. [19] MP is an Asian plant native to India, China, Bangladesh, etc. [20] Numerous research groups have examined *M. paniculata* for its bioactive compounds. Numerous chemicals, including terpenoids, flavonoids, phenols, coumarins, and indole alkaloids, have been discovered thus far. [21]

2. Method and materials:

2.1 Plant material:

MP leaves were collected from the G.B. Pant University of Agriculture & Technology Pantnagar, Uttarakhand, INDIA. These were shade dried, and their specimen sample had been deposited in the CSIR-NISCAIR, New Delhi.

Kernels of PA were collected from the Dhanachuli band, Uttarakhand, INDIA. The plant samples were dried under shade and their specimen sample had been deposited in the CSIR-NISCAIR, New Delhi.

2.2 Extraction of plants:

Fresh fruits (*Prunus armeniaca*) were collected. They were brought to the lab, where the seed kernel was extracted by hammering the individual stones. The skin was then peeled off, the kernels were shade-dried, and finally, mechanically ground. The fresh leaves (MP) were collected. They were fetched to the laboratory and after shade drying of the aerial parts of the plant for two weeks, it was crushed to make powder.

2.2.1 Cold Maceration: Plant materials were extracted by using the cold maceration method; plant samples (leaves and kernels) were collected, washed, rinsed, and dried properly. The plant material was extracted in powder form over 4-5 days using several

organic solvents (petroleum ether, ethyl acetate, and methanol). The filter paper was used to remove any non-extractable components from the extract, including cellular components and other elements that are insoluble in the extraction solvent.

The extract was then removed in a beaker and evaporated to remove the excessive moisture and an airtight container was used to collect the extract [28].

The extraction yield of all extracts was calculated using the following equation below:

$$\text{Percentage Yield} = \frac{\text{Actual yield} \times 100}{\text{Theoretical yield}}$$

2.3 Neuroprotective activity:

2.3.1 Drugs used in the experimental study

Aluminum chloride (AlCl₃) was used as a negative control. Piracetam was used as a positive control. All chemicals used in the present study were of analytical grade and were obtained from Sigma Chemical Co. and Merck.

2.3.2 Animals used in the study.

The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Raj Kumar Goel Institute of technology (Pharmacy) (Reg. no- 992/PO/RE/S/06/CCSEA) per the national guidelines on the use of laboratory animals with the protocol number: RKGIT/IAEC/2024/13. A total number of 24 healthy Swiss albino mice weighing 30±5g were divided randomly into four groups. They were housed and kept in a controlled room temperature environment (22± 20C) and light with alternate 12-hour light/dark cycles. The animals had free access to rat pellets and water ad libitum.

2.3.3 Experimental design

For acclimatization, the animals were kept in a laboratory room for a week. 4 groups of six animals each (n=06 animals) were formed from the animals. Neurodegeneration was induced by intraperitoneal administration of AlCl₃ (40 mg/kg b.w/day). [22] Piracetam (150 mg/kg body weight) [23] was used as the standard drug.

2.3.4 Model I - Rota rod test

Experiment set up: The rota rod, also known as the rota rod test, is used as a basic assessment tool for coordination and balance in rodents and provides one measure of locomotor ability [24, 25]. In its most basic form, the rota rod is comprised of a rotating cylinder upon which an animal is placed. As the cylinder rotates, the animal must move forward to keep from falling off the cylinder. The cylinder is elevated above a padded landing area to reduce the risk of injury to animals that fall. Animals with deficits affecting balance or coordination fall from the apparatus more quickly than animals with normal motor function. [24, 26, 27]. The Rota-rod (Techno) test was used to assess the impact of extract and

aluminum treatment on muscle functioning. To keep their posture on the Rota-rod (3 cm in diameter and rotating at a steady 25 rev/min), all of the rats received two initial training sessions of 300s, spaced around 10 minutes apart. Following the first training trials, a 120-second baseline trial was carried out. Each animal's duration on the Rotarod was noted. A maximum score of 120 seconds was awarded to the animals that managed to stay on the Rotarod.

2.3.5 Model II- Actophotometer

Experiment set up: The Actophotometer, as defined by Dews P.B (1953), was used to track the locomotor behaviour of the animals. To quantify the animals' locomotor activity (horizontal movement), an actophotometer (Dolphin) equipped with a digital counter, photocell, and light source was utilized. After five minutes of each animal being in an Actophotometer, the basal activity score for each animal was noted. Following 30 minutes and an hour of treatment with the appropriate drug, each animal's activity score was noted. The decreased activity score was taken as an index of neurodegeneration. [28]

2.4 Biochemical analysis for the estimation of oxidative damage markers: Antioxidant activity:

2.4.1 Production of TBARS from brain tissue

TBARS production was assessed using a modified methodology. [29] Following their decapitation and chloroform anesthesia, the mice's brains were immediately removed and placed on ice. After homogenizing one gram of brain tissue in cold 100 mM Tris buffer pH 7.4 (1:10 w/v), the sample was centrifuged. The homogenates (100 µl) were incubated at 37°C for one hour with or without 50 µl of the freshly made oxidant (iron), various plant extract concentrations, and a suitable volume of de-ionized water. This resulted in a total volume of 300 µl. 200 µl, 500 µl, and 500 µl of 8.1% sodium dodecyl sulfate (SDS), acetic acid (pH 3.4), and 0.6% TBA were added, respectively, to perform the color reaction. The reaction mixtures were incubated for one hour at 97 degrees Celsius, including those containing serial dilutions of 0.03 mM standard MDA. In a spectrophotometer, the absorbance was measured at 532 nm after the tubes had cooled.

2.4.2 Estimation of the superoxide dismutase (SOD) activity

A slightly modified version of Magnani's approach was used to determine the amount of superoxide dismutase in mouse brain tissue. [30] The test was conducted in three repetitions, and the brain homogenate was produced in phosphate buffer. Each sample test tube received 1 milliliter of Tris buffer before the homogenate was added. The brain homogenate was substituted with distilled water in the control. The pyrogallol solution was then added right before the absorbance was measured. The following formula was used to

calculate the findings after the absorbance was measured at 420 nm.

SOD activity (U/ml) = % Inhibition of pyrogallol autoxidation/ 50

2.5 Statistical significance

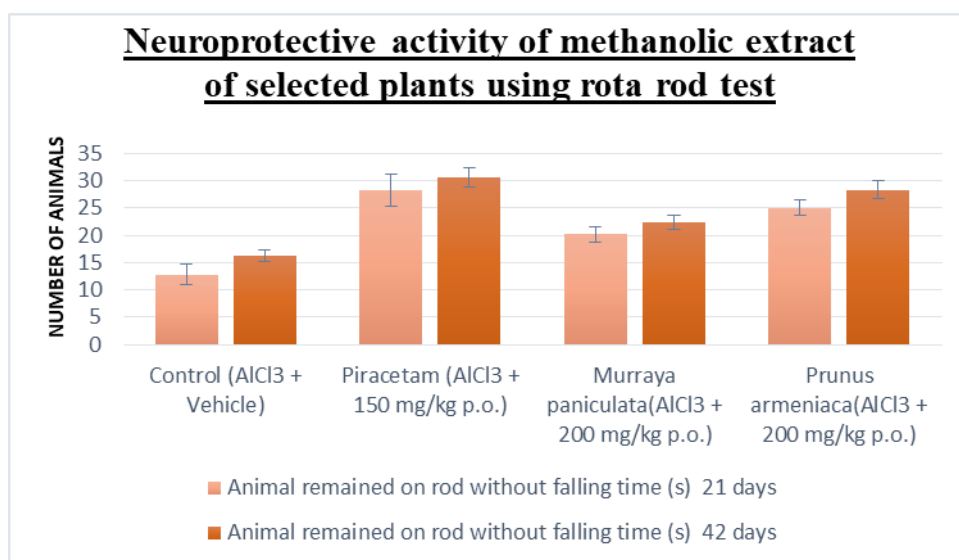
The values are observed and expressed as mean ± SEM. Statistical differences in means were analyzed using one-way ANOVA followed by the Student-Newman-Keuls test. *P<0.5, **P<0.01, ***P<0.001 was considered statistically significant.

3. Results

3.1 Rota rod test: In the present investigation; it was observed that AlCl3 significantly decreased (16.33 ± 1.03) time the animal remained on the rod without

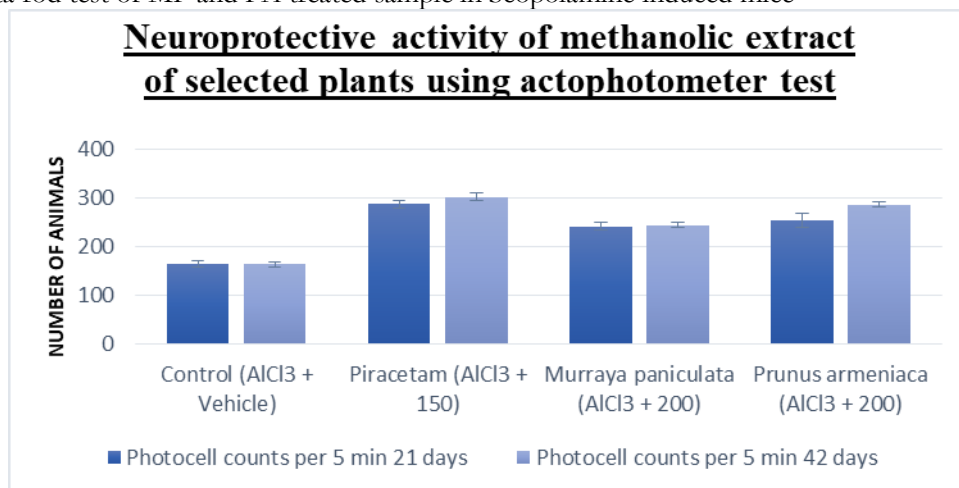
% Inhibition of pyrogallol autoxidation = A test / A control × 100

falling (s) at 40mg/kg b.w/day, i.p. in rota rod test, and this was considered as a symptom of motor dysfunction and neurodegeneration in animals. In both treatment groups of extracts MP and PA time animals remained on the rod without falling (s) was found to increase (22.33 ± 1.36*a & 28.33 ± 1.63*) as compared to AlCl3-treated animals. Thus, the extract was considered to decrease motor dysfunction due to AlCl3. The PA formulation-treated group was found to be most effective (29.25 ± 2.58*); in time, the animal remained on the rod without falling (s) was significantly increased; even Piracetam (30.66 ± 1.75*) also had a significant effect as compared to the AlCl3 group.



Values are in Mean±SD (n=6). * p<0.05 as compared to AlCl3 treated mice

Figure 1: Rota rod test of MP and PA treated sample in Scopolamine induced mice



Values are in Mean±SD (n=6). * p<0.05 as compared to AlCl3 treated mice

Figure 2: Actophotometer test of MP and PA treated sample in AlCl3-treated mice

3.2 Actophotometer test: In the present investigation, it was observed that AlCl3 significantly decreases (163.16 ± 4.91^a) Photocell counts per 5 min at 40 mg/kg b.w/day, i.p., in the actophotometer test, and this was considered as a symptom of motor

dysfunction and neurodegeneration in animals. In both treatment groups of extracts MP and PA, Photocell counts per 5 min were found to increase (244.16 ± 5.84*a, 86.16 ± 5.98*a) as compared to AlCl3-treated animals. Thus, the extract was considered to decrease motor dysfunction due to

AlCl₃ injection. PA formulation-treated groups found to be most effective ($299.25 \pm 15.25^*$) in Photocell counts per 5 min was significantly increased; even Piracetam ($301.66 \pm 8.16^*$) also had a significant effect as compared to the AlCl₃ group.

3.3 Lipid peroxidation activity in the brain tissue of animals

Figure 3 illustrates the marker of lipid peroxidation, TBARS, from the brains of control and experimental mice. The level of TBARS was significantly increased in the Control group animals. Administration of PA and MP extracts significantly decreased the levels of lipid peroxidation marker, which was comparable with Piracetam

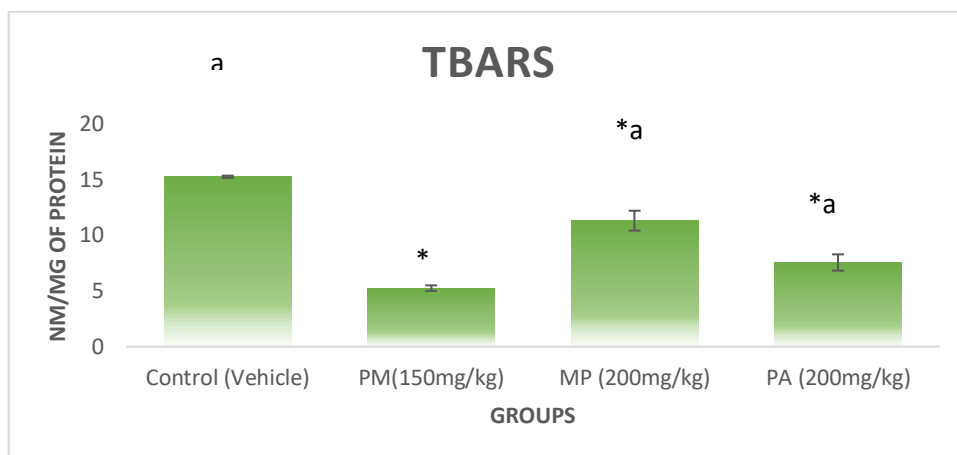


Figure 3: Graphical presentation of lipid peroxidation activity in terms of TBARS

3.4 Superoxide dismutase activity in brain tissue of animals

Figure 4 illustrates superoxide dismutase activity in the brain of experimental animals among the groups. Results reveal PA extract administration caused a significant increase in SOD levels ($1.4 \pm 0.04^{*a}$) as compared to the vehicle-treated group (0.7 ± 0.07^a).

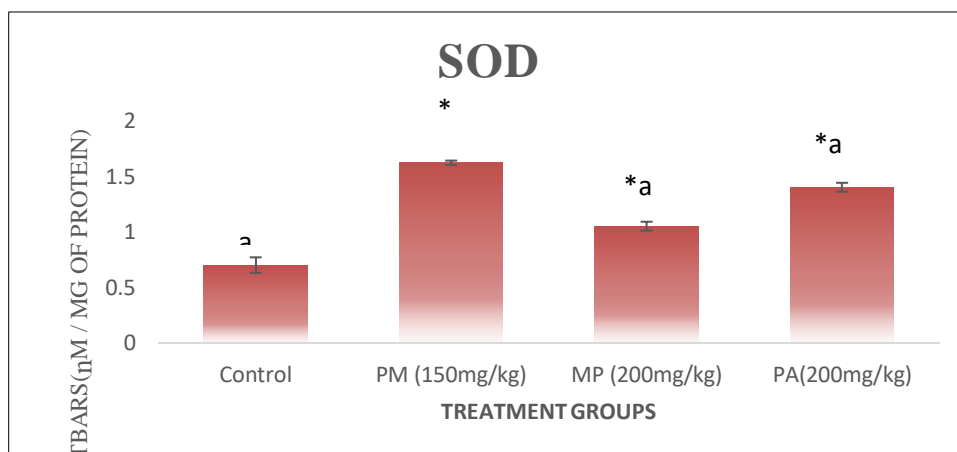


Figure 4: Graphical presentation of superoxide dismutase activity in terms of SOD

Discussion: The present study investigated the neuroprotective and memory enhancing effects of extracts of PA and MP kernels and leaves on the AlCl₃-induced neurodegeneration in mice. Of the two, PA showed better functional recovery, as indicated by the higher fall-off time and increased locomotor activity. These ameliorations indicate the involvement of the extract in maintenance of neuromuscular coordination and cognitive function that are generally reduced in neurodegenerative conditions. The antioxidant capabilities of the extracts were studied by lipid peroxidation (LPO)

and superoxide dismutase (SOD) assays, confirmed that PA exerted more pronounced antioxidant than MP. Oxidative stress plays a key role in the pathogenesis of neurodegenerative diseases, including AD, and the therapeutic potential of *P. armeniaca* in preventing oxidative damage indicates that this agent might be a useful neuroprotective agent. Taken together, the results of this study indicate that PA has potential as a powerful neuroprotective and memory-improving agent and this could be related to its antioxidant properties and cholinesterase inhibition.

4. **Summary and conclusion:** Results of the present study showed that the methanolic extracts of PA promoted significant neuro-protection and memory enhancement than MP. These effects might be due to the antioxidant activity and the inhibition of acetylcholinesterase.

5. **Declaration of interest:** None

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