



STUDY OF THE EFFECT OF *VINCA MINOR* LEAVES EXTRACT AGAINST CHEMICALLY INDUCED COGNITIVE IMPAIRMENT IN EXPERIMENTAL ANIMALS

Keshav Bondre*, Sakshi Nagrikar, Akansha Ramteke, Gauri Tatewar, Divyabharati Biradar

Institute of Pharmaceutical Education and Research, Borgoan (Meghe), Wardha, Maharashtra, India, 44201.

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*Corresponding Author

Keshav Bondre

Institute of Pharmaceutical Education
and Research, Borgoan (Meghe),
Wardha, Maharashtra, India, 44201.



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ABSTRACT

Background: Alzheimer's disease (AD) is a progressive neurodegenerative condition primarily marked by a decline in cognitive function and currently has few effective treatment options. Current treatments offer only symptomatic relief and are often associated with adverse effects. In the search for alternative therapies, *Vinca minor*, a medicinal plant rich in alkaloids and antioxidants, has shown promising neuroprotective potential. **Objective:** The aim of the study is to evaluate the neuroprotective effects of *Vinca minor* leaves extract against chemically induced Alzheimer's disease in experimental animals. **Methods:** Male Wistar rats were divided into five groups, including normal control, disease control, standard drug, and two test groups receiving *Vinca minor* extract. A behavioral assessment was conducted using the radial arm maze and the novel object recognition test. Biochemical estimations included acetylcholinesterase (AChE),

glutathione (GSH), nitric oxide (NO), and catalase (CAT) levels. Histopathological analysis of the brain tissue was performed to assess neuronal integrity. **Results:** Treatment with *Vinca minor* significantly improved behavioral performance, enhanced antioxidant enzyme levels, reduced AChE and NO levels, and preserved neuronal architecture in hippocampal regions compared to the disease control group. **Conclusion:** The findings suggest that *Vinca minor* leaf

extract exerts cognitive-enhancing and neuroprotective effects, potentially through antioxidant and anticholinesterase mechanisms, supporting its use as a natural therapeutic option in AD management.

KEYWORDS: *Vinca minor*, Alzheimer's disease, Cognitive impairment, Neuroprotection, Acetylcholinesterase, Oxidative stress, Behavioral assessment, Histopathology.

INTRODUCTION

Cognitive Impairment

Mild cognitive impairment (MCI) is a condition characterized by cognitive decline with minimal impact on instrumental activities of daily living (IADL).^[1] MCI is often viewed as the transitional stage between age-related cognitive decline and early dementia. However, despite its conceptual clarity, it poses challenges in clinical practice.^[2] In 1986, a National Institute of Mental Health workgroup proposed "age-associated memory impairment" (AAMI) to characterize memory changes believed to be part of normal aging. To address the limitations of AAMI, the International Psychogeriatric Association later introduced the term "age-associated cognitive decline".^[3] MCI is a clinical diagnosis, and its neuropathological characteristics are still being defined, with no established criteria.^[4] The clinical spectrum of AD now extends from dementia back to MCI and even to preclinical AD, where individuals remain cognitively normal but exhibit underlying biological markers of the disease.^[5] While MCI can be an early cognitive manifestation of AD, it may also result from other neurological, neurodegenerative, systemic, or psychiatric conditions.^[6]

Background on Alzheimer's disease

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by cognitive decline, progressively impairing memory, orientation, and reasoning. While its exact cause remains unknown, extensive research has explored its pathophysiology and potential treatment strategies.^[7] AD is characterized by a gradual deterioration in cognitive and functional abilities, accompanied by behavioral changes, and is linked to the accumulation of amyloid plaques and tau protein deposits in the brain.^[8]

AD is responsible for 60% to 70% of progressive cognitive impairment cases in elderly individuals.^[6] The most common cognitive symptoms include short-term memory deficits, executive dysfunction, visuospatial impairment, and praxis difficulties. However, some rarer variants of AD present with relatively preserved memory function.^[8] In 2011, the National

Institute on Aging and the Alzheimer's Association (NIA-AA) introduced distinct diagnostic criteria for the preclinical, mild cognitive impairment, and dementia stages of AD. Advances in research led to a subsequent initiative to update and unify these guidelines, resulting in a "research framework" designed specifically for observational and interventional studies rather than routine clinical practice. According to this framework, AD is defined by its underlying pathological processes, which can be identified through postmortem examination or in vivo biomarkers.^[9]

Limitations of current treatments

Current treatments for Alzheimer's disease (AD) primarily provide symptomatic relief without halting or reversing disease progression. Cholinesterase inhibitors like donepezil, rivastigmine, and NMDA receptor antagonists such as memantine may offer modest cognitive improvement but fail to address the underlying neurodegeneration.^[10] Recently approved disease-modifying therapies targeting amyloid- β , such as aducanumab, have shown limited clinical efficacy and raised safety concerns, including amyloid-related imaging abnormalities (ARIA).^[11] Additionally, treatments often cause adverse effects and are expensive, limiting accessibility, especially in resource-limited settings.^[12] The multifactorial nature of AD pathophysiology, involving amyloid plaques, tau tangles, neuroinflammation, and oxidative stress, also poses challenges to the effectiveness of single-target therapies.^[13]

Medicinal potential of *Vinca Minor* (alkaloids like vincamine)

Vinca Minor, commonly known as lesser periwinkle, is a low-growing, evergreen plant native to Europe but now found in many parts of the world. It belongs to the Apocynaceae family and is often used as a ground cover due to its trailing stems and attractive violet-blue flowers.¹⁴ Traditionally, *Vinca Minor* is well-known for its relaxing, tonic, and vasodilator properties. It also possesses antitumor^[15,16], cytotoxic^[17], antihyperlipidemic^[18], and antimicrobial activities.^[15] By increasing blood flow and localized glucose absorption, *Vinca Minor* improves brain metabolism and protects against hypoxia and ischemia. *Vinca Minor* has been used in herbal medicine for its potential benefits in improving blood circulation and cognitive function.^[19] The plant contains active alkaloids such as vincamine, which have been studied for their neuroprotective and vasodilatory effects, making it of interest in the treatment of conditions like Alzheimer's disease and dementia.^[20]

Rationale for the study

Alzheimer's disease (AD) is a major public health concern with rising global prevalence and

limited therapeutic options. Current pharmacological treatments primarily provide symptomatic relief without halting the disease's progression. Moreover, these therapies often have side effects and limited efficacy in later stages. Recent attention has turned toward plant-based therapies for their potential neuroprotective and anti-inflammatory effects. *Vinca Minor*, traditionally used in folk medicine, contains alkaloids such as vincamine, which have shown cognitive-enhancing properties. Since its therapeutic effects remain unexplored in experimental animals. This study aims to evaluate the effect of *Vinca Minor* leaf extract in a rat model of AD, thereby contributing to the search for safer, more effective alternatives in AD management.

MATERIALS AND METHODS

Chemicals and Reagents

The chemicals and reagents used in the study are Scopolamine, Rivastigmine, Ethanol, Chloroform, 5,5-dithiobis (2-nitrobenzoic acid), Trichloroacetic acid (TCA), Sodium phosphate dibasic, Potassium dihydrogen phosphate, Sodium Chloride, Sulphanilamide, N-(1-naphthyl)-ethylenediamine dihydrochloride, Hydrogen peroxide, Thiobarbituric acid (TBA), Pyridine, Formaldehyde, Sodium dodecyl sulphate (SDS).

Plant Materials

The plant was collected from the Matoshri Plant Nursery, Wardha. An expert taxonomist identified and authenticated it. A reference sample of the specimen was submitted to the institute.

Plant Extraction

The leaves of *Vinca Minor* were dried in the shade, crushed into coarse powder, and used for extraction. Coarsely ground leaves of *Vinca Minor* were defatted with petroleum ether and then extracted with ethanol by continuous Soxhlet extraction. Ethanolic extract was obtained by using 200g of coarse leaves with ethanol: water (7:3) in a Soxhlet apparatus at 60-70°C temperature. After the extraction procedure was completed in the Soxhlet apparatus, the solvent ethanol was distilled, followed by filtration of the extract through Whatman No. 3 filter paper. The extract was then sun-dried for 10-15 days.

Preliminary phytochemical screening of the plant extract

The extracts were tested to determine the presence of various phytochemicals, including alkaloids, carbohydrates, tannins, phenolic compounds, flavonoids, amino acids, proteins,

saponins, steroids, and fats.

Experimental Animals

The Albino Wistar rats, of either sex and weighing 200-250g, were obtained from the animal house of the Institute of Pharmaceutical Education and Research, Borgaon (Meghe), Wardha. The animals were kept in polypropylene cages under standard laboratory conditions with a 12-hour light and dark cycle. They had free access to commercial pellet diet and water ad libitum. The temperature was maintained at $25\pm 2^{\circ}\text{C}$ with a relative humidity of $50\pm 15\%$. The experiments were conducted after the protocol was approved by the Ethical Committee of the institution, and every effort was made to minimize animal suffering.

Experimental Design

Alzheimer's disease was induced in all animals except the normal group using the inducer Scopolamine (1 mg/kg, i.p.) in saline water, administered for 14 days via the intraperitoneal route. All drugs were freshly prepared at the time of administration. Ethanolic extract of *Vinca Minor* (EEVM) was dissolved in saline water at doses of 200 mg/kg and 400 mg/kg. Scopolamine (1 mg/kg) and Rivastigmine (2.5 mg/kg) were dissolved in saline water.

All rats were randomly assigned to 5 groups of 6 animals each as follows: (1) Normal group: Rats received only saline water at a dose of 10 ml/kg, p.o.; (2) Control group: Rats received only scopolamine as an inducer at 1 mg/kg, i.p.; (3) Standard group: Rats received scopolamine (1 mg/kg, i.p.) and rivastigmine (2.5 mg/kg, p.o.) as a standard drug; (4) Test I group: Rats received scopolamine (1 mg/kg, i.p.) and EEVM at a dose of 200 mg/kg, p.o.; (5) Test II group: Rats received scopolamine (1 mg/kg, i.p.) and EEVM at a dose of 400 mg/kg, p.o. Learning and memory were evaluated using various behavioral models and biochemical parameters following treatment with the test and standard drugs.

BEHAVIORAL ASSESSMENTS

Radial Arm Maze^[21,22]

The 8-arm radial maze is a widely used behavioral test to assess spatial learning and memory in rodents. It is based on the idea that animals depend on spatial cues to navigate the maze, remember their previous arm choices, and maximize food collection while minimizing errors. This test differentiates between working memory, which involves recalling recently visited arms during a session, and reference memory, which involves remembering consistently baited arms across multiple sessions. The radial arm maze features a central octagonal

platform (26 cm in diameter) with eight evenly spaced arms, each measuring 60 cm in length, 10 cm in width, and 12 cm in height, often containing food rewards at their ends. The maze is elevated 70 cm above the floor to prevent escape and reduce external disturbances. Experiments are conducted in a well-lit room enriched with spatial cues, such as wall posters and objects, to aid the animals in navigation and orientation. In the radial arm maze, a working memory error occurs when an animal re-enters an arm where food has already been collected. In contrast, a reference memory error involves entering an arm that was never baited, indicating long-term memory deficits. Latency to complete the task refers to the total time the animal takes to retrieve all available rewards.

Novel Object Recognition Test^[23,24,25]

The Novel Object Recognition (NOR) test is a widely used behavioral task to assess recognition memory in rodents. It is based on the principle that animals have an innate tendency to explore novel objects more than familiar ones. When an animal remembers a previously encountered object, it will spend more time exploring the new object, indicating intact recognition memory. This test primarily evaluates short-term and long-term memory, which are dependent on different brain regions, including the hippocampus, perirhinal cortex, and prefrontal cortex. The testing arena consists of a square open-field chamber (40 × 40 cm) enclosed by walls to prevent escape. It contains two or more distinct, non-edible, odorless objects of similar size but varying in shape and texture, made from materials like plastic, glass, or metal. An overhead camera system is used to record and monitor exploratory behavior. Recognition Index (RI) is observed, i.e., the time spent exploring the novel object relative to total exploration time.

BIOCHEMICAL ANALYSIS

AD is a chronic neurodegenerative disease that leads to progressive memory loss and deterioration in cognitive function. Several biochemical parameters are altered in AD, reflecting oxidative stress, neuroinflammation, neurotransmitter dysfunction, and protein aggregation. Measuring these parameters helps in understanding disease progression and evaluating potential therapies.

Determination of Acetylcholinesterase (AChE)^[26]

Ellman's assay (1961) is the most widely used method for determining acetylcholinesterase (AChE) activity. This method quantifies AChE activity by measuring the rate of hydrolysis of acetylthiocholine iodide (ATCI), which produces thiocholine. Thiocholine reacts with 5,5'-

dithiobis (2-nitrobenzoic acid) (DTNB), forming a yellow colored compound (5-thio-2-nitrobenzoic acid), spectrophotometrically measured at 412 nm.

Procedure

- Pipette 2.6 mL of 0.1 M phosphate buffer (pH 7.4) into a cuvette/microplate well.
- Add 100 μ L of DTNB (0.01 M, Ellman's reagent).
- Add 100 μ L of sample homogenate.
- Pre-incubate for 5 minutes at 25°C.
- Add 100 μ L of acetylthiocholine iodide (ATCI, 0.075 M) to start the reaction.
- Measure the absorbance at 412 nm immediately and at every 10-minute interval for 20 minutes.
- Calculate the rate of increase in absorbance per minute ($\Delta A/\text{min}$).

Determination of reduced glutathione (GSH)^[27,28]

Reduced glutathione (GSH) is an essential intracellular antioxidant that safeguards cells against oxidative damage. Its concentration can be determined with Ellman's reagent—5,5'-dithiobis(2-nitrobenzoic acid, DTNB)—which reacts with GSH to yield the yellow chromophore 5-thio-2-nitrobenzoic acid (TNB) displaying peak absorbance at 412 nm.

Procedure

- Take 0.5 mL of the deproteinized supernatant.
- Add 2 mL of 0.1 M phosphate buffer (pH 7.4 or 8.0).
- Add 0.5 mL of DTNB reagent (0.01 M, freshly prepared).
- Incubate for 5 minutes at room temperature.
- Measure the absorbance at 412 nm using a spectrophotometer.

Determination of nitric oxide (NO)^[29]

Nitric oxide (NO) is an essential signaling molecule consisting physiological and pathological processes. Since NO is highly unstable, its levels are indirectly measured by quantifying nitrite (NO_2^-) and nitrate (NO_3^-), its stable metabolites, using the Griess reagent assay.

Procedure

- Pipette 100 μ L of sample or standard into a 96-well plate or test tube.
- Add 100 μ L of Griess reagent [containing 1% sulfanilamide in 5% phosphoric acid

and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride (NED)]

- Absorbance is measured at 540 nm (or 550 nm) using a spectrophotometer or microplate reader.

Determination of catalase (CAT)^[30]

Catalase (CAT) is a crucial antioxidant enzyme that breaks down hydrogen peroxide (H₂O₂) into water (H₂O) and oxygen (O₂), preventing oxidative damage. The catalase activity can be measured spectrophotometrically by monitoring the decomposition of H₂O₂ at 240 nm.

Procedure

- Take 2.9 mL of 30 mM H₂O₂ solution in a cuvette. [10 mM H₂O₂ in 50 mM potassium phosphate buffer (pH 7)]
- Add 0.1 mL of the sample (tissue homogenate or serum).
- Immediately mix and record the absorbance at 240 nm every 3 minutes using a spectrophotometer.
- Determine the rate of decrease in absorbance (ΔA per minute)

HISTOPATHOLOGICAL STUDIES

After 28 days, the rats of each group were sacrificed, and the brain parts were separated. The brain regions were cleaned to eliminate excess tissue and fat. The cleaned brains were placed in a container with a 10% formalin solution. The samples were sent to Kanhere Histopathology Laboratory, Plot No. 102A, Shrihari Nagar, Near NIT Garden, Manewada, Nagpur.

Statistical Analysis

Data were analyzed using GraphPad Prism 5.01 for Windows. Results were expressed as Mean and S.D. The Dunnett test (non-parametric) was used to test the significance of the difference between the variables in various groups. Results with P-values less than 0.05 were interpreted as statistically significant.

RESULTS

Behavioral Results

Radial Arm Maze

- a) Determination of correct arm entries:** The accuracy of arm entries serves as an indicator of working memory performance in experimental subjects.

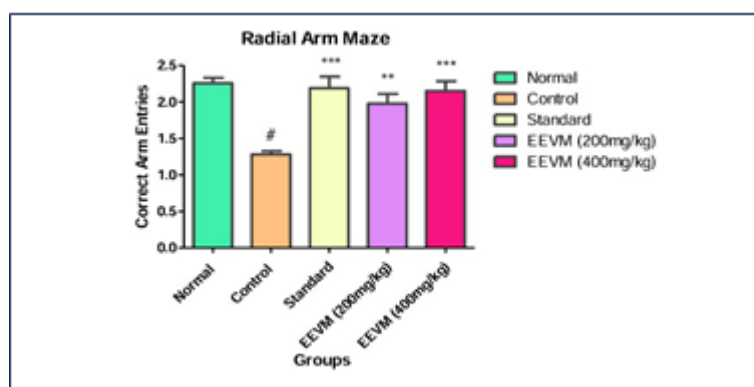


Fig. No. 1: Result of the effect of EEVM on correct arm entries.

The above observation shows that correct arm entries decrease after receiving scopolamine in all group animals except the normal group. However, there was a significant increase in correct arm entries after 28 days of treatment with EEVM 200mg/kg and 400 mg/kg due to the presence of phytoconstituents in the extract, which may help reduce the oxidative stress in rats, which may lead to improvement in memory.

b) Determination of total number of errors: The number of errors indicates the correct functioning of the brain. If errors increase, it means brain functioning is affected, which can be used to determine the functioning of the brain. A reduction in errors suggests that memory retention has occurred.

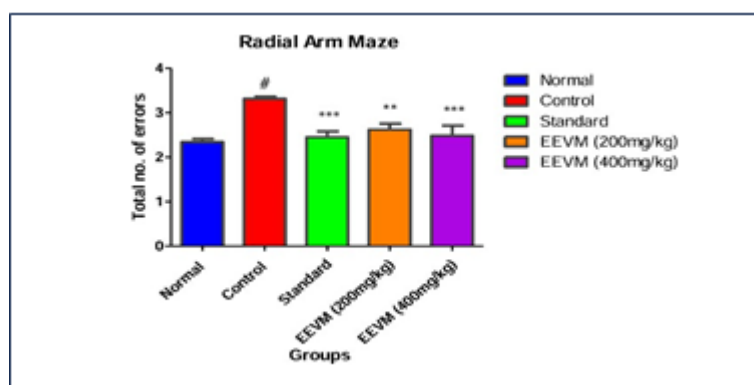


Fig. No. 2: Result of the effect of EEVM on the total number of errors.

The above observation shows that the total number of errors increases after receiving scopolamine in all group animals except the normal group. However, there was a significant decrease in the total number of errors after 28 days of receiving treatment with EEVM 200mg/kg and 400 mg/kg due to the presence of phytoconstituents in the extract, which may help reduce the oxidative stress in rats, which may lead to improvement in memory.

Novel Object Recognition Test

The NOR test is performed quickly with a single learning phase. When exposed to a familiar object alongside a novel object, rats spend more time exploring the novel object than the familiar object.

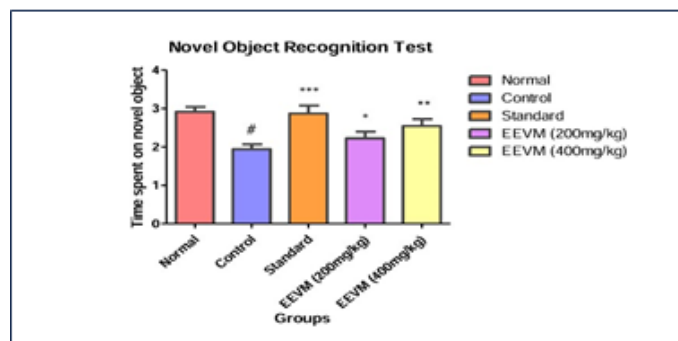


Fig. No. 3: Result of the effect of EEVM on time spent on novel object.

The above observation shows that the total number of errors increases after receiving scopolamine in all group animals except the normal group. However, there was a significant decrease in the total number of errors after receiving treatment with EEVM 200mg/kg and 400 mg/kg due to the presence of phytoconstituents in the extract, which may help reduce oxidative stress in rats, which may lead to improvement in memory.

Biochemical Results

Biochemical parameter is used to determine the actual level of biochemicals present in the body organs, which helps in the determination or diagnosis of disease states.

Determination of Acetylcholinesterase (AChE)

Acetylcholinesterase plays a key role in ending the cholinergic signal by breaking down acetylcholine. AChE hydrolyses and inactivates ACh. Increased AChE activity leads to a lack of ACh and thus memory deficits, which is observed in the brains of AD patients.

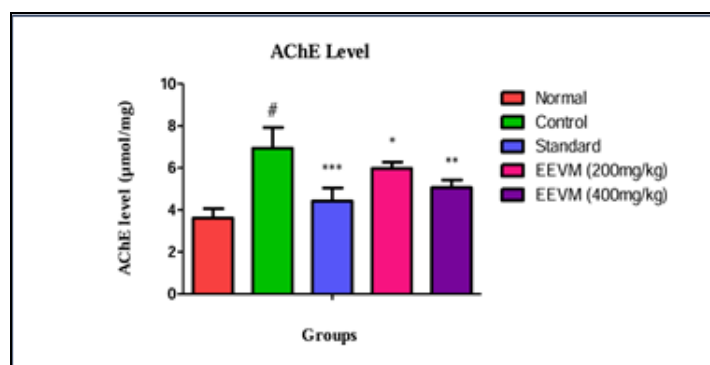


Fig. No. 4: Result of the effect of EEVM on AChE level

From the above graph, it is observed that the level of AChE increases in animals after receiving scopolamine as compared to the normal group. Whereas the AChE level decreases in animals receiving standard drug and test EEVM extract, due to the presence of phytochemicals in the extract, which may help reduce the oxidative stress in rats, which may lead to a decrease in AChE, which ultimately leads to improvement in Acetylcholine.

Determination of reduced glutathione (GSH)

Glutathione (GSH) is a highly prevalent non-protein thiol found within cells. As a natural antioxidant, it helps regulate oxidative stress and serves a vital protective function in both neuronal and non-neuronal cells.

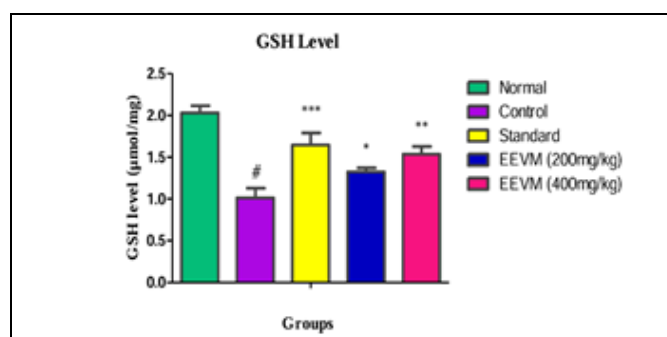


Fig. No. 5: Result of the effect of EEVM on the GSH level.

The above graph indicates that the level of reduced glutathione in animals decreases after the induction period when compared to the normal group. However, a significant increase in the level of reduced glutathione was observed after treatment with the standard drug, as well as with EEVM extract. This increase is likely due to the antioxidant properties of the extract, which leads to an increase in the level of GSH, as GSH is an antioxidant.

Determination of nitric oxide (NO)

Nitric oxide (NO) functions as a neurotransmitter at synapses, facilitating cerebral blood flow and playing a crucial role in neuronal intracellular signaling.

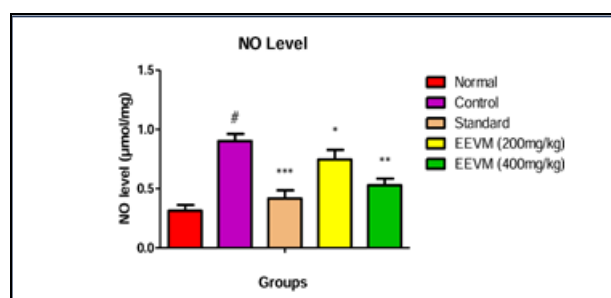


Fig. No. 16: Result of the effect of EEVM on the NO level.

The above graph shows that the group II animals had higher NO levels than the normal group. NO levels in animals belonging to groups III, IV, and V considerably dropped compared to the control group after receiving conventional medication and EEVM at the doses of 200mg/kg and 400mg/kg. This might be because EEVM has antioxidant properties, which lower the amounts of free radicals, which in turn lowers NO levels.

Determination of catalase (CAT)

Catalase, an enzyme found in cells, plays a role in breaking down hydrogen peroxide into water and oxygen, thereby protecting cells from oxidative damage.

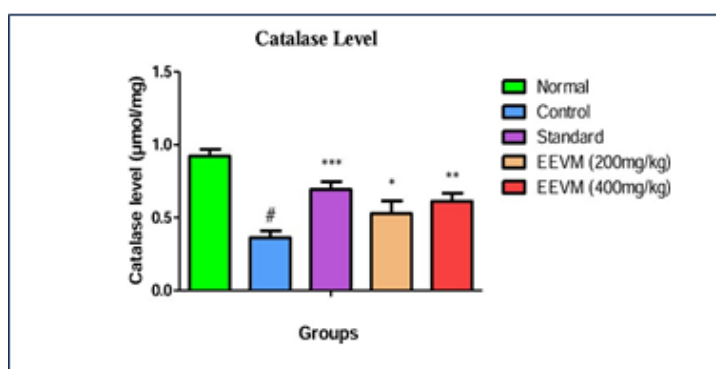
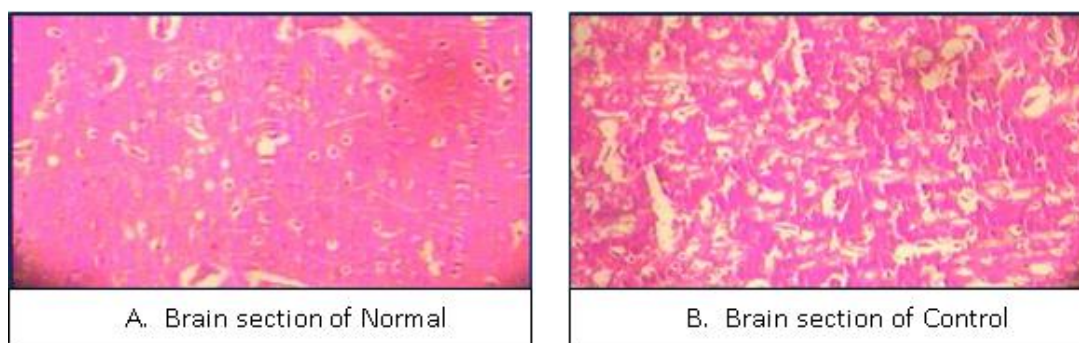


Fig. No. 7: Result of the effect of EEVM on the catalase level.

According to the results above, catalase levels fall during scopolamine administration while it is rise during the administration of conventional treatment and the EEVM extract. Perhaps because of its antioxidant capacity, which raises brain catalase levels and reduces oxidative stress. 3.92 7.41.

Histological Results



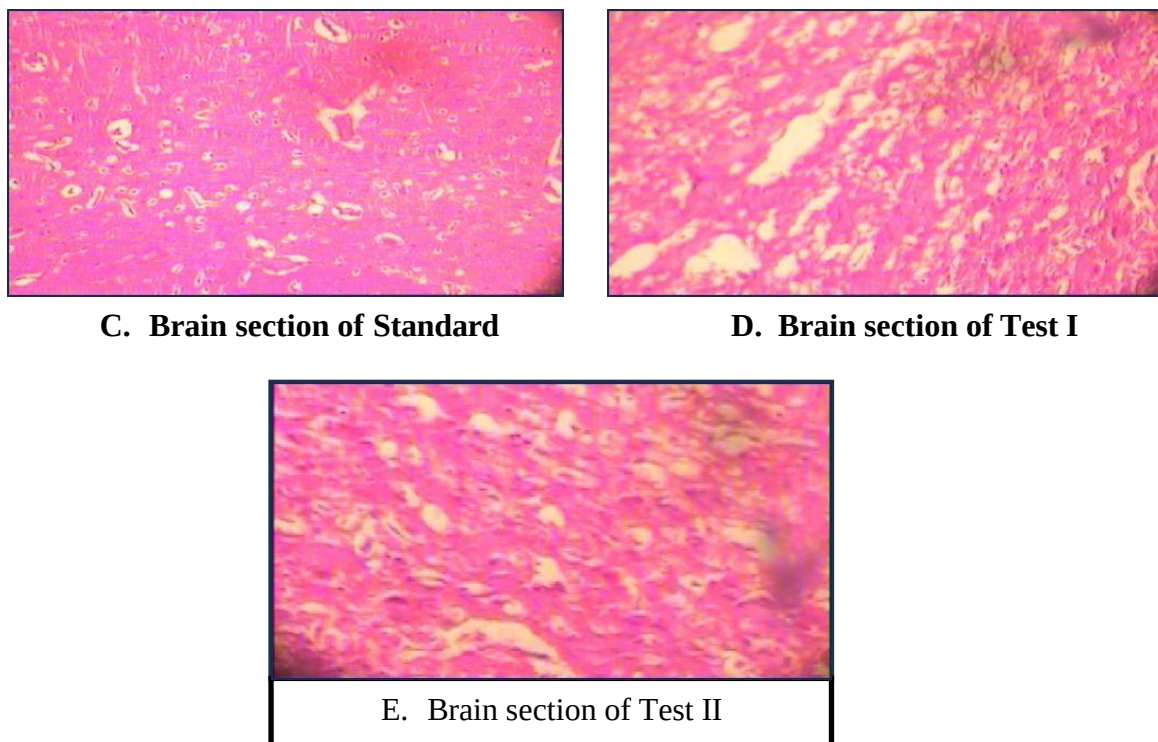


Fig. No. 8: Histopathological Studies.

- A. Normal Group:** The brain tissue from animals in the normal group exhibits a typical cellular structure.
- B. Control Group:** Scopolamine-induced control group animal showed loss of neuron cells, amyloid plaque (AP) deposition, and neurofibrillary tangles.
- C. Standard Group:** Animal treated with standard drug Rivastigmine 2.5 mg/kg displayed improvement in brain region with low neuronal degeneration (ND) and diminished the Amyloid plaque (AP) and neurofibrillary tangles (NFT).
- D. Test Groups IV and V:** Animals treated with the test drug EEVM 200mg/kg and 400 mg/kg showed normal histology with mild degeneration of neuronal cells (NC) and disappearance of the Amyloid plaque (AP) and Neurofibrillary tangles.

DISCUSSION

Alzheimer's disease (AD) is a neurodegenerative condition characterized by a gradual decline in cognitive functions, including memory, orientation, and reasoning.^[31] While treatments like cholinergic inhibitors and glutamate NMDA antagonists are available, they often lead to adverse side effects.^[32] To mitigate these issues, traditional herbal formulations are being explored as alternative therapies.^[33,34]

A thorough review of existing literature and traditional medicinal knowledge reveals that *Vinca Minor* leaf extract possesses numerous therapeutic properties, including antitumor, cytotoxic, antihyperlipidemic, and antimicrobial activities, as well as protection against hypoxia and ischemia.^[33,34] Despite the widespread prevalence and severity of Alzheimer's disease, the allopathic medical system has yet to offer a fully effective treatment. Therefore, this study aims to investigate the memory-enhancing potential of *Vinca Minor* in a scopolamine- induced Alzheimer's-like model in rats.

Literature reviews indicate that *Vinca Minor* leaf extract contains various bioactive compounds, including alkaloids, carbohydrates, phenols, tannins, flavonoids, and amino acids.^[35,36] These components function as natural antioxidants and may contribute to memory enhancement.^[36,37]

Accordingly, various chemical tests were conducted, revealing the presence of alkaloids, carbohydrates, phenols, tannins, flavonoids, amino acids, and proteins in the extract.

In this study, scopolamine was used to induce an Alzheimer-like condition. Research indicates that scopolamine causes neuropathological changes and cognitive impairment in experimental animals, mimicking the effects of Alzheimer's disease. It disrupts cholinergic and noradrenergic neurotransmission and promotes free radical generation. Scopolamine accelerates A β production by facilitating the formation of amyloid oligomers. Its administration in rats significantly increases brain AChE activity, impairing mitochondrial function over time due to excessive free radical production, ultimately leading to cell death.^[38]

The induction of Alzheimer's disease in animals was achieved by administering scopolamine intravenously at a dose of 1 mg/kg. This resulted in increased levels of AChE and nitric oxide, while reducing the levels of reduced glutathione and brain catalase. These biochemical alterations confirm the successful induction of Alzheimer's disease in the experimental rats.

In this study, rivastigmine (2.5 mg/kg) was used as a standard drug to treat scopolamine-induced Alzheimer's disease in rats and to compare its effects with those of *Vinca Minor* leaf extract. Being highly lipid-soluble, rivastigmine easily crosses the blood- brain barrier and works by inhibiting the breakdown of acetylcholine, a key neurotransmitter involved in learning and memory. Treatment with rivastigmine led to a decrease in AChE and nitric

oxide (NO) levels while increasing glutathione (GSH) and catalase levels. These findings indicate that rivastigmine effectively counteracts Alzheimer's disease.

The ethanolic extract of *Vinca minor* (EEVM) was given to the test group in two different doses: 200 mg/kg and 400 mg/kg. This extract contains the phytoconstituents as mentioned above, which act as natural antioxidants and may contribute to the treatment of Alzheimer's disease.

In this study, the Radial Arm Maze (RAM) and Novel Object Recognition Test (NORT) models were used to assess behavioral changes after treatment with the test and standard drugs in experimental animals. In addition to behavioral analysis, biochemical evaluations were conducted by measuring the levels of key neurotransmitters and biomarkers, including AChE, GSH, NO, and catalase. Each of these plays a crucial role in addressing cognitive impairment.

The Radial Arm Maze (RAM) is a sensitive and reliable method for evaluating learning and memory enhancement in rats.^[39] However, following treatment with EEVM at different doses, there is a noticeable reduction in errors, with animals making more correct arm entries. The 400 mg/kg dose demonstrated a more significant improvement in memory performance compared to the 200 mg/kg dose.

The Novel Object Recognition Test (NORT) is a valuable paradigm for evaluating cognitive function in rodents. The ability to differentiate between familiar and novel objects relies on intact memory of previously encountered items. This test measures the duration of time spent exploring a novel object.^[40] Animals treated with rivastigmine and EEVM showed increased exploration of the novel object, suggesting improved memory function. Among the test doses, EEVM 400 mg/kg exhibited a more significant effect than EEVM 200 mg/kg.

Acetylcholine (ACh) is a key neurotransmitter involved in memory and cognition, while acetylcholinesterase (AChE) is the enzyme responsible for its breakdown.^[41] In this study, findings indicate that AChE levels significantly increase in Group II animals treated with scopolamine, reflecting cognitive impairment. However, animals in Groups IV and V, which received EEVM at doses of 200 mg/kg and 400 mg/kg, respectively, exhibited a notable decrease in AChE levels. The reduction was more pronounced in the 400 mg/kg group, suggesting a stronger neuroprotective effect at the higher dose.

Glutathione (GSH) is a natural antioxidant that regulates oxidative stress in the body and plays a crucial role in preventing neurodegenerative disorders, including Alzheimer's disease (AD). A decline in GSH levels is associated with increased oxidative damage, contributing to the progression of AD.^[42] Treatment with the standard drug rivastigmine, as well as EEVM at doses of 200 mg/kg and 400 mg/kg, led to a marked increase in GSH levels. Among these, EEVM at 400 mg/kg exhibited a more significant effect than EEVM at 200 mg/kg, demonstrating its potential as a protective agent against AD.

Nitric oxide (NO) is an enzymatic byproduct associated with various neurological disorders, including Alzheimer's disease (AD). Due to its free radical properties, NO contributes to neuroinflammation and oxidative stress, leading to neuronal damage. Therefore, assessing NO levels is crucial for diagnosing Alzheimer's conditions.^[43] Treatment with EEVM at doses of 200 mg/kg and 400 mg/kg resulted in a notable reduction in NO levels compared to the control group. The 400 mg/kg EEVM dose demonstrated a more significant decrease in NO levels, potentially due to the presence of flavonoids, which possess strong antioxidant properties.

Catalase is a crucial antioxidant enzyme that helps mitigate oxidative stress by breaking down cellular hydrogen peroxide. A deficiency in catalase is linked to the development of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), and schizophrenia.^[44] However, administration of the standard drug rivastigmine and EEVM at doses of 200 mg/kg and 400 mg/kg led to a significant increase in catalase levels. This rise in catalase activity highlights the neuroprotective potential of the extract, likely due to its antioxidant properties. Among the tested doses, EEVM 400 mg/kg exhibited a more pronounced effect than EEVM 200 mg/kg.

Histopathological examination revealed a normal cellular arrangement in the brain tissue of animals in the normal group. In contrast, scopolamine-induced control group animals exhibited significant neuronal cell loss, along with the deposition of amyloid plaques (AP) and neurofibrillary tangles (NFT), hallmarks of Alzheimer's disease.^[45] Sections from animals treated with the standard drug rivastigmine (2.5 mg/kg) demonstrated notable improvement in brain histology. Similarly, animals treated with EEVM at 400 mg/kg showed largely normal brain histology, with only mild neuronal degeneration and the disappearance of plaques, indicating its potential neuroprotective effects.

The overall findings suggest that administration of *Vinca Minor* extract (EEVM) may help improve working memory and cognitive impairment caused by scopolamine. The results indicate a significant neuroprotective effect, particularly at the 400 mg/kg dose. The therapeutic potential of *Vinca Minor* extract in treating Alzheimer's disease may be attributed to its antioxidant activity, free radical scavenging properties, AChE inhibition, and other neuroprotective mechanisms.

CONCLUSION

The present study demonstrates that Ethanolic Extract of *Vinca Minor* (EEVM) significantly mitigates scopolamine-induced dementia and oxidative stress by enhancing learning, memory, antioxidant potential, and anti-acetylcholinesterase activity. The extract effectively promotes cholinergic neurotransmission by inhibiting AChE while strengthening the antioxidant defense system through increased GSH and catalase activity. Additionally, it reduces oxidative damage by lowering nitric oxide (NO) levels, further supporting its neuroprotective role against Alzheimer's disease.

EEVM, with its neurobiological activity, presents a promising novel therapeutic approach for preventing and treating Alzheimer's disease (AD). Its regulatory effects on cholinergic function, neurotransmitters, and inflammation are comparable to those of rivastigmine, highlighting its potential as an alternative therapeutic strategy. By modulating key biochemical markers and reducing neurodegeneration, EEVM could serve as an effective intervention for managing Alzheimer's disease and other neurodegenerative dementias.

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