ABSTRACT

*Mentha arvensis* Linn is a plant that is commonly called as *Pudina* and used in our daily cuisines in India. It belongs to family Lamiaceae that is native to the temperate regions of Europe and western and central Asia, east to the Himalaya and eastern Siberia, and America. The plant is a herbaceous perennial plant growing to 10–60 cm (rarely to 100 cm) tall. Its leaves are in opposite pairs, simple, 2–6.5 cm long and 1–2 cm broad, hairy, and with a coarsely serrated margin and the flowers of the plant are pale purple (occasionally white or pink), in clusters on the stem, each flower 3–4 mm long. The plant is widely distributed all over India. It has a number of pharmacological activities. Reported pharmacological action include antiulcerogenic effect, Inflammatory bowel syndrome (IBS), Antibacterial, Antifungal, Anticancer, Hepatoprotective, Antidiabetic, Anti Candida, Analgesic, Antiallergic and Anti-inflammatory. Very little literature is available regarding its memory enhancing activity. The present study was undertaken to study its memory enhancing activity.

KEYWORDS: Mentha arvensis, herb.

INTRODUCTION

The Central nervous system (CNS) comprising of the brain and spinal cord process information with the help of chemical messengers viz. neurotransmitters, neuromodulators, neuroregulators, neuromediators and neurotropic factors which act via specific mechanism to mediate neurotransmission. Neurotransmitter viz. Adrenaline, Nor-adrenaline, Dopamine,
Gamma Amino Butyric Acid (GABA), Glutamate, Acetylcholine, 5-Hydroxytryptamine (5-HT), Peptides viz. Endorphins, Serotonin, Glycogen and Vasoactive Intestinal Polypeptides (VIP) etc. and Neuromodulator viz. Prostaglandins (PG’s), Purines and Neuropeptides interact with their recognition sites i.e. receptors and regulate the function of CNS.[1]

Memory is the ability of an individual to record sensory stimuli, events, information etc., retain them for short or long periods of time and recall the same later when needed. Memory is the most important function of the brain.[2]

Memory loss, also referred to as amnesia, dementia or memory impairment, is an abnormal degree of forgetfulness and/or inability to recall past events. It is a disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Although the normal aging process can result in difficulty in learning and retaining new material, normal aging itself is not a cause of significant memory loss unless there is accompanying disease that is responsible for the memory loss. Memory deficits have long been recognized as severe and consistent neurological disorders associated with numerous psychiatric and neurodegenerative diseases, such as Alzheimer’s disease, Senile dementia, Parkinson’s disease, Huntington’s disease, Trauma, Chronic insomnia, Epileptic disorder and Attention deficit disorders etc.[3] however, the most common cause of memory loss has been found to be Alzheimer’s disease.

Alzheimer’s Disease (AD)
AD is a neurodegenerative disorder that destroys cells in the brain, leading cause of dementia, a condition that involves gradual memory loss, decline in the ability to perform routine tasks, disorientation, difficulty in learning, loss of language skills, impairment of judgment and personality changes. As the disease progress, people with Alzheimer’s disease fail to care for themselves and the loss of brain cells eventually lead to the failure of regions. This loss results in gross atrophy of the affected regions, including degeneration in the temporal lobe, parietal lobe, parts of the frontal cortex other systems of the body. It is typically found in people over the age of 65 years. It was first described by German psychiatrist and neuropathologist, Alois Alzheimer in 1906 and was named after him.[4] The ultimate cause of this disease is unknown but the clinical sign is progressive cognition deterioration. AD is characterized by loss of neurons and synapses in the cerebral cortex and certain sub cortical and cingulate gyrus.[5] There is an overall shrinkage of brain tissue, the grooves or furrows in
the brain, called sulci (plural of sulcus), are noticeably widened and there is shrinkage of the gyri (plural of gyrus), the well-developed folds of the brain's outer layer.

**Risk factors for AD**[^6]

There are a number of risk factors associated with Alzheimer’s disease which have been divided into two types.

- Modified risk factors, which means they can be changed.
- Unmodified risk factors, which means they cannot be changed.

**Modified Risk Factors**

- Smoking, High blood pressure, Diabetes, High cholesterol levels in the blood, Obesity and lack of physical activity, Alcohol, Depression and Head injuries are the major modifiable risk factors for both Alzheimer’s disease and vascular dementia. These risk factors are more common in older age groups.

- **Smoking**

  Cigarette smoking is causally related to a wide range of diseases including many forms of cancer, Alzheimer’s disease, cardiovascular disease and diabetes. The evidence is strong and consistent that smokers (vs. non-smokers or ex-smokers) are at a 45% higher risk of developing Alzheimer’s disease.

- **High blood pressure**

  People who have high blood pressure (hypertension) in midlife are on average more likely to develop dementia compared to those with normal blood pressure. Research has shown that treating high blood pressure with physical activity and improvements in diet can bring the risk down, if this is not successful, appropriate medications can also help.

- **Diabetes**

  Research has shown that type 2 diabetes in midlife is associated with increased risk of Alzheimer’s disease, vascular dementia and cognitive impairment. In fact, people who have type 2 diabetes are, on average, twice as likely to develop dementia compared to those without diabetes.

- **High Cholesterol**

  People with high total cholesterol levels in midlife are on average more likely to develop dementia compared to those with normal total cholesterol. Research has shown that people
who have their high cholesterol treated with drugs called “statins” have a lower risk of dementia. So treating high cholesterol is important for both heart and brain health.

- **Obesity and lack of Physical activity**
  Both obesity and lack of physical activity in midlife may increase the risk of dementia and Alzheimer’s disease and, for that reason, should also be addressed.

- **Alcohol**
  Alcohol is ranked fifth among the most important risk factors for death and disability worldwide and it has been implicated as a causal factor for more than 200 diseases and injuries, including major non-communicable diseases such as liver cirrhosis, some cancers dementia and cardiovascular disease.

- **Depression**
  People who experience depression in later life or have a history of depression may also develop dementia. However, the relationship between depression and dementia is still unclear. Many researchers believe that depression is a risk factor for dementia, whereas others believe it may be an early symptom of the disease.

- **Head injuries**
  People who experience severe or repeated head injuries are at increased risk of developing dementia. It is possible that deposits that form in the brain as a result of the injury may be linked to the onset of dementia.

Non-Modified Risk Factors
Age and gender are the most common non-modified risk factors for Alzheimer’s disease.

- **Age**
  Alzheimer’s disease is not a normal part of aging but age is the strongest known risk factor for Alzheimer’s disease. This does not mean that most people develop the disease as they become aged. Some younger people, in their 40s or 50s, are diagnosed with the young (early) onset form of the disease. After the age of 65, the risk of developing Alzheimer’s disease doubles approximately every five years.
It is well-established that aging can impair the body’s self-repair mechanisms, including in the brain. And, many of the cardiovascular risk factors increase with age, such as high blood pressure, heart disease, and high cholesterol.

> **Gender**

There has been some debate that women may be more likely to develop Alzheimer’s disease than men. The international evidence has not consistently shown this to be true. More research is required to determine if other factors than age may heighten a woman’s chances of developing Alzheimer’s disease.

> **Others**

Other medical conditions that can increase a person’s chances of developing dementia include Parkinson’s disease, multiple sclerosis, chronic kidney disease and HIV, Down syndrome and some other learning disabilities also increase a person’s risk of dementia.

Despite significant progress in characterization and understanding of Alzheimer’s disease, presently there is no cure. Many methods of treatment of Alzheimer’s disease have been explored and currently Tacrine, Donepizal, Galantamine, Cholinomimetic drugs (AChEI) etc are in use. However due to their significant side effects like hepatotoxicity and high cost, their use has been limited. Other Nootropics (e.g. Piracetam) have been widely used but the resulting chemophobia and other effects associated with it have also limited its use. So it is worthwhile to explore medicines from the nature for the treatment of these cognitive effects. Our valley of Kashmir being a rich source of medicinal plants possesses a great potential that could be exploited for the welfare of the mankind. More than 50% of plant species described in British pharmacopoeia is reported to grow in Kashmir Valley and it is established that 570 plant species are of medical importance.[7] Plant drugs are considered to be less toxic and free from side effects. Because of their effectiveness, minimal side effects and relatively low costs, herbal drugs are prescribed widely[8-11] Some of the plants and herbs that have been proved to possess memory enhancing activity include, *Bacopa monerra*, *Azadirachta indica*, *Ginkgo biloba*, *Crocos sativus* Linn., *Curcuma longa* Linn., *Zingiber officinale* Roscoe, *Allium sativum*, *Prunus amygdalus*, *Rheum spp.* Linn., *Citrus aurantium* Linn., *Emblica officinalis*, *Mentha aquatic*, etc.

In the present study one such herb, *Mentha arvensis* Linn. has been used. Different activities like Sedative-Hypnotic, Anti-inflammatory, Anti-ulcerogenic, Anti-fungal, Anti-helmentic,
Hepatoprotective\(^{12-30}\) have already been reported for *Mentha arvensis* Linn, but no Memory Enhancing Activity has been reported till date except one of its species, *Mentha aquatic*, which has been scientifically evaluated for Nootropic activity. Therefore the current study was an attempt to evaluate the Memory enhancing activity of this locally available plant, *Mentha arvensis* Linn. against Scopolamine induced Memory impairment in albino rats.

**MATERIALS AND METHODS**

Identification and Collection of the Plant material

Aerial parts of *Mentha arvensis* Linn. were collected from Chadoora area of Kashmir in the month of April-May. It was identified and authenticated by Dr. Anzar Khuroo, taxonomist, at Centre for Biodiversity & Taxonomy, University of Kashmir, Srinagar. A sample of the plant material was deposited in the herbarium of the Department of Taxonomy, University of Kashmir under VOUCHER SPECIMEN NUMBER 2227-KASH for future reference. The bulk collection of the plant was then proceeded after its proper identification and authentication.

**Preparation of the extracts**

Following extracts of the plant of *Mentha arvensis* Linn. were prepared for the current study:
- Aqueous extract of the aerial parts of *Mentha arvensis* Linn.
- 70% Ethanolic extract of the aerial parts of *Mentha arvensis* Linn.

Preparation of aqueous extract of the aerial parts of *Mentha arvensis* linn.

Aqueous extracts of the aerial parts of *Mentha arvensis* Linn. was prepared according to the method.\(^31\) Dried aerial parts of *Mentha arvensis* Linn were pulverized and the powdered material (600 g) was macerated in distilled water for 48 hours with occasional shaking and then it was allowed to stand for 18 hours. The contents were kept for elution and then filtered. Filtrate was concentrated on a water bath at a temperature of 40-50°C. The residue so obtained was air dried and then weighed to calculate the percentage yield. The residue obtained was then stored in a cool and dry place for further use in the experimental studies.

Preparation of hydroalcoholic extract of the aerial parts of *Mentha arvensis* linn.

Alcoholic extract (70% v/v ethanol) of the aerial parts of *Mentha arvensis* Linn. was prepared by the method.\(^31\) The leaves were pulverized and the powdered material (550 g) was macerated in 70% ethanol for 48 hours with occasional shaking and then it was allowed to stand for 18 hours. The contents were kept for elution and then filtered. Filtrate was
concentrated on a water bath at a temperature of 40-50°C. The residue so obtained was weighed to calculate the percentage yield, and then stored in a cool and dry place for further use in the experimental studies.

Preliminary Phytochemical Screening
The aqueous and ethanolic extracts of the aerial parts of *Mentha arvensis* Linn. were subjected to preliminary phytochemical screening. The presence of important organic chemical constituents was determined by the standard qualitative methods.[32-33]

Tests for carbohydrates
Molisch’s test (General test): To 2 ml of test solution 2-3 drops of α-naphthol and the 1 ml of conc. H$_2$SO$_4$ was added from the sides of test tube to form two layers. Violet ring at the junction of two liquids indicate the presence of carbohydrates.

i) *Test for Reducing Sugars*

a. Fehling’s Test: 1 ml each of Fehling’s A and Fehling’s B solutions were mixed and boiled for 1 min. Equal volume of test solution was added to the test tube and boiled for 5 min. Formation of yellow ppt. which turns brick red, indicates the presence of reducing sugars.

b. Benedict’s Test: To 2 ml of Benedict’s reagent, 1 ml of test solution was added and boiled for 2 min and allowed to stand. Presence of sugar is indicated by red ppt.

ii) *Test for Monosaccharides*

*Barfoed’s Test*: To 1 ml of test solution 2 ml of Barfoed’s reagent was added and boiled for 2 min and allowed to stand. Presence of sugar is indicated by red ppt.

iii) *Test for Pentose Sugar*

Mix equal amount of test solution and HCl. Heat, add a crystal of phloroglucinol. Formation of red colour indicates the presence of pentose sugar.

iv) *Test for Hexose Sugar*

*Selwinoff’s Test*: 3 ml Selwinoff’s reagent and 1 ml test solution was mixed and heated on water bath for 1-2 min. Red colour indicates the presence of hexose sugar.

v) *Test for Non-Reducing Polysaccharide (Starch)*

*Iodine Test*: 3 ml test solution and few drops of dilute iodine were mixed. Blue colour appears which disappears on heating and reappears on cooling indicates presence of starch.
Tests for Proteins

a). Biuret Test: To 3 ml test solution, 2 ml of 4% NaOH and few drops of 1% CuSO₄ was added. Violet or pink colour indicates presence of proteins.

b). Ninhydrin Test: Mixture of 3 ml test solution and 3 drops of 5% Ninhydrin solution was heated in boiling water bath for 10 min. Appearance of purplish or bluish colour indicates the presence of amino acids.

Test for Steroids

Salkowski’s Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of conc. sulphuric acid, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Test for terpenoids

Libermann Buchard’s Test: Extracts were treated with chloroform and filtered. The filtrates were treated with few drops of acetic anhydride, boiled and cooled. Conc. sulphuric acid was added. Formation of brown ring at the junction indicates the presence of terpenoid.

Tests for glycosides

i. Tests for Cardiac Glycosides

a. Keller Killiani Test: To 2 ml of extract, glacial acetic acid, one drop of 5% FeCl₃ and conc. H₂SO₄ was added. Presence of cardiac glycosides is indicated by the formation of reddish brown colour at junction of two liquid layers and upper layer appeared bluish green.

b. Legal’s Test: To extract, 1 ml pyridine and 1 ml sodium nitroprusside was added. Presence of cardiac glycosides is indicated by formation of pink to red colour.

ii. Test for Anthraquinone Glycosides

Borntrager’s Test: To 3 ml extract, add dilute H₂SO₄, boil and filter. To the cold filtrate add equal volume of chloroform. Shaken well and the organic solvent was separated, ammonia was added. The ammoniacal layer turns pink or red, which indicates the presence of anthraquinone glycosides.

iii. Test for Reduced Anthraquinone Glycosides

Modified Borntrager’s Test: Extracts were treated with ferric chloride solution and immersed in boiling water for 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of
rose-pink colour in the ammoniacal layer indicates the presence of anthranol glycosides.

iv. Test for Saponin Glycosides
*Foam Test*: Shaken the extract with water vigorously in a test tube. Persistent foam indicates the presence of saponin glycosides.

v. Test for Flavonoids
*Shinoda Test*: To dry extract, 5 ml of 95% ethanol, 3 drops of HCl and 0.5 g of magnesium turnings were added. Pink colour formation indicates the presence of flavonoids.

Tests for alkaloids
The extract was evaporated. To the residue dilute HCl was added, shaken well and filtered. With the filtrate following tests were performed.

a. *Dragendroff’s Test*: To 2-3 ml of filtrate, few drops of Dragendroff’s reagent were added. Presence of alkaloids is indicated by formation of red precipitate.

b. *Mayer’s Test*: To 2-3 ml of filtrate, few drops of Mayer’s reagent were added. Presence of alkaloids is indicated by formation of white precipitate.

c. *Wagner’s Test*: To 2-3 ml of filtrate, few drops of Wagner’s reagent were added. Presence of alkaloids is indicated by formation of reddish brown precipitate.

Tests for tannins and phenolic compounds
To 2-3 ml of aqueous extract, few drops of following reagents were added

a. *FeCl₃ (5%) Solution*: Deep blue-black colour indicated the presence of tannins and phenolic compounds.

b. *Lead Acetate Solution*: White precipitate indicated the presence of tannins and phenolics.

Animals and exposure conditions
Albino rats of Wistar strain of both sexes, weighing 120-200 gm, were procured from the animal house of Institute of Integrative Medicine (IIIM) Canal road, Jammu. The animals were kept in polypropylene cages (6 in each cage) under standard laboratory conditions (12 hour light and 12 hour dark: day and night cycle) and had a free access to commercial pelleted diet (Ashirwad Industries) and tap water ad libitum. All studies were performed in accordance with the guidelines of CPCSEA and after approval of the Institutional Animal Ethics Committee (IAEC), Department of Pharmaceutical Sciences, University of Kashmir NO:F(IAEC-Approval)KU/2015/. All the experimental work was carried out in the
temperature range of 15-20°C and relative humidity in the range of 50-80%.

Pharmacological studies
In the present study, the effect of Aqueous and 70% Ethanolic extract of aerial parts of *Mentha arvensis* Linn. was studied against Scopolamine induced amnesia in albino rats. From the literature survey no data regarding LD<sub>50</sub> of this plant was available, so Acute Oral Toxicity studies on *Mentha arvensis* Linn. were carried out. In vitro antioxidant parameters were also evaluated. The following studies were conducted on *Mentha arvensis* Linn.

- **Acute oral toxicity studies:**
  a. Aqueous extract of the aerial parts of *Mentha arvensis* Linn.
  b. 70% Ethanolic extracts of the aerial parts of *Mentha arvensis* Linn.

- **Evaluation of memory enhancing potential**
The evaluation of Memory impairment was carried out as follows.

  - **Behavioral Paradigm.**
    - Elevated Plus Maze
  - **Biochemical Estimations.**

Estimation of Brain Acetyl cholinesterase (AChE) Activity.

Acute oral toxicity studies.
Studies were carried out in order to check the toxic effects of the extracts. The study was performed as per Organization for Economic Cooperation and Development (OECD) guidelines no 425. Rats were used for this purpose. The animals were fasted overnight, providing only water, after which the extract was administered to the respective groups orally at the dose level of 2000 mg/kg body weight by gastric intubation and the groups were observed continuously for 24 hours for behavioral, neurological and autonomic profiles, and then at 24 hours and 72 hours for any lethality. The animals were further observed for toxic symptoms for 14 days. According to the guidelines if mortality is observed in 2 or 3 animals, then the dose administered is assigned as a toxic dose. If mortality is observed in one animal, then the same dose is repeated again to confirm the toxic dose. If mortality is observed in one animal, then the same dose is repeated again to confirm the toxic dose. If mortality is not observed at all, the plant extract is considered as non-toxic. Alternatively, the toxicity test is started with a dose of 100 mg/kg body weight and repeated for further other doses such as 250, 500, and 1000 and finally 2000 mg/kg body weight. In this study, parameters like
grooming, hyperactivity, sedation, respiratory arrest, convulsions, motor activity and mortality were observed.

Evaluation of memory enhancing potential
In the present study, Aqueous and 70% Ethanolic extract of the aerial parts of *Mentha arvensis* Linn were evaluated for the memory enhancing potential against Scopolamine Hydrobromide (1 mg/kg/i.p) induced Memory impairment in Wistar albino rats and Piracetam (200 mg/kg/i.p ) was used as standard Nootropic agent. The Memory Enhancing Activity was assessed by Elevated Plus Maze (EPM) task as a behavioral paradigm to record the Transfer Latency (TL) of each animal as an indicator for acquisition and retention of Memory. Biochemical estimation of Acetylcholinesterase was also carried out on the brain homogenate of same animals.

Experimental design
A total of 54 rats were employed in the present study. They were divided into nine different groups (n=6) and the experimental study was conducted for a period of 15 days. Seven days prior to behavioral study, the rats were acclimatized to the standard laboratory conditions and on day 1st, animals were trained for the Elevated Plus Maze (EPM) task. All the groups were administered different extracts except rats of Group I (received vehicle only), rats of Group II (received Scopolamine Hydrobromide) and rats of Group III (received Piracetam 200mg/kg/p.o) for successive 14 days. Scopolamine Hydrobromide (1 mg/kg body weight) was administered intraperitoneally (i.p.) in a single dose-only once, 90 minutes after administration of the last dose of respective extract except rats of group I.[34] Rats of Group I served as Normal control and received only vehicle 2% v/v acacia (10ml/kg/p.o) during 14 days study. Rats of Group II received Scopolamine Hydrobromide (1mg/kg b.w) and served as Toxic control. Rats of Group III received standard Nootropic agent, Piracetam (piracetam injection from UCB Ind. Pvt. Ltd.) in a dose of 200 mg/kg/p.o. Rats of Groups IV-VI received Aqueous extract of the aerial parts of *Mentha arvensis* Linn. at three dose levels of 100, 200,400 mg/kg/day p.o, and rats of Groups of VII-IX received 70% Ethanolic extracts of the aerial parts of *Mentha arvensis* Linn. at three dose levels of 100, 200,400 mg/kg/day p.o respectively as per the following protocol (Table 1). Transfer Latency (TL) was then recorded 45 minutes after the injection of Scopolamine Hydrobromide and retention was recorded after 24 hours (on Day 15th). The rats were then sacrificed immediately after the behavioral test for various biochemical estimations.
Table 1: Treatment Schedule.

<table>
<thead>
<tr>
<th>GROUP</th>
<th>TREATMENT</th>
<th>DOSE</th>
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<tbody>
<tr>
<td>I</td>
<td>Normal Control-Vehicle only Daily single dose of 2% Acacia</td>
<td>10mg/kg: p.o</td>
</tr>
<tr>
<td>II</td>
<td>Toxic Control Scopolamine Hydrobromide</td>
<td>1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>III</td>
<td>Standard Piracetam + Scopolamine hydrobromide</td>
<td>200mg/kg: p.o + 1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>IV</td>
<td>AMA1: Aqueous Extract of the aerial parts of Mentha arvensis + Scopolamine hydrobromide</td>
<td>100mg/kg: p.o + 1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>V</td>
<td>AMA2: Aqueous Extract of the aerial parts of Mentha arvensis + Scopolamine hydrobromide</td>
<td>200mg/kg: p.o + 1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>VI</td>
<td>AMA3: Aqueous Extract of the aerial parts of Mentha arvensis + Scopolamine hydrobromide</td>
<td>400mg/kg: p.o + 1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>VII</td>
<td>EMA1: Ethanolic Extract of the aerial parts of Mentha arvensis + Scopolamine Hydrobromide</td>
<td>100mg/kg: p.o + 1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>VIII</td>
<td>EMA2: Ethanolic Extract of the aerial parts of Mentha arvensis + Scopolamine Hydrobromide</td>
<td>200mg/kg: p.o + 1 mg/kg b.w: i.p</td>
</tr>
<tr>
<td>IX</td>
<td>EMA3: Ethanolic Extract of the aerial parts of Mentha arvensis + Scopolamine Hydrobromide</td>
<td>400mg/kg: p.o + 1 mg/kg b.w: I.P</td>
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</table>

Behavioral Paradigm Evaluation of Transfer Latency – Elevated Plus Maze (EPM) task.

The Elevated Plus Maze was described as tool for testing memory by the investigator working in the field of psychopharmacology. Elevated Plus Maze served as exteroceptive behavioral model to evaluate learning and memory in rats.

The Elevated plus Maze consists of two open arms and two closed arm which are opposite to each other. The maze was elevated to a height of 50 cm. On the 15th day respectively each rat was placed at end of the open arm, facing away from the central platform. Transfer Latency (TL) was time taken by the rats to move in to the covered arm with all its four paws, Transfer Latency was recorded by using stop watch. If the animals did not enter into one of the covered arms with in 90s, it was gently pushed in to one of the two covered arms and transfer latency was assigned as 90s. The rat was allowed to explore the maze for 10s and returned to the home cage. Twenty four hours later i.e. on 16th day Transfer Latency was recorded again. The measurement of Transfer Latency on the day 15 served as parameter for acquisition and those on day 16 served as parameter for retention of memor.\(^{[35]}\)
Biochemical Evaluation

On the day 15th the biochemical estimations were carried out on brain homogenates. For preparation of homogenate, the animals were sacrificed by cervical dislocation according to the method of [36] and the brain was carefully removed and weighed. The removed brains were washed carefully with 0.9% normal saline and homogenized in ice cold 0.1M phosphate buffer (pH 7.2, 10% w/v) using a Teflon homogenizer. The clear supernatant, obtained after centrifugation at 3000 rpm for 15 min at 4°C, was used to estimate Acetylcholinesterase (AChE) activity.

- Estimation of Brain Acetylcholinesterase (AChE) Activity.

Principle

The assay is based on measurement of the change in absorbance at 412 nm. The assay uses the thiol ester acetylthiocholine instead of the oxy ester acetylcholine. AChE hydrolyses the acetyl-thiocholine to produce thiocholine and acetate. The thiocholine in turn reduces the Dithiobiis-Nitrobenzoic Acid (DTNB) liberating Nitro-Benzoate, which absorbs at 412 nm.

The reaction is shown below.

\[
\text{Acetylthiocholine} \xrightarrow{\text{enzyme}} \text{Thiocholine + Acetate}
\]

\[
\text{Thiocholine + Dithiobisnitrobenzoate} \rightarrow \text{yellow color}
\]

Reagents

- Sodium phosphate buffer (pH 7.2, 0.1M)
- Ellman’s reagent (DTNB)
- Acetylthiocholine iodide

Preparation of reagents

1. Sodium phosphate buffer (pH 7.2, 0.1M): 3.42ml of 1M Na\textsubscript{2}HPO\textsubscript{4} and 1.58 ml of 1M NaH\textsubscript{2}PO\textsubscript{4} were added and volume was made upto 45ml with water. pH was then adjusted by NaOH/phosphoric acid and final volume was made upto 50ml with water.
2. Ellmans reagent: 47.53 mg of Ellmans reagent were taken and added to 12 ml of distilled water.

Procedure: (Ellman GL, 1961)

1. A 0.4-ml of prepared homogenate was added to a cuvette containing 2.6 ml of phosphate buffer (pH 7.2, 0.1 M).
2. 100 µl of Ellman’s reagent (DTNB 0.01 M) reagent was added and taken into a photocell. The absorbance was measured at 412 nm; when this had stopped increasing, the photometer slit was opened, so that the absorbance was set to zero.

3. Of the substrate (Acetylthiocholine iodide 0.075M), 20 µl were added. Changes in absorbance were recorded and the change in absorbance per min. was calculated.

4. The rates were calculated as follows.

\[
R = \frac{\Delta A}{1.36 \times 10^3} \times \frac{1}{\left( \frac{400}{3120} \right)C_0} = 5.74 \times 10^{-4} \frac{\Delta A}{C_0}
\]

Where,

- \( R \) = rate, in moles substrate hydrolyzed per min per g of tissue;
- \( A \) = change in absorbance per min;
- \( C_0 \) = original concentration of tissue (mg/ml).

**RESULTS**

Physical characteristics and percentage yield of different extracts.

The Aqueous (AMA) and Ethanolic 70% (EMA) extracts of the aerial parts of *Mentha arvensis* Linn. showed the following observations.

- **a) Aqueous extract (AMA)**
  - Amount of powdered leaves taken = 600 g.
  - Weight of the extract obtained = 50.4 g.
  - % age yield = 11.90%.

<table>
<thead>
<tr>
<th>Table 2: Colour, Odour and % Extractive value of AMA.</th>
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<tbody>
<tr>
<td>Extract</td>
</tr>
<tr>
<td>---------</td>
</tr>
<tr>
<td>Aqueous</td>
</tr>
</tbody>
</table>

- **b) 70% Ethanolic extract (EMA)**
  - Amount of powdered leaves taken = 550 g
  - Weight of the extract obtained = 45 g
  - % age yield = 12.22%.

<table>
<thead>
<tr>
<th>Table 3: Colour, Odour and % Extractive value of EMA.</th>
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<tbody>
<tr>
<td>Extract</td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Ethanolic</td>
</tr>
</tbody>
</table>
Preliminary Phytochemical Screening Tests

The Preliminary Phytochemical screening conducted on the Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. gave positive results for Alkaloids, Tannins, Saponins, Glycosides, Terpenes, Flavonoids, Fats, Carbohydrates, Steroids, Proteins (Table 1).

**Table 4: Results of Preliminary Phytochemical screening of the aerial parts of *Mentha arvensis* Linn.**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>PHYTOCONSTITUENTS</th>
<th>AMA</th>
<th>EMA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Flavonoids</td>
<td>++</td>
<td>+++</td>
</tr>
<tr>
<td>2</td>
<td>Phenolics</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>3</td>
<td>Proteins</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>4</td>
<td>Saponins</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Terpenes</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Carbohydrates</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Tannins</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

AMA: Aqueous extract of the aerial parts of *Mentha arvensis.*

EMA: 70% ethanolic extract of aerial parts of *Mentha arvensis.*

(-) = Absent; (+) = Slight coloration; (++) = Deep coloration; (+++) = Very deep coloration.

Acute oral toxicity testing

Acute Oral Toxicity studies revealed that the Aqueous and 70% Ethanolic extracts of *Mentha arvensis* Linn. were safe up to 2000 mg/kg of body weight and approximate LD$_{50}$ is more than 2000 mg/kg b.w. No lethality or any toxic reactions or morbidity was observed up to the end of the study period.

Results of memory enhancing study of the aerial parts of *mentha arvensis* linn. using scopolamine hydrobromide as a memory impairing agent in rats.

Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. were evaluated for Memory Enhancing Potential using Scopolamine Hydrobromide as a Memory impairing agent in rats. Both the Aqueous and 70% Ethanoic extract of the aerial parts of *Mentha arvensis* Linn. were administered orally to different groups of Wistar albino rats (either sex) at the dose levels of 100, 200 and 400 mg/kg b.w daily for 14 days to study
their effect against Scopolamine Hydrobromide induced Memory impairment.. Scopolamine Hydrobromide (1mg/kg i.p) was given only once at 14th day, 90 min. after the last dose of extracts/Piracetam. The evaluation of Memory Enhancing Potential was done by behavioral paradigm using Elevated Plus Maze (EPM) as exteroceptive model to record the Transfer Latency (TL) on the 14th day, 45 min. after the Scopolamine Hydrobromide administration which indicates the Acquisition of Memory and after 24 hrs (on 15th day) which indicates the Retention of Memory in experimental animals (Albino rats). The decrease in TL in tested groups compared to Toxic control represents the reversal of scopolamine induced memory impairment.

The evaluation of Memory Enhancing Potential was also done by conducting different Biochemical Estimations (Acetyl cholinesterase (AChE) activity in the brain homogenates of treated animals (Rats) in each group. All the experiment work was carried out in the ideal temperature and humanity range of 15-20°C and 70-75 % respectively.

Results of behavioral paradigm (elevated plus maze)

Effects of Aqueous (AMA) and 70% Ethanolic (EMA) extracts of the aerial parts of Mentha arvensis Linn. (Table 15, Fig. 11).

Aqueous and Ethanolic (AMA and EMA) extract of the aerial parts of Mentha arvensis Linn. showed dose dependent reversal of Scopolamine Hydrobromide induced memory impairment in wistar albino rats, when administered orally for 14 days.

A highly significant increase (p< 0.01) in the TL of the rats of Group II (64.87±1.45sec), administered 2% Acacia+ Scopolamine Hydrobromide (1mg/kg i.p on 14th day after last dose of vehicle) was observed when compared to TL (38.13±1.69sec) of the rats of Group I (normal control received 2% Acacia (10mg/kg p.o) only). Moreover, a highly significant (p<0.01) increase was also observed in TL (59.39±3.33 sec) of the rats of Group II on 15th day (retention) as compared to the TL of the rats of Group I (27.36±0.97 sec) on 15th day.

The dose of 100mg/kg b.w/day, p.o of Aqueous extract (AMA1) of the aerial parts of Mentha arvensis Linn. when administered along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day) to the rats of Group IV showed a significant decrease (p<0.05) in TL (44.81±1.32 sec) on 14th day (Acquisition) and TL (21.39±0.59 Sec) on 15th day (Retention)
as compared to TL of the rats of Group II (64.87±1.45 sec) on 14th day (Acquisition) and TL (59.39±3.33 sec) on 15th day (Retention).

At the dose of 400mg/kg b.w/day, p.o of 70% Ethanolic extract (EMA3) the aerial parts of *Mentha arvensis* Linn. when administered along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day) to the rats of Group X showed a very highly significant decrease (P<0.001) in TL (30.60 ± 0.73 Sec) on 14th day and TL (13.41 ±0.44 Sec) on 15th day as compared to TL of the rats of Group II (64.87±1.45 sec) on 14th day (Acquisition) and TL (59.39±3.33 sec) on 15th day (Retention), indicating a significant reversal of Memory impairment induced by Scopolamine Hydrobromide in experimental animals.

Effect of Aqueous and 70% Ethanolic extracts of *Mentha arvensis* Linn.on Transfer Latency against Scopolamine Hydrobromide induced Memory impairment.

Biochemical Evaluation; Acetylcholinesterase (AChE) activity.

Effects of Aqueous (AMA) and 70% Ethanolic (EMA) extract of the aerial parts of *Mentha arvensis* Linn. (AMA) on Acetylcholinesterase (AChE) activity.

Both Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. showed dose-dependent decrease in AChE activity in the brains of animals, which were administered Aqueous and 70% Ethanolic extracts at different doses for 14-days along with Scopolamine Hydrobromide at 14th day after last dose of extracts when compared to the rats of toxic control group (Group II) that received scopolamine Hydrobromide only.
A very highly significant increase (p< 0.001) in the AChE activity of the rats of Group II (2.38 ± 0.189 µmol/min/g tissue), compared to the rats of Group I (1.56 ± 0.062 µmol/min/g tissue) that received 2% Acacia (10 ml/kg p.o) only.

The rats of Group III, treated with Piracetam, 200mg/kg/day (along with Scopolamine Hydrobromide 1mg/kg b.w, i.p once at 14th day) for 14 days showed a very highly significant decrease (p<0.001) in AChE activity (1.00 ± 0.078 µmol/min/g tissue) when compared to AChE activity of the rats of Group II (2.38 ± 0.189 µmol/min/g tissue) and showed a non significant decrease (p>0.05) in AChE activity as that of the rats of Group I (1.56 ± 0.062 µmol/min/g tissue).

The dose of 100mg/kg b/w/day, p.o of Aqueous extract (AMA1) of the aerial parts of Mentha arvensis Linn. when administered Along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day) to rats of Group IV showed an extremely significant decrease (p<0.001) in AChE activity (2.05 ± 0.216 µmol/min/g tissue) compared to AChE activity of the rats of Group II (2.38± 0.189 µmol/min/g tissue) and non-significant increase (p>0.05) in AChE activity compared to the rats of Group III (1.00 ± 0.078 µmol/min/g tissue).

At the dose of 200mg/kg b/w/day, p.o of Aqueous extract (AMA2) of the aerial parts of Mentha arvensis Linn. when administered Along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day) to the rats of Group V showed a very highly significant decrease (p<0.01) in AChE activity (1.98 ± 0.120 µmol/min/g tissue) as compared to AChE activity of the rats of Group II (0.2.38 ± 0.189 µmol/min/g tissue) and non-significant increase (P>0.05) in AChE activity compared to the rats of Group III (1.00 ± 0.078µmol/min/g tissue).

At the dose of 400mg/kg b/w/day, p.o of Aqueous extract (AMA3) of the aerial parts of Mentha arvensis Linn. when administered along with Scopolamine hydrobromide (1mg/kg b.w, i.p once at 14th day) to rats of Group VI showed a very highly significant decrease (p<0.001) in AChE activity (1.86 ± 0.077 µmol/min/g tissue) when compared to AChE activity of the rats of Group II (0.2.38 ± 0.189 µmol/min/g tissue) and non-significant increase (p>0.05) in AChE activity compared to the rats of Group III (1.00 ± 0.078µmol/min/g tissue).

The dose of 100mg/kg b.w/day, p.o of 70% Ethanolic extract (EMA1) of the aerial parts of Mentha arvensis Linn. when administered along with Scopolamine hydrobromide (1mg/kg
b.w, i.p once at 14th day) to rats of Group VII showed a very highly significant decrease (p<0.001) in AChE activity (2.03 ± 0.076 µmol/min/g tissue) compared to the AChE activity of the rats of Group II (2.38 ± 0.189 µmol/min/g tissue) and also found to be non significant (p>0.05) as that of the AChE activity of the rats of Group I (1.56 ± 0.062 µmol/min/g tissue). A dose of 200mg/kg b.w/day, p.o of 70% ethanolic extract (EMA2) of the aerial parts of *Mentha arvensis* Linn. when administered along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day) to the rats of Group VIII showed a very highly significant decrease (p<0.001) in AChE activity (1.69 ± 0.046 µmol/min/g tissue) compared to AChE activity of the rats of Group II (2.38 ± 0.189 µmol/min/g tissue) and also found to be non significant (p>0.05) as that of the AChE activity of the rats of Group I (1.56 ± 0.062 µmol/min/g tissue).

A dose 400mg/kg b.w/day, p.o of 70% Ethanolic extract (EMA3) of the aerial parts of *Mentha arvensis* Linn. when administered along with Scopolamine Hydrobromide (1mg/kg b.w, i.p once at 14th day) to the rats of Group IX showed a very highly significant decrease (p<0.001) in AChE activity (1.50 ± 0.047 µmol/min/g tissue) when compared to AChE activity of the rats of Group II (2.38 ± 0.189 µmol/min/g tissue) and also found to be non significant (p>0.05) as that of the AChE activity of the rats of Group I (1.56 ± 0.062 µmol/min/g tissue).

![Fig. 2: Effect of Aqueous and 70% Ethanol (AMA and EMA) extracts of the aerial parts of *Mentha arvensis* Linn. on Acetylcholinesterase (AChE) activity against Scopolamine Hydrobromide induced memory impairment.](image)
CONCLUSION

The utilization of herbal plants has drawn immense interest as they could accommodate therapeutic response and are promising candidates to be developed as pharmaceutical products like antioxidants, antibacterial, hepatoprotective, anti-inflammatory agents etc. Mentha arvensis is one of these herbal plants which has proved beneficial in a number of fields and research is going on.

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