Evaluation of In Vitro Anti-Oxidant Activity of Mentha arvensis Linn in Memory Enhancing Study

Nazima Ameen, Sabeeha Shafi*

Department of Pharmaceutical Sciences, University of Kashmir, Hazratbal, Srinagar, Kashmir, Jammu & Kashmir (India)

Received: 8th June, 17; Revised 22nd June, 17, Accepted: 15th Aug, 7; Available Online:25th Aug, 17

ABSTRACT
The main objective of the study was to evaluate the in vitro anti-oxidant parameters for the study of memory enhancing activity of Mentha arvensis Linn closely related to Alzheimers disease. Mentha arvensis Linn is a plant commonly known as Pudina. It is used in our daily cuisines in India. The study included the aerial parts of Mentha arvensis belonging to family Lamiaceae. Two extracts were taken as the aqueous and ethanolic extract. The phytochemical analysis of plant of Mentha arvensis Linn showed the presence of alkaloids, tannins, saponins, glycosides, terpenes, flavonoids, fats, carbohydrates, steroids and proteins. Oxidative compounds are responsible for causing many diseases like ischemic heart disease, Parkinson disease, hyperlipidemia, atherosclerosis and Alzheimers disease. In order to cure these diseases anti-oxidants are used. Keeping these facts in view, the anti-oxidant activity of Mentha arvensis Linn was evaluated in memory enhancing activity.

Keywords: Mentha arvensis, herb.

INTRODUCTION
The has been involvement of oxidative stress and free radical damage to the pathogenesis of Alzheimers Disease. Under normal conditions, damage by oxygen radicals is controlled by anti-oxidants for example the metabolism of H2O2 by catalase and glutathione peroxidase. During pathological conditions, the oxidant versus anti-oxidant balance is necessarily changed, either primarily or secondarily. Oxidative damage occurs when the oxidative balance is disturbed such that reactive oxygen species production exceeds cellular anti-oxidant defences. The oxidative damage found in the pathogenesis of Alzheimers Disease includes advanced glycation end products, nitration, lipid peroxidation adduction products, carbonyl-modified neurofilament protein and free carbonyls. This kind of damage selectively involves all neurons in populations vulnerable to death in Alzheimers disease. Memory is that ability of an person to record sensory stimuli, events, information etc., and retain them for short or long periods of time. These recordings are recorded and recalled later when needed. Memory is the most important function of the brain.

Memory loss, which is also referred to as amnesia, dementia or memory impairment, is an abnormal degree of forgetfulness and/or inability to recall past events. It has been described as 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 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. 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. There is an overall shrinkage of

*Author for Correspondence: sabeehashafi@gmail.com
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 include modified risk factors such as Smoking, High blood pressure, Diabetes, High cholesterol levels in the blood, Obesitiy 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. Age and gender are the most common non-modified risk factors for Alzheimer’s disease. 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 many drugs like Tacrine, Donepezil, 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.

Kashmir called as Switzerland of Asia being a rich source of medicinal plants which 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. 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. Some of the plants and herbs that have been proved to possess memory enhancing activity include, Bacoppa monniera, Azadirachta indica, Ginkgo biloba, Crocus sativus Linn., Curcuma longa Linn., Zingiber officinal Roscoe, Allium sativum, Prunus amygdalus, Rheum spp. Linn., Citrus aurantium Linn., Emblica officinalis, Mentha aquatica, etc.

In the present study one such herb, Mentha arvensis Linn. has been used. Different activities of Sedative-Hypnotic, Anti-inflammatory, Anti-ulcerogenic, Antifungal, Anti-helmentic, Hepatoprotective 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 in vitro anti-oxidant activity of 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:

1. Aqueous extract of the aerial parts of Mentha arvensis Linn.
2. 70% Ethanolic extract of the aerial parts of Mentha arvensis Linn.
3. Preparation of aqueous extract of the aerial parts of Mentha arvensis linn.
4. Aqueous extracts of the aerial parts of Mentha arvensis Linn. was prepared according to the method. 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. 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.

**Biochemical Estimations**

**Estimation of Brain Acetyl cholinesterase (AChE) Activity**

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\textsuperscript{14}. 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). The rats were then sacrificed immediately after the behavioral test for various biochemical estimations.

**Biochemical Evaluation**

On the day 15\textsuperscript{th} 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 and the brain was carefully removed and weighed. The removed brains were washed carefully with 0.9% normal saline and dislocation according to the method of 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, 0.1M) 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 10 minutes, 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.

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.

The rates were calculated as follows:

\[
R = \frac{\Delta A}{1.36 \times 10^4 \times \frac{1}{400} \times 3120} = 5.74 \times 10^{-5} \frac{\Delta A}{C_0}
\]

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).

**In Vitro antioxidant studies**\textsuperscript{16,37}

The in-vitro antioxidant studies were conducted on the Aqueous and 70% Ethanolic extracts of aerial parts of Mentha arvensis Linn. by using Ascorbic acid as a standard reference. The following methods were used:

- **Diphenyl picryl hydrazyl (DPPH) radical scavenging activity**

  The free Radical Scavenging Capacity of Ethanolic and Aqueous aerial extract of Mentha arvensis Linn was determined by the standard method. Freshly prepared DPPH (2,2-diphenyl-1-picyrlhydrazyl), solution was taken in test tubes and extract were added followed by serial dilutions (50μg/ml to 250μg/ml) to every test tube so that the final volume was 3 ml and after 30 min, the absorbance was read at 517 nm using a spectrophotometer. Ascorbic acid was used as standard. Control sample was prepared containing the same volume without any extract and standard and the absorbance was read at 517 nm using a spectrophotometer. Methanol served as blank. The percent inhibition was determined as follows:

  \[
  IC_{50} = \frac{Abs C - Abs S}{Abs C} \times 100
  \]

  Where:

  - \( Abs C \) = Absorbance of Control
  - \( Abs S \) = Absorbance of Standard/ Sample

**Determination of reducing activity**

The reductive capability of the Ethanolic and Aqueous aerial extract of Mentha arvensis Linn was quantified by the standard method. One ml of the extracts (100, 200, 300, 400 and 500μg/ml) was mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide [K\textsubscript{3} Fe (CN)\textsubscript{6}]. Similar concentrations of standard ascorbic acid were used as standard. The mixture was incubated at 50°C for 20 min. Then, the
reaction was terminated by adding 2.5 ml of 10% trichloroacetic acid. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.5 ml of 0.1% FeCl$_3$. Blank reagent is prepared as above without adding extract. The absorbance was measured at 700 nm in a spectrophotometer against a blank sample. Increased absorbance of the reaction mixture indicated greater reducing power.

The percent inhibition was determined as follows:

\[
\text{Percent Inhibition} = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \times 100
\]

Where:

\- \text{Abs C} = \text{Absorbance of Control}
\- \text{Abs S} = \text{Absorbance of Standard/Sample}

Nitric oxide radical inhibition assay

Nitric oxide radical inhibition can be estimated by the use of Griess Illosvoy reaction. In this assay, Griess Illosvoy reagent was modified by using naphthyl ethylene diamine dihydrochloride (0.1% w/v) instead of 1-napthylamine (5%). The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml) and Mentha arvensis Linn extracts (25 to 125 mg/ml) or standard solution (rutin, 0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture mixed with 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylen diamine dihydrochloride was added, mixed and allowed to stand for 30 min at 25°C. A pink coloured chromophore is formed in diffused light. The absorbance of these solutions was measured at 540 nm against the corresponding blank solutions. Rutin was used as a standard.

The percent inhibition was determined as follows:

\[
\text{Percent Inhibition} = \frac{\text{Abs C} - \text{Abs S}}{\text{Abs C}} \times 100
\]

Where:

\- \text{Abs C} = \text{Absorbance of Control}
\- \text{Abs S} = \text{Absorbance of Standard/Sample}

Statistical analysis

The data obtained from the biochemical evaluations was expressed as MEAN ± SEM for each group. The statistical analysis was carried out using one way analysis of variance (ANOVA) followed by Student’s ‘t’ test. Values of p<0.05 were considered statistically significant and values of p<0.01 were considered very highly significant.
statistically highly significant and a very highly significant respectively while as p>0.05 was considered as non-significant.

**RESULTS AND CONCLUSION**

Alzheimer’s Disease 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 other systems of the body. The administration of Antimuscarinic agent scopolamine produces transient memory deficit. Scopolamine Hydrobromide amnesia test is widely used as primary

Table 3: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of Mentha arvensis Linn. on Reducing Power.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/ml)</th>
<th>Percent (%) Inhibition (MEAN ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard (Ascorbic acid) I (Std)</td>
<td>Aqueous extract (Aerial Parts) II (AMA)</td>
</tr>
<tr>
<td>01</td>
<td>50</td>
<td>0.71±0.03</td>
</tr>
<tr>
<td>02</td>
<td>100</td>
<td>0.83±0.03</td>
</tr>
<tr>
<td>03</td>
<td>150</td>
<td>1.62±0.08</td>
</tr>
<tr>
<td>04</td>
<td>200</td>
<td>1.20±0.22</td>
</tr>
<tr>
<td>05</td>
<td>250</td>
<td>2.70±0.13</td>
</tr>
</tbody>
</table>

p- Value Statistically Compared Groups

II v/s I
III v/s I
p>0.05 Non-Significant; p<0.05* Significant; p < 0.01** Highly Significant; p <0.001*** very highly Significant

Table 4: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of Mentha arvensis Linn. on Nitric Oxide Scavenging Property.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Concentration (µg/ml)</th>
<th>Percent (%) Inhibition (MEAN ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard (Rutin) I (Std)</td>
<td>Aqueous extract (Aerial Parts) II (AMA)</td>
</tr>
<tr>
<td>01</td>
<td>50</td>
<td>40.12±0.81</td>
</tr>
<tr>
<td>02</td>
<td>100</td>
<td>62.33±1.19</td>
</tr>
<tr>
<td>03</td>
<td>150</td>
<td>72.55±1.52</td>
</tr>
<tr>
<td>04</td>
<td>200</td>
<td>83.12±1.79</td>
</tr>
<tr>
<td>05</td>
<td>250</td>
<td>96.77±1.17</td>
</tr>
</tbody>
</table>

p- Value Statistically Compared Groups

II vs I
III vs I
p>0.05 Non-Significant; p<0.05* Significant; p < 0.01** Highly Significant; p <0.001*** very highly Significant

statistically highly significant and a very highly significant respectively while as p>0.05 was considered as non-significant.

Alzheimer’s Disease 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 progresses, people with Alzheimer’s disease fail to care for themselves and the loss of brain cells eventually lead to the failure of other systems of the body. The administration of Antimuscarinic agent scopolamine produces transient memory deficit. Scopolamine Hydrobromide amnesia test is widely used as primary

**Figure 1:** Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of Mentha arvensis Linn. on Acetylcholinesterase (AChE) activity against Scopolamine Hydrobromide induced memory impairment.
screening test for anti-Alzheimer drug. Acetylcholine is considered as the most important Neurotransmitter involved in the regulation of Cognitive functions. Cholinergic neurons play an important role in Cognitive

Figure 2: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the leaves of the aerial parts of Mentha arvensis Linn. on DPPH Radical Scavenging.

Figure 3: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of Mentha arvensis Linn. on Nitric Oxide Scavenging Property.

Figure 4: Effect of Aqueous and 70% Ethanolic (AMA and EMA) extracts of the aerial parts of Mentha arvensis Linn. on Nitric Oxide Scavenging Property.
deficit associated with Alzheimer’s disease and Neurodegenerative diseases.\textsuperscript{38-43}

In the present study, Preliminary Phytochemical Screening of the Aqueous and Ethanolic extracts of Mentha arvensis Linn. revealed the presence of Flavonoids, Phenolics, Proteins, Saponins, Terpenes, Carbohydrates, Steroids, Glycosides, Tannins and Alkaloids in Aqueous extract of the aerial parts of Mentha arvensis Linn. while the Ethanolic extract of aerial parts of Mentha arvensis Linn. contains all the above constituents except Tannins.

Scopolamine is a centrally acting cholinergic agent which causes impairment in learning. The treatment with drugs which increase Cholinergic Neurotransmission causes an improvement in Cognitive deficits in AD\textsuperscript{44}

Both Aqueous and 70% Ethanolic extract of Mentha arvensis Linn. inhibited Acetylcholinesterase enzyme in a dose dependent manner, there by elevating Acetylcholine concentration in the brain homogenate and ultimately improved memory in rats. Both the extracts at the dose of 400 mg/kg b.w showed highest inhibition of Acetyl cholinesterase enzyme than the other two respective doses (200, 100mg/kg b.w). The present study were carried at lower doses level of 100 and 200 and 400 mg/kg b.w/day of each extract (1/20, 1/10, 1/5\textsuperscript{th} of 2000mg). In addition to this Antioxidant parameters (DPPH Radical Scavenging Activity, Nitrous Oxide Scavenging Property and Reducing Power) were also evaluated. The Antioxidant Parameters like DPPH Radical Scavenging Activity, Nitrous Oxide Scavenging Property and Reducing Power were also evaluated. The results shown by Aqueous and 70% Ethanolic extracts of the aerial parts of Mentha arvensis Linn. for DPPH Radical Scavenging Activity was found to be concentration dependent, with 70% Ethanolic extract having a greater DPPH Radical Scavenging Activity as compared to Aqueous extract at the same concentration. The Reducing Power Activity shown by Aqueous and 70% Ethanolic extracts of the aerial parts of Mentha arvensis Linn. was found to be concentration dependent with 70% Ethanolic extract having a greater Reducing Power Activity as compared to Aqueous extract at the same concentration.

The Nitric Oxide Scavenging Property shown by Aqueous and 70% Ethanolic extracts of the aerial parts of Mentha arvensis Linn. was found to be concentration dependent with 70% Ethanolic extract having a greater Nitric Oxide Scavenging Activity as compared to Aqueous extract at the same concentration. The Aqueous and 70% Ethanolic extract of the aerial parts of Mentha arvensis Linn. also showed concentration dependent increase in the Antioxidant Activity (DPPH, Reducing Power and Nitric oxide Scavenging Activity). The Concentration of 250μg/ml showed very highly significant decrease in the mean % inhibition as compared to the concentration of 200,150,100,50 μg/ml. Furthermore 70% Ethanolic extract was found to possess more antioxidant activity as compared to the Aqueous extract.

From the study it can be concluded that the aerial parts of Mentha arvensis Linn. possess good Memory Enhancing Activity, but the effect was found to be more pronounced in case of 70% Ethanolic extract than the Aqueous extract of the plant. However, further studies need to be conducted on this plant to isolate the active constituent(s) responsible for its Memory Enhancing action and for elucidating the mechanism of action of the isolated compound.

More plants need to be evaluated for their memory enhancing activity\textsuperscript{45-46}.

REFERENCES

11. Dr.Duke’s phytochemical and Ethnobotanical Databases.
29. Kumbhar, Umesh R. Screening of Mentha arvensis Linn. for anthelmintic activity. /hdl.handle.net/123456789/2376.
43. Sujith K, Ronald DC, Sathish, Suba V. (2012). Memory-enhancing activity of Anacyclus pyrethrum...

