PRELIMINARY PHYTOCHEMICAL SCREENING AND ACETYLCHOLINESTERASE INHIBITORY ACTIVITY OF ARTEMISIA AMGDALINA

Nasreena Sajjad1, Abubakar Wani2, Rohaya Ali1, Sumaya Hassan1,

*Bashir Ahmed Gani3, Rabia Hamid1

1Department of Biochemistry, University of Kashmir, (India)
2Indian Institute of Integrative Medicine, Jammu, (India)
3Centre of Research for Development, University of Kashmir, (India)

ABSTRACT

The main strategy against Alzheimer’s Disease (AD) is to inhibit acetylcholinesterase and it is still considered one of the important therapeutic strategy. There are various phytochemicals that have been reported to exhibit acetylcholinesterase inhibitory activity. Moreover, many acetylcholinesterase inhibitors (AChEIs) are used for the symptomatic treatment of AD. In the present study we screen methanolic extract of Artemisia amgdalina for acetylcholinesterase inhibitory activity. This assay is based on Ellman’s method. The extract showed 0% ±0, 14% ±1.46, 29% ±1.33, 51% ±2.65, 70% ±4.54 percent AChE inhibition at 0, 25, 50 and 100 μg/mL concentrations respectively. Qualitative phytochemical Analysis demonstrated the presence of secondary metabolites like alkaloids, cardiac glycosides, phenolics. Considering the complex multifactorial etiology of AD, the plant extracts will be safer and better candidates for the future disease modifying therapies against this devastating disease.

Keywords: Alzheimer's disease, Acetylcholinesterase inhibition, Acetylcholine, Medicinal plants, Secondary metabolites,

Abbreviations: AChE, Acetylcholinesterase; AChEIs, Acetylcholinesterase inhibitors; Ach, Acetylcholine; AD, Alzheimer disease

1. INTRODUCTION

Alzheimer’s disease (AD) is the most common form of dementia. [1] The nature of this disease is very complex, it is of multifactorial nature. [2] There are various hypothesis given from time to time to explain the complex nature of AD. The treatment of people suffering from AD poses a great challenge. [3] There is continuous ongoing research in
the development of new therapies and drugs in the treatment of AD. [4] Cholinergic deficit hypothesis has received the greatest attention. [5] Acetylcholine is very important neurotransmitter which is responsible for signal transmission in the brain. [6] There occur degeneration of acetylcholine (Ach) synthesizing neurons in subcortical nuclei of the human basal forebrain. [7] Acetylcholinesterase is one of the essential enzyme which catalyses the hydrolysis of neurotransmitter acetylcholine thereby terminating the impulse transmission. [8] Therefore, AChE inhibition has emerged as the major therapeutic target. There are many cholinesterase inhibitors which have been in market as drugs like rivastigmine, donepezil and galantamine. [9] Many of which are plant derived. But these drugs result in many side effects like abdominal pain, dizziness, weight loss, syncope etc [10] Therefore there is need of better options. Herbal medicine has documented many natural plant derived Acetylcholiesterase inhibitors (AchIs). They are basically plant derived secondary metabolites usually belonging to group alkaloids. [11] Considering that the naturally occurring compounds from plants can be considered as potential source of new inhibitors. Plants have been screened for cholinesterase inhibitory activity. [12] Ayurvedic system of medicine of India has been using various plant species since 4000 years for treating central nervous system disorders as well as to improve the memory and cognitive function. [13]

*Artemisia amgdalina*, a medicinally important plant of Kashmir valley, has been taken for the present study. It is an endemic plant of the valley belonging to family Asteraceae. [14] It is locally known as “veer tethven” in Kashmir. This plant grows in subalpine region of Kashmir Himalaya. [15] The plant extract has been used locally in the treatment of various ailments like piles, nervous disorders, epilepsy and pain. The women folk of the valley use it for amenorrhea and dysmenorrhea. [16] In India, *Artemisia* species are mainly used in traditional system of medicine, *Unani-tibb* and *Ayurveda* for the management of various ailments [17]. In the present study phytochemical screening was carried out to identify major biologically active phytoconstituents and the Acetylcholinesterae inhibitory activity was screened.

II MATERIALS & METHODS

2.1. Chemicals

Acetylthiocholine iodide (ATCI), AChE from electric eel (type VI-S lyophilized powder), bovine serum albumin (BSA), 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB), Donepezil, were purchased from Sigma Aldrich. All other reagents used were of analytical grade and obtained locally.

2.2. Plant Material and Samples Preparation

*Artemisia amgdalina* was collected from Botanical garden, Department of Botany, University of Kashmir and identified in the Centre of Plant Taxonomy (COPT). The whole plant material was collected, dried and pulverized into coarse powder and extracted successively using hexane, ethyl acetate, methanol, respectively by soxhlet extraction. The solvents were allowed to evaporate in a rotary evaporator at 40°C–45°C, and the extracts obtained
were stored in a refrigerator at 4°C. The entire study was conducted using single batch of each plant extract to avoid batch-to-batch variation and maximize the product constancy.

2.3. Phytochemical Screening
The methanolic extract was subjected to phytochemical analysis via various qualitative tests, to ascertain the presence of secondary metabolites like alkaloid, flavonoids, tannins, steroids, saponins, terpenes. The phytochemical screening was carried out by using standard qualitative methods described by Trease and Evans [18].

2.4. Evaluation of AChE Inhibitory Activity Using Ellman’s Method
The methanolic extract of Artemisia amgdalina was tested for AChE inhibitory activity using modified method as described by Ellman’s colorimetric method [19] in 96-welled microplate. The enzyme acetylcholinesterase hydrolyzes the substrate ATCI to thiocholine and acetic acid. Thiocholine is allowed to react with DTNB, and this reaction resulted in the development of a yellow color. The color intensity of the product is measured at 412 nm, and it is proportional to the enzyme activity. Donepezil, a standard AChE inhibitor, was used as positive control. The percent of acetylcholinesterase inhibition was calculated as following:

\[
\% \text{ Inhibition} = 100 - \left[\frac{\text{Absorbance of the test compound}}{\text{Absorbance of the control}}\right] \times 100
\]

2.5. Statistical analysis: Data are expressed as mean ± standard deviation (SD). All analysis was carried out in at least three replicates for each sample. Results were analyzed statistically using SPSS 15.0, Sigma plot 10.0, and GraphPad Prism 5 software (San Diego, CA, USA). A value of \( p < 0.05 \) was considered statistically significant.

III RESULTS

3.1. Phytochemical Screening of plant extract
The phytochemical analysis of Artemisia amgdalina revealed the presence of terpenes, alkaloids, phenolics, cardiac glycosides in methanolic extract of Artemisia amgdalina. (TABLE 1)

3.2. Acetylcholinesterase Inhibitory Activity of the Extracts
The plant extract of Artemisia amgdalina showed AChE inhibitory activity in a dose-dependent manner. The extract showed 0% ±0, 14% ±1.46, 29% ±3.13, 51% ±2.65, 70% ±4.54 percent AChE inhibition at 0, 25, 50 and 100 μg/mL concentrations respectively. (Fig a) The results of this study confirm the potent acetylcholinesterase inhibition of the plant extract.
DISCUSSION

Alzheimer’s disease is the common form of dementia. Various pathways are involved in the pathogenesis of Alzheimer disease [20]. Progressive loss of cholinergic synapses takes place in the brain of AD patients. Many treatment strategies have been developed. [21] One of the major targets of treatment strategy, is the inhibition of acetylcholinesterase. In fact many of the drugs in the market are basically acetylcholinesterase inhibitors like we have tacrine, serine, donepezil, rivastigmine and galanthamine.[22] Several studies revealed that cholinesterase inhibitors could act on multiple therapeutic targets such as prevention of the formation of b-amyloid plaques, antioxidant activity and modulation of APP processing[23]. Acetylcholine is very important neurotransmitter which helps in signal transmission in the brain. The decrease in the levels of this neurotransmitter is one the causes that leads to Alzheimer disease. [24] Therefore, inhibition of acetylcholinesterase is the main therapeutic strategy against AD. [25] Many plants have been screened for acetylcholinesterase inhibitory activity.[26] There has been a long history of using various medicinal plants to increase cognitive function.[27] Various acetylcholinesterase inhibitors are used as drugs but they suffer from one major drawback and that is they produce various side effects.[28] Therefore there is need of potent inhibitors from natural resources. In this study, we investigated the plant extract of Artemisia. amgdalina for AChE inhibition potential. Based on the results of this study it can be concluded that the extract of Artemisia. amgdalina possesses AChE inhibitory potential. The plant extract showed potent AChE inhibition in concentration dependent manner.

V FIGURES AND TABLES

**TABLE 1: Artemisia amgdalina crude extract fractions, phytochemical screening.**

<table>
<thead>
<tr>
<th>S.NO.1</th>
<th>Phytochemicals</th>
<th>Hexane</th>
<th>Methanol</th>
<th>Ethyl acetate</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Terpenes</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>02</td>
<td>Tannins</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>03</td>
<td>Flavonoids</td>
<td>–</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>04</td>
<td>Saponins</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>05</td>
<td>Alkaloids</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>06</td>
<td>Phenolics</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>07</td>
<td>Steroids</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>08</td>
<td>Cardiac Glycosides</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Fig a: Acetylcholinesterase inhibitory activity of *Artemisia amgdalina*. Values represent the mean ± SD (n=3)

VI CONCLUSION

Inhibition of acetylcholinesterase is the main therapeutic strategy against AD. Many plants have been screened for acetylcholinesterase inhibitory activity. The extracts of *Artemisia amgdalina* were proved to have a great potential and should be considered for further studies to identify the constituents responsible for the AChE inhibitory activity, which can be eventually utilized in the prevention and treatment of AD. Furthermore, isolation purification and characterization of the phytochemicals found present will make interesting studies.

ACKNOWLEDGEMENT:

The authors thank financial supports from UGC.

CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

REFERENCES


[27] Hachiro Sugimoto,Yoshiharu Yamanishi, Youichi Iimura and Yoshiyuki Kawakami. Donepezil Hydrochloride (E2020) and Other Acetylcholinesterase Inhibitors. *Current Medicinal Chemistry, 2000, 7, 303-339*