Screening of Indian Medicinal Plants for Acetylcholinesterase Inhibitory Activity

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The cholinergic hypothesis of Alzheimer’s disease (AD) has provided the rationale for the current pharmacotherapy of this disease, in an attempt to reduce the cognitive decline caused by cholinergic deficits. Nevertheless, the search for potent and long-acting acetylcholinesterase (AChE) inhibitors that exert minimal side effects in AD patients is still ongoing. AChE inhibitors are currently the only approved therapy for the treatment of Alzheimer’s disease; only a limited number of drugs are commercially available. Hydroalcohol extracts of six herbs, Andrographis paniculata, Centella asiatica, Evalvulus alsinoides, Nardostachys jatamansi, Nelumbo nucifera, Myristica fragrans used in Indian systems of medicine, were tested for in vitro acetylcholinesterase inhibitory activity based on Ellman’s method in 96-well microplates using AChE obtained from bovine erythrocytes. The results showed that the hydroalcohol extract from Centella asiatica, Nardostachys jatamansi, Myristica fragrans, Evalvulus alsinoides inhibited 50% of AChE activity at concentrations of 100–150 μg/mL. Andrographis paniculata and Nelumbo nucifera extracts showed a weak inhibition of acetylcholinesterase with IC50 values of 222.41 ± 19.87 μg/mL and 185.55 ± 21.24 μg/mL, respectively. Physostigmine was used as a standard and showed inhibition of acetylcholinesterase with an IC50 value of 0.076 ± 0.0042 μg/mL. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: acetylcholinesterase; microplate assay; Indian medicinal plants; Nardostachys jatamansi; Myristica fragrans; Evalvulus alsinoides.

INTRODUCTION

Alzheimer’s disease (AD) is a chronic and progressive neurodegenerative disease that is characterized symptomatically by progressive deterioration of the activities of daily living, behavioral disturbances and cognitive loss (Johnson et al., 2000). The neurodegenerative features of AD include pathological changes in the brain, such as the formation of β-amyloid plaques and neurofibrillary tangles. An important approach for treating AD is the inhibition of acetylcholinesterase (AChE). Based on the cholinergic hypothesis, a defect in the cholinergic system is involved in AD (Greig et al., 2001; Perry, 1986). Among the possible strategies for enhancing brain cholinergic activity, acetylcholinesterase inhibitors (AChEIs) have been used most extensively for the symptomatic treatment of AD. Physostigmine and tacrine are the only AChEIs reasonably evaluated in AD patients, even though their use is limited by the short half-life and peripheral cholinergic side-effects of physostigmine, and the dose-dependent hepatotoxicity of tacrine (Nordberg and Svensson, 1998; Mukherjee et al., 2007a).

Galanthamine, a long-acting, selective, reversible and competitive AChE inhibitor is considered to be more effective in the treatment of AD and to have fewer limitations (Bores et al., 1996). Some other dehydroevodiamine (Park et al., 1996) type AChE inhibitors have been reported, but because of bioavailability problems and possible side-effects, there still is great interest in finding better AChE inhibitors. In this present study, the inhibitory activities on AChE of six plant materials were investigated: Andrographis paniculata Nees, Centella asiatica (L.) Urban, Evalvulus alsinoides L., Nardostachys jatamansi DC, Nelumbo nucifera Gaertn, Myristica fragrans Houtt. These plants are commonly used to improve memory function traditionally as central nervous system active plants in the Indian system of medicine and there is no report for their anticholinesterase activity (Kirtikar and Basu, 1933; Murthy, 1998).

MATERIALS AND METHODS

Plant materials. Andrographis paniculata aerial part (SNPS – 1011), Centella asiatica whole plant (SNPS – 1010), Evalvulus alsinoides whole plant (SNPS – 1009), Nardostachys jatamansi rhizome (SNPS – 1006), Nelumbo nucifera rhizome (SNPS – 1007), Myristica fragrans seed (SNPS – 1008) were collected from Purulia district, West Bengal, India and Nilgiri hills, Tamil Nadu, India. Plant materials were authenticated by Dr S. Rajan, Field Botanist, Survey of Medicinal Plants and Collection Unit, Government of India, Udhagamandalam. A voucher specimen of each of these plant materials has been retained in the School of Natural Products Studies, Department of Pharmaceutical Technology, Jadavpur University, Kolkata 700 032.
Enzyme was then added and the absorbance was again measured. A range of concentrations was used so that the rates for the sample to the blank (10% MeOH in water) could be calculated. The percentage of inhibition was calculated by comparing the slope of the line so obtained and expressed as a percentage compared with an assay using a buffer (10% MeOH in water). Any increase in absorbance due to the enzyme from the rate after adding the enzyme was subtracted from the rate of the reaction before adding any inhibitor. Any increase in absorbance due to spontaneous hydrolysis of substrate was corrected by subtracting the rate of the reaction before adding the enzyme from the rate after adding the enzyme. Percentage of inhibition was calculated by comparing the rates for the sample to the blank (10% MeOH in buffer). A range of concentrations was used so that the IC_{50} value could be calculated.

**Extraction of plant materials.** Freshly collected plant materials were dried in shade, powdered (500 g), extracted with ethanol (95%), and freeze-dried which were further used for screening purposes. The percentage yields were Andrographis paniculata – 12.65% w/w, Centella asiatica – 21.3% w/w, Nardostachys jatamansi – 10.95% w/w, Nelumbo nucifera – 8.72% w/w, Myristica fragrans – 16.65% w/w.

**Chemicals.** Acetylthiocholine iodide (ATCI), acetylcholinesterase enzyme (AChE) from bovine erythrocytes, 5,5′-dithiobis [2-nitrobenzoic acid] (DTNB) and physostigmine were obtained from Sigma (Poole, UK). Methanol and all other organic solvents (analytical grade) were purchased from Merck.

**Microplate assay.** AChE activity was measured using a 96-well microplate reader (Ren et al., 2006; Mukherjee et al., 2007b) based on Ellman’s method (Ellman et al., 1961). The enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman’s reagent (DTNB) and physostigmine to elicit its effect on the nervous system. Any increase in absorbance due to spontaneous hydrolysis of substrate was corrected by subtracting the rate of the reaction before adding the enzyme from the rate after adding the enzyme. Percentage of inhibition was calculated by comparing the rates for the sample to the blank (10% MeOH in buffer). A range of concentrations was used so that the IC_{50} value could be calculated.

**RESULTS AND DISCUSSION**

IC_{50} values (concentration of sample required to inhibit 50% of acetylcholinesterase enzyme) were calculated from the regression equations prepared from the concentrations of the samples (Table 1). The extract of the aerial parts of Andrographis paniculata showed some inhibitory activity with the IC_{50} value of 222.41 ± 19.87 μg/mL. Andrographis paniculata Nees. (Acanthaceae) is an important plant in Ayurvedic medicine. Psychopharmacological studies on A. paniculata extract to elicit its effect on the nervous system showed that it gave a significant alteration in behaviour pattern and a reduction in spontaneous motility as well as prolonging pentobarbitone-induced sleeping time in experimental animals (Mandal et al., 2001; Maiti et al., 2006). Although indicating an effect on the central nervous system, these activities are not easily associated with enhanced CNS cholinergic activity.

The extract of Centella asiatica showed some enzyme inhibitory activity with IC_{50} value 106.55 ± 9.96 μg/mL. *C. asiatica* (Umbelliferae) extract showed CNS depressant activity (Sakina and Dandiya, 1990). The whole plant of C. asiatica has been shown to be beneficial in improving memory (Vaidyaratnam, 1994) and is also reported to improve the general mental ability of mentally retarded children (Apparao et al., 1973). Nalini et al. (1992) showed that fresh leaf juice improves the passive avoidance task in rats. Veerendra Kumar and Gupta (2002) demonstrated that the aqueous extract of *C. asiatica* has cognition-enhancing properties with an associated decrease in the brain oxidative stress parameters of normal rats. Treatment with *C. asiatica* aqueous extract during the postnatal period influenced the neuronal morphology, and promoted the higher brain function of juvenile and young adult mice and also enhanced learning and memory (Rao et al., 2005). *C. asiatica* ethanol extract and its components accelerated nerve regeneration and increased neurite elongation via ERK activation (Soumyanath et al., 2005).

Gupta et al. (2003) reported that the aqueous extract of *C. asiatica* possessed antiepileptic, cognitive-enhancing and antioxidant properties on the course of kindling development, kindling-induced learning deficit and oxidative stress markers in pentyleneetetrazole (PTZ) kindled rats. All these reports are associated with increased levels of acetycholine (ACh) in the CNS. Asiaticoside, a bioactive triterpenoid compound, was isolated from *C. asiatica*. Chen et al. (2006) examined the putative anxiolytic activity of asiaticoside in mice.

**Table 1. The inhibitory activity on acetylcholinesterase of the hydroalcohol extract of some Indian medicinal plants and physostigmine**

<table>
<thead>
<tr>
<th>Serial No.</th>
<th>Sample</th>
<th>Family</th>
<th>Part used</th>
<th>IC_{50} value (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Andrographis paniculata</td>
<td>Acanthaceae</td>
<td>Aerial part</td>
<td>222.41 ± 19.87</td>
</tr>
<tr>
<td>2</td>
<td>Centella asiatica (L.)</td>
<td>Umbelliferae</td>
<td>Whole plant</td>
<td>106.55 ± 9.96</td>
</tr>
<tr>
<td>3</td>
<td>Nardostachys jatamansi</td>
<td>Valerianaceae</td>
<td>Rhizome</td>
<td>130.11 ± 12.97</td>
</tr>
<tr>
<td>5</td>
<td>Myristica fragrans Hoult.</td>
<td>Myristicaceae</td>
<td>Seed</td>
<td>133.28 ± 11.26</td>
</tr>
<tr>
<td>6</td>
<td>Evalvulus alsinoides L.</td>
<td>Convolvulaceae</td>
<td>Whole plant</td>
<td>141.76 ± 16.27</td>
</tr>
<tr>
<td>7</td>
<td>Physostigmine (positive control)</td>
<td>–</td>
<td>–</td>
<td>0.076 ± 0.0042</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD (n = 3).

by using a number of experimental paradigms of anxiety. Asiaticoside at doses of 5 or 10 mg/kg showed anxiolytic-like effects in mice. Total triterpenes isolated from *C. asiatica* reduced the immobility time and ameliorated the imbalance of amino acid levels in forced swimming mice and proved the antidepressant activity of total triterpenes (Chen et al., 2003).

The hydroalcoholic extract of *Nardostachys jatamansi* rhizome showed some inhibitory activity with an IC$_{50}$ value of 130.11 ± 12.92 μg/mL. In Ayurveda the rhizomes of *N. jatamansi* are used to treat epilepsy, hysteria and convulsions and it possesses many CNS properties (Gupta et al., 1994). *N. jatamansi* shows GABA enhancing effects (Prabhu et al., 1994) and useful in ischemic stroke (Green et al., 2000). It has been shown to protect against the cerebral ischemia (Salim et al., 2003). Jatamansone, an active phytoconstituent of *N. jatamansi*, was shown to have tranquilizing activity in mice and monkeys. Jatamansone was also found to impair biosynthesis of serotonin in the rabbit brain leading to a reduction in 5-hydroxytryptamine level (Anonymous, 1992).

*Nelumbo nucifera* Gaertn. (Nymphaeaceae) is a large aquatic herb widely found in India. Different pharmacological activities of the methanol extract of the rhizomes have been reported including psychopharmacological effects (Mukherjee et al., 1996). Mukherjee et al. (1996) reported that the extract caused a significant reduction in spontaneous activity, a decrease in exploratory behavioural pattern in the head dip and Y-maze tests, a reduction in muscle relaxant activity by rotarod test and the 30 °C inclined screen and traction tests and also potentiated the pentobarbitone induced sleeping time in mice. In this study a hydroalcoholic extract of *Nelumbo nucifera* rhizome showed weak AChE inhibitory activity with an IC$_{50}$ value of 185.55 ± 21.24 μg/mL.

The extract from *Myristica fragrans* seeds showed an inhibitory effect on AChE with IC$_{50}$ value 133.28 ± 11.26 μg/mL. *Myristica fragrans* is known to be psychotomimetic and to possess both stimulatory and depressant activities (Kelly et al., 2003). The plant has been reported to improve the memory of mice (Parle et al., 2004), to possess a psychotropic action (Forrest and Heacock, 1972). The preliminary studies on the neuropharmacological actions of the *n*-hexane extract produced twitches, palpebral closure, hypersensitivity to touch, vocalization when being approached and decreased rearing. Several reports suggest the anxiogenic activity of *M. fragrans* (Sonavane et al., 2002). Most of these activities could be due to an elevation of ACh levels in the CNS, which could be due to AChE inhibition. *Evovulus alsinoides* whole plant is used in India as a psychotropop for neuropharmacological disorders (Anonymous, 1992). The plant, as an Ayurvedic preparation, potentates the action of phenytoin (Dandekar et al., 1992). An ethanol extract of *E. alsinoides* showed adaptogenic and memory enhancing properties in rats (Siripurapu et al., 2005). *E. alsinoides* extract showed a strong inhibitory effect on AChE with an IC$_{50}$ value of 141.76 ± 16.27 μg/mL (Table 1). Physostigmine, the positive control, gave an IC$_{50}$ of 0.076 ± 0.0042 μg/mL. Thus from this study it is evident that out of six extracts evaluated for the AChE inhibitory activity, at least four have stronger activity than others, while the others showed a comparatively weak effect but may contain more active compounds.

Acknowledgements

The authors wish to express their gratitude to the Commonwealth Scholarship Commission, Association of Commonwealth Universities, UK, for the Commonwealth Academic Staff Fellowship Award to Dr Pulok K. Mukherjee through the selection made from the University Grants Commission (UGC), India. Thanks are also due to the Department of Biotechnology (DBT), Government of India for providing financial assistance through research project to the School of Natural Product Studies, Jadavpur University.

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