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Original article

Acetylcholinesterase inhibitory effects of some plants from Rosaceae

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Abstract

Background and objectives: Alzheimer's disease (AD) is an age dependent disorder. AD is associated with decrease of brain acetylcholine level. Nowadays, one of the methods for progression inhibition of AD is using acetylcholinesterase inhibitors. Rosaceae is a large plant family. Different biological effects of some species of this family have been reported. The aim of the present study was to assess the acetylcholinesterase inhibitory (AChEI) activity of the selected plants belonging to Rosaceae family. **Methods:** AChEI activity of six species from Rosaceae including *Cotoneaster nummularia, Cerasus microcarpa, Amygdalus scoparia, Agrimonia eupatoria, Rosa canina* and *Rosa damascena* were evaluated based on Ellman's method in concentration of 300 µg/mL using total extracts and methanol fractions which were obtained by maceration. **Results:** The results showed that the total extract and methanol fraction of the aerial parts of *A. eupatoria* demonstrated significant AChEI activity with 46.5% and 56.2% inhibition of the enzyme, respectively. **Conclusion:** According to the results of the AChEI activity of the methanol fraction of *A. eupatoria*, it seems that the polar components of the species such as flavonoids may be responsible for its effectiveness.

Keywords: Acetylcholinesterase inhibition, *Agrimonia eupatoria*, Alzheimer, Ellman's method, Rosaceae

Introduction

Rosaceae family comprises about 100 genus and 2000 species which are wide spread all over the world [1]. The plants of this family are used in traditional and folklore medicine of many countries and also in modern medicine [2]. Because of the presence of different secondary metabolites in Rosaceae species, various biological effects have been reported from them [3-5]. One of the activities that many researchers are interested in, is inhibition of acetylcholinesterase enzyme (AChE). Acetylcholine is involved in the process of Alzheimer's disease and it is decreased during the disorder. It is destroyed by the enzyme acetylcholinesterase, so one of the methods to prevent the reduction of acetylcholine in the brain in Alzheimer's disease is preventing its destruction. Some drugs such as donepezil work

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with this mechanism [6]. Researchers are constantly trying to find new stronger drugs that have fewer side effects. Numerous studies have been carried out on plants and some of them have shown acetylcholinesterase inhibitory effects and could be considered as good candidates for further Alzheimer studies [7-10]. AChEI activity of the plants is evaluated by different techniques, among them Ellman's method is more common. In this method, the enzyme hydrolyses the substrate acetylthiocholine iodide (ATCI) resulting in the formation of thiocholine which reacts with Ellman's reagent or 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitro-benzoate which can be detected at 405 nm. The rate of yellow color production is measured in 405 nm by an ELISA reader which shows the enzyme activity [11].

In the present investigation, AChEI activity of some plants of Rosaceae family including *Cotoneaster nummularia, Cerasus microcarpa, Amygdalus scoparia, Agrimonia eupatoria, Rosa canina* and *Rosa damascena* were evaluated by using Ellman's method in 96-wells plate.

Experimental

Plant material

Rosaceae species included aerial parts of *Cotoneaster nummulari* Fisch. & C.A.Mey. collected from Tehran province, whole plant of *Agrimonia eupatoria* L. from Golestan province, flowers of *Rosa damascena* Mill. from Isfahan province, aerial parts of *Amygdalus scoparia* Spach and fruits of *Rosa canina* L. from Kohgiluyeh and Boyerahmad province, *Cerasus microcarpa* (C.A.Mey.) Boiss. from Hamedan province. The species were identified at the Herbarium of Traditional Medicine and Materia Medica Research Center (TMRC), Shahid Beheshti University of Medical Sciences, Tehran, Iran. The voucher numbers were registered as 1248, 1854, 1489, 3259, 1998 and 2343, respectively.

The plant materials were dried in shade and ground.

Chemicals

Acetylthiocholine iodide (ATCI) was prepared from Fluka (Germany). Acetylcholinesterase enzyme (AChE) from bovine erythrocytes was purchased from Sigma (Germany). 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB) was obtained from Merck (Germany). Methanol and all other solvents were provided from Merck (Germany).

Plants extraction

10 g of each plant powder was macerated with methanol:water 80:20 (1:10) for three days. Every 24 h, the mixture was filtered and fresh solvent was added to the plant powder. The combined extracts were concentrated and dried using rotary evaporator and freeze dryer. In order to provide different fractions, 50 g of each powder was macerated with *n*-hexane for 3 days and each day the solvent was replaced with fresh solvent. Then, the residue of the plant was dried and then extracted with chloroform and methanol, respectively with the same process. The filtrates were combined and concentrated under reduced pressure.

Acetylcholinesterase inhibitory assay

AChEI activity was determined using a 96-well microplate reader based on Ellman's method [12]. Briefly, 125 µL of 3 Mm DTNB, 25 µL of 15 mM ATCI and 50 µL of phosphate buffer pH 8, and 25 µL of sample dissolved in methanol (3 mg/mL) were added to 96-well plates. The absorbance was measured at 405 nm every 13 sec for 65 sec. 25 µL of 0.22 U/mL of AChE enzyme was then added and the absorbance was again read every 13 sec for 104 sec. Absorbance was plotted against time and enzyme activity was calculated from the slope of the line. Any increase in the absorbance due to the non-enzymatic hydrolysis of substrate was corrected by subtracting the rate of reaction before addition of the enzyme from the rate after addition of the enzyme. Percentage of enzyme inhibition was

calculated by comparing the rates for the sample to the blank (using methanol without extract). Donepezil was used as the positive control.

Results and Discussion

Due to the increased incidence of Alzheimer's disease among the elder people and the effects of acetylcholinesterase inhibitors in patients, new drugs that inhibit this enzyme are of particular importance [6]. Plants of Rosaceae family have shown various biological effects. They are rich in phenolic compounds which have been demonstrated to prevent Alzheimer's progression [13]. In the present investigation, the AChEI activity of total extracts of *Agrimonia eupatoria, Amygdalus scoparia, Cerasus microcarpa, Cotoneaster nummularia, Rosa canina* and *Rosa damascena* have been studied. The results showed that total extract of *A. eupatoria* could inhibit the enzyme more than other samples examined in this study (table 1). Therefore, fractionation was done on this species in order to find the most effective fraction. Since, hexane and chloroform fractions were not soluble in methanol, AChEI assay was done only on methanol fraction of the plant. The results showed 56.2% inhibition of AChE by methanol fraction of *A. eupatoria*. *Amygdalus scoparia* and *Cerasus microcarpa* with 47.5% and 41.4% enzyme inhibition, showed considerable results as well. The total extract of *Rosa damascena* demonstrated the least inhibition (10.9%) (table 1). IC_{50} of donepezil ,positive control, was found 0.015 µg/mL.

Table 1. The acetylcholinesterase inhibitory activity of Rosaceae species in concentration 300 µg/mL

Name	acetylcholinesterase inhibiton %	
	Total extract	Methanol fraction
Agrimonia eupatoria	49.5±3.7	56.2±1.6
Amygdalus scoparia	47.5±3.5	-
Cerasus microcarpa	41.4±1.5	-
Cotoneaster nummularia	32.8±4.7	-
Rosa canina	31.3±1.5	-
Rosa damascena	10.9±1.2	-

-: no test was performed

Several investigations have been carried out to find new compounds for AChEI effect among them herbal components are more interesting. Physostigmin and galantamin with alkaloid structure which have been extracted from plants are able to inhibit AChE and are used for AD [14,15]. New researches have proved that other secondary metabolites such as flavonoids and phenolic compounds can inhibit AChE as well. This class of compounds with antioxidant and inhibitory activities of the acetylcholinesterase enzyme could be effective in the treatment of AD [13]. During an investigation on 180 medicinal plants using bioassay-guided fractionation, AChEI activity of *Agrimonia pilosa* which contained flavonoids was established and quercetin was found as one of the active components [16]. Flavonoid components have been reported from other species of *Agrimonia* which are responsible for specific biological effects including inhibition of AChE [16]. Since, in the present study, the methanol fraction of *A. eupatoria* demonstrated considerable inhibitory effect (56.18%) and regarding to the fact that methanol fractions usually contain polar compounds such as flavonoids, it could be concluded that the enzyme inhibition activity of the plant might be mainly due to the flavonoids components of the species.

The presence of flavonoids has been established in *Amygdalus* species as well. These flavonoids have been found as antiradical agents [5,17]. Fruits of *A. communis* are used as a nutrient nut all over the world but in a recent investigation, aerial parts of the species have been evaluated and demonstrated considerable AChEI activity. It seems that *A. communis* is an especial plant which not only has the economic importance but also different parts of the plant appear diverse biological effects. The role of flavonoids and phenolics in AD has been mentioned in other studies [13]. Most of them are powerful antioxidant agents and some of them can inhibit AChE, therefore, the plants containing these components such as Rosaceae species might be considered as alternative treatment in AD mainly

with the two mentioned mechanisms along with other mechanisms which need to be investigated.

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Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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