ACETYCHOLINESTERASE and BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES of Salvia aethiopis L. (Lamiaceae) from Turkey

Ilham EROZ POYRAZ
Anadolu University, Faculty of Pharmacy, Department of Pharmaceutical Botany, 26470 Tepebasi, Eskisehir, TURKEY

ABSTRACT
The objective of this study is to determine the acetylcholinesterase and butyrylcholinesterase inhibitory activities of ethanolextract of Salvia aethiopis L. (Lamiaceae) from Turkey. The extraction yield of ethanolextract of S. Aethiopis was 6.23%. Cholinesterase inhibitory activity of the extracts was tested against AchE and BChE at 800, 400, 200, 100 and 50 µg/mL concentrations. The inhibition potential of AchE and BChE of the plant extract, in a dose-dependent manner, possibly an alterative effect on the AD, supports by clinical studies for the future studies.

Keywords: Salvia aethiopis L., Acetylcholinesterase inhibition, Butyrylcholinesterase inhibition, Alzheimer’s disease.

1. Introduction
Salvia L. (Lamiaceae) genus is to present by nearly 1,000 species all around the world (Walker and Sytsma 2007) and nearly 100 species in Turkey (Kahraman et al. 2011). The genus members have been used from ancient times against perspiration, fever; as carminative, spasmylytic, antiseptic, astringent and for skin and hair care (infusion). It is also used in nervous conditions, trembling, depression and vertigo (Dweck2000). In Turkey Salvia species are used ethnobotanically for the treatment of asthma, cold, sore throat, stomachache, inflamed wounds (Cansaran and Kaya 2010, Koyuncu et al. 2010, Tuzlaci and Erol 1999, Tuzlaci and Senkardes 2011). It is reported that Salvia species have a usage especially in Europe for age related central nervous system (CNS) disorders, to shiver and suffer the effects of stroke and strengthen weak minds and memories. Nowadays it has discovered the plant might prove useful in struggle against Alzheimer’s disease (AD) (Adams et al. 2007). In the progression of AD, researches are based on

Along with this study, it is evaluated the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activity of *S. aethiopis* ethanol extract to make a suggestion about possible link between ethnobotanical usage results and inhibition potential of AChE and BChE an important determiner on the AD.

2. MATERIAL AND METHODS

2.1. Plant Material

*S. aethiopis* L. (Lamiaceae) was collected from Turkey, Eskisehir, Anadolu University, Yunus Emre Campus, 750 m, 17.06.2011. Collected plants were deposited in Anadolu University Faculty of Pharmacy Herbarium (ESSE 14584). Species were identified according to Flora of Turkey and the East Aegean Islands (Hedge 1982).

2.2. Preparation of the extract

20 g herbawas extracted with petroleum ether in a Soxhlet apparatus. The fat-free material was air dried and macerated with 200 ml ethanol at 40°C, 30 min for four times. Extracts were filtered and then dried under vacuum. The yield of soluble constituents was calculated (Ozturk et al. 2009).

2.3. AChE and BChE Inhibitory Activity

Acetylcholinesterase (AChE, E.C.3.1.1.7, from electric eel) and butyrylcholinesterase (BChE, E.C. 3.1.1.8, from equine serum) were purchased from Sigma-Aldrich (Steinheim, Germany) and used as enzyme sources. Acetylthiocholine iodide (ATC) and butyrylthiocholine iodide (BTC) were obtained from Fluka (Germany) and employed as substrates of reaction. 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, Sigma-Aldrich, Steinheim, Germany) was used as chromogenic reagent for the measurement of the cholinesterase activity. AChE and BChE inhibitory activity of the extract was determined by modified Ellman’s spectrophotometric method (Ellman et al. 1961) using galantamine as reference. Sample solutions were prepared using 2% DMSO. All assay procedures were performed by using BioTek-Synergy H1 microplate reader (USA) spectrophotometer. Briefly, 140 µL phosphate buffer (0.1 M, pH=8), 20 µL extract solution, 20 µL enzyme solution (2.5 U/mL), 20 µL DTNB (0.01 M) were added by Biotek Precision XS robotic system (USA) in a 96-well microplate and incubated at 25°C for 15 min. After the incubation 10 µL substrate solution (0.075 M acetylthiocholine iodide-ATC or butyrylthiocholine iodide-BTC) were added to the enzyme-inhibitor mixture. The production of the yellow anion (5-thio-2-nitrobenzoic acid) was recorded for 5 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor was processed. Control and inhibitor readings were corrected with blank-reading. Percentage inhibition of AChE or BChE was determined by comparison of the reaction rates of the samples relative to blank sample using the formula (E-S)/Ex100, where E is the activity of enzyme without test sample and S is the activity of enzyme with test sample. Each concentration was analyzed in quadruplicate.

4. RESULTS and DISCUSSION

The objective of this study is to determine the acetylcholinesterase and butyrylcholinesterase inhibitory activities of ethanol extract of *S. aethiopis* from Turkey. The extraction yield of ethanol extract of *S. aethiopis* was calculated as 6.23%. Cholinesterase inhibitory activity of the extracts was tested against AChE and BChE at 800, 400, 200, 100 and 50 µg/mL concentrations using BioTek-Synergy H1 microplate reader (USA) spectrophotometer. Percentage inhibitions of AChE and BChE were summarized in Table 1.
Table 1. Inhibitory effect of the *S. aethiopis* ethanol extract against AChE and BChE.

<table>
<thead>
<tr>
<th>Extract</th>
<th>AChE Inhibition %</th>
<th>BChE Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>800 µg/mL</td>
<td>400 µg/mL</td>
</tr>
<tr>
<td></td>
<td>µg/mL</td>
<td>µg/mL</td>
</tr>
<tr>
<td><em>S. aethiopis</em> (ethanol extract)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galantamine</td>
<td>99.87±1.64%</td>
<td>99.15±1.55%</td>
</tr>
<tr>
<td></td>
<td>±1.64</td>
<td>±1.55</td>
</tr>
</tbody>
</table>

At 800 µg/mL concentration galantamine had 99.87±1.64% inhibition whereas the ethanol extract of *S. aethiopis* had 83.51±1.16% inhibition against AChE. In terms of BChE the extract showed the most inhibition (58.19±1.10%) at 800 µg/mL.

Some investigations have been carried out on *Salvia* species have effects on acetylcholinesterase inhibition. Ferreira et al. (2006) screened some medicinal plants from Portugal for their AChE inhibition activity. It was noted *S. officinalis* is usages for the treatment of condiment, anorexia, flatulence, climacterium, menopause, nerves and anti-impotence. Decoction of *S. officinalis* effect on AChE activity (% inhibition) was studied at 0.5, 1 and 5 mg/mL. At first dose it was not detected any inhibition (inhibition ≤ 5%); the inhibition was 6.0 ± 8.1% at second dose and at third one the inhibition was 57.2 ± 15.9%. AChE inhibition of the ethanolic extract of the plant was 16.4 ± 5.4% for 0.5 mg/mL while not determined any value for 1 mg/mL. Sezer Senol et al. (2010) studied AChE inhibitory activity at 25, 50 and 100 µg/ml of the dichloromethane and ethyl acetate extracts of 55 Turkish *Salvia* taxa. The highest yield percentage (w/w) of the extracts were 13.41 for ethyl acetate extract of *S. pachystachys* while *S. aethiopis* dichloromethane extract were 4.70. The highest AChE inhibition percentage was obtained from dichloromethane extract of *S. fruticosa* (51.07±1.31 at 100 µg/ml) while galantamine was 98.97 ± 0.24 at 100 µg/ml. *S. aethiopis* AChE inhibition percentage was 3.40 ± 0.05 with same solvent’ extract.

Alzheimer’s disease, an advancing type of dementia, have abnormally low acetylcholine concentrations. Acetylcholine, a neurotransmitter, has a key role in cognitive function and reasoning. Alzheimer’s patients have an acetylcholine deficiency. Also, it is evidence the factors as free radicals, and inflammation of the brain tissue along with the disease. As might be expected, any compound enhances the cholinergic system in the brain may be useful in treating Alzheimer’s disease and similar brain malfunctions (Singhal et al., 2012). Herbs like sage, turmeric, Saint John’s wort etc. have anti-inflammatory and antioxidant activities that may be used for the treatment of AD (Jivad and Rabiei 2014, Singhal et al., 2012). *Salvia* species have been used against several disorders including constipation, colds, fevers, cholera, liver problems, epilepsy and nervous disorders and memory-enhancing an anti-inflammatory, sedative, antioxidant, anti-inflammatory, oestrogenic, antidepressive (Perry et al., 2000). It was reported the anti-inflammatory efficacy (Hernández-Pérez et al. 1995)and antioxidant potential (Firuzi et al. 2013, Sezer Senol et al. 2011, Tepe et al. 2006, Tosun et al. 2009) of *S. aethiopis*. Along with the present study, ethanol extract of *S. aethiopis* was showed 83.51±1.16% inhibition of AChE at 800 µg/mL concentration while galantamine with 99.87±1.64% inhibition. At the same dose, the BChE inhibition of the extract was 58.19±1.10%. The ethanol extract of *S. aethiopis* performed more potent inhibitory activity against AChE enzyme as regards BChE enzyme.
5. CONCLUSION

It is evaluated the acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition activity of *S. aethiopis* ethanol extract. The inhibition potential of AChE and BChE of the plant extract, in a dose-dependent manner, possibly an alternative effect on the AD, supports by clinical studies for the future studies.

6. ACKNOWLEDGEMENT

The author would like to thank to Prof. Dr. Nilgun OZTURK, Assoc. Prof. Dr. Yusuf OZKAY and Research Assistant Begum Nurpelin SAGLIK for their kindly supports.

7. REFERENCES


