In vitro Effects of Certain Plant Extracts on Acetylcholinesterase (EC 3.1.1.7) Enzyme in Lake Van Fish Liver and Brain

Van Gölü Balığı Beyin ve Karaciğerindeki Asetilkolinesteraz (EC 3.1.1.7) Enzimi Üzerine Bazı Bitki Özütlerinin *İn vitro* Etkileri

Research Article

Zehra Başı*, Vedat Türkoğlu, Muhammet Güler, M. Rıza Kıvanç
Yüzüncü Yıl University, Faculty of Arts and Sciences, Department of Chemistry, Van, Turkey.

ABSTRACT

In this study, the effect of *Glycyrrhiza glabra* (Leguminosae), *Pimpinella anisum* (Umbelliferae) and *Matricaria chamomilla* (Compositae) extracts on both Lake Van fish (Chalcalburnus tarichi P.1811) brain and liver acetylcholinesterase enzymes (AChE) activity, responsible for transformation of nerve stimulations, has been investigated. While activating fish liver AChE, these extracts inhibited fish brain AChE activity. The plants used in the showed noncompetitive inhibition on the fish brain AChE. I_{50} values were estimated 1.714 mg.mL^{-1} for *Glycyrrhiza glabra*, 0.604 mg.mL^{-1} for *Matricaria chamomilla* and 0.394 mg.mL^{-1} for *Pimpinella anisum*. The optimum temperature for liver and brain AChE was found to be 30°C and optimum pH of 6-8.5 and 6-8 respectively. In addition, specific activities of fish brain and liver AChE were found 130.5 EU.mg^{-1} and 13.59 EU.mg^{-1} respectively.

Key Words
Fish liver, fish brain, acetylcholinesterase, plant extract, activation, inhibition.

ÖZ

Bu çalışmada; sinir uyarılarının naklinden görevli enzim olan Van Gölü balığı (Chalcalburnus tarichi P.1811) karaciğer ve beyin asetilkolinesteraz (EC 3.1.1.7) enzimi üzerine meyan [*Glycyrrhiza glabra* L. (Leguminosae)], anason [*Pimpinella anisum* L. (Umbelliferae)] ve papatya [*Matricaria chamomilla* L. (Compositae)] özütlerinin etkisi araştırılmıştır. Bu özütler balık karaciğer AChE'ni active ederken balık beyin AChE'ni inhibe etmiştir. Kullanılan bitkiler balık beyni AChE üzerine yarışmasız inhibisyon göstermiştir. I_{50} değerleri meyan için 1,714 mg.ml^{-1}, anason için 0,394 mg.ml^{-1} ve papatya için 0,604 mg.ml^{-1} olarak bulunmuştur. Karaciğer ve beyin AcHE için optimum sıcaklık 30°C ve optimum pH sırasıyla 6-8 ve 6-8,5 olarak bulunmuştur. Ek olarak balık beyin ve karaciğer AChE'nin spesifik aktiviteleri sırasıyla 130,5 EU.mg^{-1} ve 13,59 EU.mg^{-1} olarak bulunmuştur.

Anahtar Kelimeler
Balık karaciğeri, balık beyni, asetilkolinesteraz enzimi, bitki özütü, aktivasyon, inhibisyon.

Article History: Received: Jun 15, 2016; Revised: Jul 07, 2016; Accepted: Oct 20, 2016; Available Online: Dec 31, 2016.

DOI: 10.15671/HJBC.2016.124

Correspondence to: Z. Başı, Yüzüncü Yıl University, Faculty of Arts and Sciences, Department of Chemistry, Van, Turkey
Tel: +90 432 225 1701 Fax: +90 432 225 1701 E-Mail: zehrabasi@hotmail.com

INTRODUCTION

Alzheimer's disease (AD) is commonly in the elderly because of malfunctioning of many biochemical pathways [1]. The amount of acetylcholine in the brain regions related to memory and learning is decreased in AD patients [2]. A significant approach to overcome this illness is to increase the acetylcholin amount in the brain by inhibiting acetylcholinesterase (AChE) taking into consideration the cholinergic hypothesis [3]. Many chemicals and plants have been used in treatment of AD. Among these, plants are widely used in traditional medicine. *Matricaria chamomilla* is a perennial herbaceous flowering plant native to Europe, Africa and Asia. It is used traditionally as a medicinal and pharmaceutical preparation because of its anti-inflammatory and antispasmodic features [4].

Pimpinella anisum is a perennial plant with white flowers indigenous to Near East and cultivated in the Mediterranean rim including Turkey, Mexico and Chile [5]. The of seeds this plant in hot water is applied to the patients suffering from carminative, antiseptic, diuretic, digestive, insomnia and constipation [6]. Moreover, several therapeutic effects, digestive disorders, gynecologic, and as well anticonvulsant, anti-asthma and dyspnea have been described for the seeds of *Pimpinella anisum* in ancient medical books [7]. *Glycyrrhiza glabra* is a ligneous annual shrub cultivating in Mediterranean region and Asia [8]. It is used in the treatment of diseases such as lurking cortisone, treatment of rheumatism, asthma, skin problems and eye diseases. There are certain effects of this plant such as softening chest, expectorant, enhancing the urine, taste correctors [9]. Licorice root also be used successfully against all cough and bronchial diseases. In traditional medicine, licorice root, is used against gastric mucosal inflammation and stomach ulcers and constipation. In addition, there are solvent effects cramps [10,11].

Many studies have been done on AChE activity, but there are not any studies related to in vitro effects of these plants extracts on AChE enzyme activity. The main purpose of this study was to investigate the in vitro effects of *Glycyrrhiza glabra*, *Pimpinella anisum* and *Matricaria chamomilla* on both fish brain and liver AChE.

MATERIALS AND METHODS

Materials

All chemicals used in here were of reagent grade and purchased from Sigma Chem. Co. (St Louis, Missouri, USA). Determination of sample species was conducted by Dr. Lutfi Behcet (YYU, Faculty of Science Department of Biology) and the plant samples were defined in YYU, Faculty of Science, Department of Biology, Herbarium of Lake Van Basin. *Glycyrrhiza glabra*, *Pimpinella anisum* and *Matricaria chamomilla* were obtained from a local market.

Extract preparation

Each solution (1 g) of *Glycyrrhiza glabra*, *Pimpinella anisum* and *Matricaria chamomilla* was dissolved in 50 mM (pH 8.0) sodium phosphate buffer solution and diluted to 100 mL with the same buffer. After that, the solutions were centrifuged, and undissolved content was filtered, dried, weighed and subtracted from the initial amount of the weighted plant to obtain the plant solution with the buffer [12].

Protein determination

Quantitative protein determination was performed by absorbance measurements at 595 nm with respect to Bradford method with bovine serum albumin used as a standard protein [13].

Measurement of AChE activity

AChE activity was carried out with the method of Ellman and co-workers using acetylthiocholine (ATCI) substrate. The reaction mixture in a final volume of 3 mL contained 0.1 mL of 10 mM 5,5′ -Dithiobis(2-nitrobenzoic acid) (DTNB; prepared in 50 mM sodium phosphate, pH 7.0) 0.1 mL of fish brain and liver supernatant as enzyme source and 2.7 mL of buffer (0.05 M sodium phosphate buffer, pH 8.0). The blank contained the substrate, DTNB and AChE. The mixture was pre-incubated for 5 min at 37°C, and the reaction was started by the addition of 3 mM ATCI. The mixture was incubated for 10 min and the increase in absorbance was determined at 412 nm [14].

Kinetic studies of AChE

In this section of the study, in vitro measurements of the enzyme activity exposed to *Glycyrrhiza*

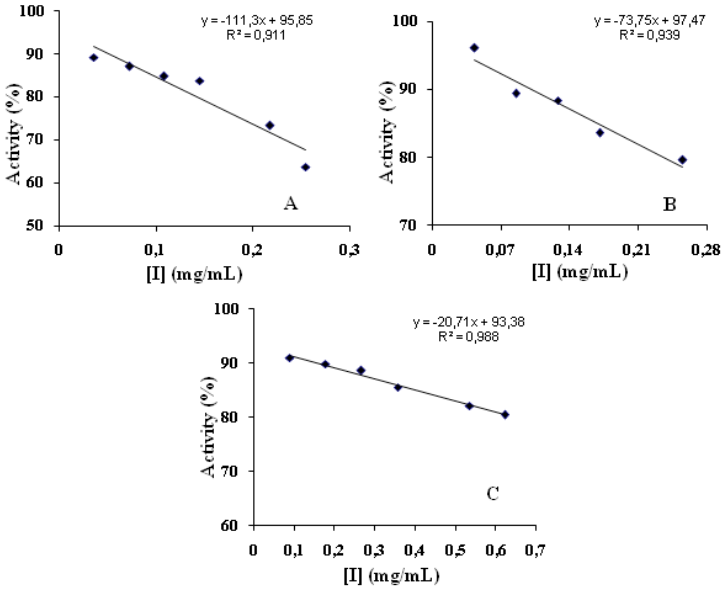

Figure 1. (A) Inhibition% of brain AChE versus *Pimpinella anisum* concentration graph. (B) Inhibition% of brain AChE versus *Matricaria chamomilla* concentration. (C) Inhibition% of brain AChE versus *Glycyrrhiza glabra* concentration graph.

glabra, *Pimpinella anisum* and *Matricaria chamomilla* extracts were performed so as to estimate I_{50} values of these extracts. For this, *Glycyrrhiza glabra* (88.9-178-260 μg/mL), *Pimpinella anisum* (36.3-72.7-109 μg/mL), and *Matricaria chamomilla* (42.6-85.2-127.8 μg/mL) were put on the reaction mixture containing 10 mM DTNB, 0.1 mL enzyme solution in 50 mM phosphate buffer (pH 8.0). After this, the mixture was incubated at 37°C for 5 min then 3 mM of ATCI was added to the mixture and the absorbance values, which are proportional to the enzyme activity, were recorded. An experiment cuvette in the absence of the extracts was used as control experiment (100% activity).

So as to determine the type of inhibition and the I_{50} values, five different concentrations of ATCI (25, 50, 100, 125 and 150 μM) and three different concentrations of each extracts were used. Lineweaver–Burk graphs were plotted and the type of inhibition was determined from this graph [15].

Optimum pH and temperature determination

The optimum pH of fish liver and brain were determined using different pH values from 3 to 10 pH of sodium phosphate buffer at constant temperature 37°C. The mixture was pre-incubated for 5 min at 37°C, and the reaction was started by the addition of 3 mM ATCI. The optimum temperature of fish liver and brain AChE was found by change range from 10 to 50°C temperatures at constant pH 8.0.

RESULTS AND DISCUSSION

Herein, the modulating effects of *Glycyrrhiza glabra*, *Pimpinella anisum* and *Matricaria chamomilla* on both brain AChE and liver AChE have been studied. The extracts which exhibit both inhibitor and activator effects on AChE have been identified in terms of the changing in the activity. The activity % versus plant extract concentration was plotted and I_{50} (extract concentration leading to loss 50% of AChE activity) values of the extracts were calculated from that graph. I_{50} values for brain AChE have been found 0.394 $mg.mL^{-1}$ for *Pimpinella anisum* (Figure 1A), 0.604 $mg.mL^{-1}$ for *Matricaria chamomilla* (Figure 1B), and 1.714 $mg.mL^{-1}$ for *Glycyrrhiza glabra* (Figure 1C). However, these three extracts have shown the activator effect on liver AChE (Figure 2). It has been studied in five different substrate and three different inhibitor concentrations for determination of the type of the inhibition. The results indicate that the plant extracts show non-competitive inhibition on AChE by means of Lineweaver-Burk graphs (Figure 3).

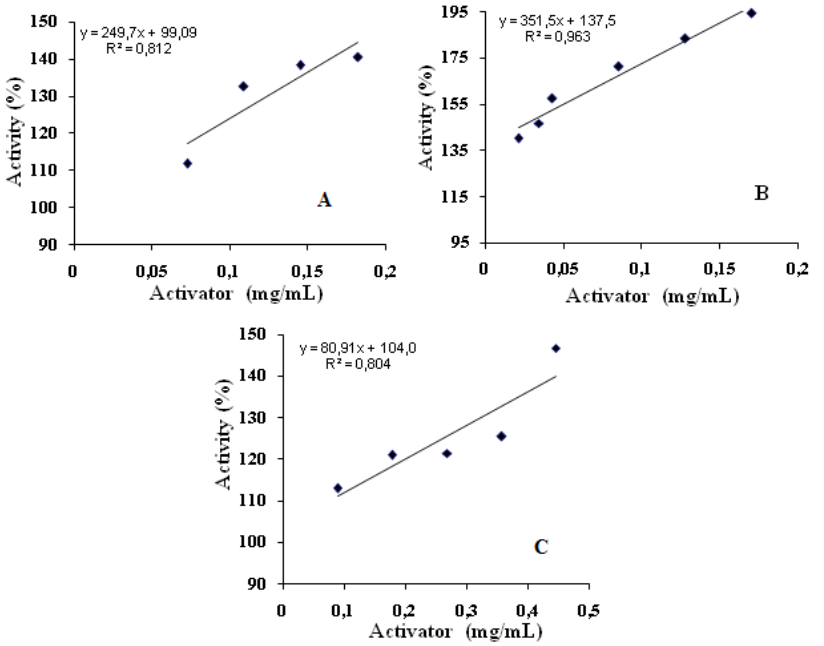

Figure 2. (A) Activation% of liver AChE versus *Pimpinella anisum* concentration. (B) Activation% of liver AChE versus Matricaria chamomilla. (C) Activation% of liver AChE versus *Glycyrrhiza glabra*.

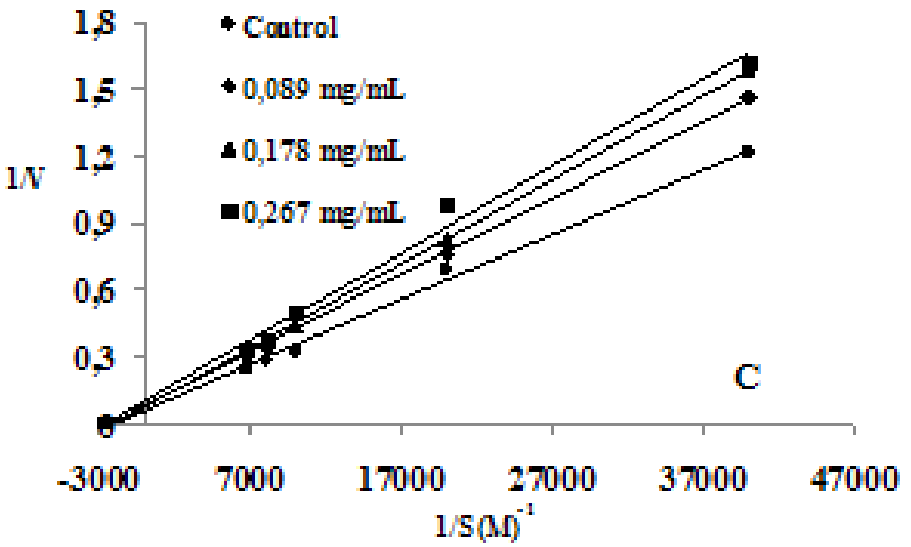

Figure 3. (A) Lineweaver-Burk graph of brain AChE using *Pimpinella anisum* extract. (B) Lineweaver-Burk graph of brain AChE versus *Matricaria chamomilla* concentration. (C) Lineweaver-Burk graph of brain AChE by the extract of dried *Glycyrrhiza glabra*.

The denaturation of proteins occurs solving and changing the structure of protein without hydrolyzing the peptide bounds. Amoung the denaturating factors, temperature and strong acids and bases are the most important parameters affecting enzyme activity [16]. In the study, the optimum temperature and pH of AChE of liver and brain homogenate has been investigated. It was found that the optimum temperature is 30°C for both brain and liver AChE (Figure 4), which is different from that reported for liver and brain AChE purified from Lake Van fish brain and liver [17,18]. Also, the optimum pH was found as 7.0 for both brain and liver AChE (Figure 5), different from those reported previously. This difference is because of the overlap of other

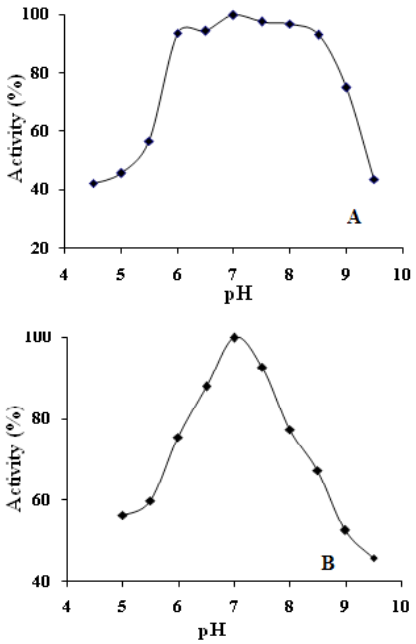

Figure 4. (A) Brain AChE activity vs temperature graph. (B) Liver AChE activity vs temperature graph.

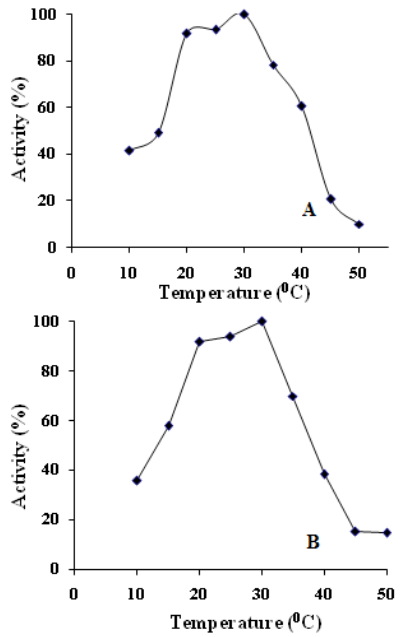

Figure 5. (A) Brain AChE activity vs pH graph. (B) Liver AChE activity vs pH graph.

proteins present in the homogenates [17-18]. In addition, specific activity for the brain has been found 130.5 EU.mg^{-1} and 13.59 EU.mg^{-1} for the liver.

CONCLUSION

In here, the modulatory effects of *Glycyrrhiza glabra* *Pimpinella anisum* and *Matricaria chamomilla* are described. According to the experimental results, these plants inhibit fish brain AChE. *Pimpinella anisum* exhibits more inhibitor effect than *Glycyrrhiza glabra* and *Matricaria chamomilla* taking into consideration their I_{50} values. However, these three plant extracts activate fish liver AChE. While cholinesterase in fish liver homogenate is pseudocholinesterase, cholinesterase in fish brain

homogenate is genuine cholinesterase. Therefore, these isoenzymes showed different effects against the same modulator compounds.

People with Alzheimer's disease have decreased brain levels of acetylcholine. The compounds used for this disease are desired to inhibit AChE activity, resulting in the increase of acetylcholine. We believe that these plants will be useful for the diseases related to the inhibition of AChE activity. Also, this study can be supported by *in vivo* studies.

References

1. M. Citron, Beta-secretase inhibition for the treatment of Alzheimer's disease-promise and chellenge, Trends in Pharmacological Sciences, 25 (2004) 92–97.
2. D.K. Lahiri, M.R. Farlow, N.H. Greig, K. Sambamurti, Current drug targets for Alzheimer's disease treatment, Drug Development Research, 56 (2002) 267–281.
3. M. Heinrich, H.L. Teoh, Galanthamine from snowdrop-the development of a modern drug against Alzheimer's disease from local Caucasian knowledge, Journal of Ethnopharmacology, 92 (2004) 147–162.
4. O. Maschi, E. Dal Cero, G.V. Gali, D. Caruso, E. Bosisio, M. Dell'Agli, Inhibition of human cAMP-Phosphodiesterase as a mechanism of the spasmolytic effect of Matricaria recutita L., Journal of Agricultural and Food Chemistry, 56 (2008) 5015–5020.
5. M.A. Boelens, Spices and condiments II In Volatile Compounds in Food and Beverages, H. Maarse (Ed.), pp. Marcel Dekker, Inc., New York, Basel, Hong Kong, (1991) 449–482.
6. N.G. Bisset (Ed.), Herbal Drugs and Phytopharmaceuticals. CRC Press, Medpharm, Stuttgart, (1994) pp. 73–75.
7. Z. Aboabrahim, Z. Kharazmshahi, vol. 2. National Works Publications, Teheran, (1970) p. 141.
8. Y. Asada, W.Li, T. Yoshikawa, Biosynthesis of the dimethylallyl moiety of glabrol in *Glycyrrhiza glabra* hairy root cultures via a non-mevalonate pathway, Phytochemistry, 55 (2000) 323–326.
9. T. Baytop, Treatment with Plants in Turkey, Past and Present, , İstanbul, 1999.
10. A.Yalçın, Encyclopedia of Medicinal Plants Home drugs/ healing Waters. Gateway Publications, 1996.
11. E.A. Demirhan, The Place of Liauorice in the History of Medicine, Its Importance from the point of Wiew of Traditinol Treatments and Some Samples from İstanbul and Bursa Herbalists, Turkey Clinics J. Med Ethics., 12 (2004) 248-252.
12. A. Ozdemir, V. Turkoglu, H. Demir, *In vitro* effect of some plant extracts on acetylcholinesterase enzyme in human erythrocytes and serum, Fresenius Environmental Bulletin, 22 (2013) 2510-2515.
13. M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding, Anal. Biochem., 72 (1976) 48-51.
14. G.L. Ellman, K.O. Courtney, V. Andres, Feather-Stone RM A new and rapid colorimetric determination of acetylcholinesterase activity, Biochem. Pharmacol., 7 (1961) 88-95.
15. H. Lineweaver, D. Burk, The determination of enzyme dissociation constants, J Am Chem Soc., 56 (1934) 658–660.
16. P. Scaps, O. Borot, Acetylcholinesterase Activity of the Polyhaete Nereis Diversicolor: of Effects Temperature, Salinity, Comp. Biochem. Physiol., 125 (2000) 377-383.
17. Ö.F. Güloğlu, V. Türkoğlu, İ. Çelik, Purification and Characterization of Acetylcholinesterase from Sheep Liver and Inhibition by some Poinkillers, Asian Journal of Chemistry, 13 (2006) 1097-1103.
18. E. Akman, V. Türkoğlu, İ. Çelik, Purification and characterization of Van lake fish (*Chalcalburnus tarichii* P.1811) liver and brain acetylcholinesterase, Hacettepe Journal of Biology and Chemistry, 37 (2009) 331-336.