

Investigation of the Effect of Sodium Selenate on Acetylcholinesterase Activity under Extremely Low Frequency Electromagnetic Field

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Abstract

Acetylcholinesterase (AChE EC 3.1.1.7) is one of the most important enzymes in nervous system, which plays a role in Alzheimer's disease. Selenium is a vital micronutrient and many investigations have been performed about the physiological, biochemical and behavioral effects of this element, such as postponing the Alzheimer's symptoms in the elderly and delaying the initiation signs of skin aging. Recent studies have shown that this element protects various enzymes against the toxicity caused by heavy metals such as; Pb, Al, Cu and Cd. AChE activity is altered under the influence of extremely low frequency electromagnetic field (ELF-EMF). In this study, the effects of ELF-EMF, with 0.3 mT field intensity and 50, 100, 217 Hz frequencies, were investigated on the AChE, in the presence of different concentrations of sodium selenate, using UV-Visible, fluorescence and circular dichroism spectroscopic techniques. The results demonstrated that the enzyme activity declined by increasing the frequency and the amount of sodium selenate. Also, significant structural changes occurred in the secondary and tertiary structures of AChE. Our results

showed that with increasing the concentration of sodium selenate transition from α -helix to β -structure was appeared in the presence of ELF-EMF. In conclusion, according to changes observed in the secondary and tertiary structure of enzyme, it is proposed that these fields are able to affect the structure and dynamics of the active site gorge of AChE.

Keywords: Acetylcholinesterase, Circular dichroism spectroscopy, Extremely low frequency electromagnetic field, Fluorescence spectroscopy, Sodium selenate, UV-visible spectroscopy

1. Introduction

Daily exposure to electromagnetic field is unavoidable as a consequence of living in a Society that depends heavily on the use of electricity. Humans are normally exposed to extremely low frequency electromagnetic fields (ELF EMFs; defined as having a frequency of less than 300 Hz) (Yaghmaei et al., 2009). Various surveys and epidemiological studies (Yaghmaei et al., 2009) have been performed to find the effects of these ELF EMFs. The exposure to ELF EMFs influences the cell proliferation rate and enzyme activities as well (Zwirska-Korczała et al., 2005). Previous studies have reported that ELF EMFs with the frequency of 75 Hz and amplitudes above 250 μ T threshold caused a 55% decrease in the enzymatic activity in the outer segment membranes of retinal rod cells (Ravera et al., 2004). Other studies have demonstrated that ELF EMF with the frequency of 75 Hz and amplitudes above a 250 μ T threshold decreases the activity of three membrane-bound blood cell- or synaptosome- enzymes: alkaline phosphatase, phosphoglycerate kinase and Acetylcholinesterase (AChE) up to 54-61%. Insignificant effects of ELF EMF have already been reported on the activities of soluble enzymes (Morelli et al., 2005). Therefore, in the present study, the hypothesis is that ELF EMF affects the activity of AChE, which occurs at high specific activity in the brain and nervous tissues. AChE is usually found in several forms. Mammalian brain AChE consists almost exclusively of globular forms, principally the tetrameric one (Dvir et al., 2004). AChE catalyzes the hydrolysis of acetylcholine (ACh) into acetate and choline at the cholinergic nerve terminals. It is, therefore, remarkable that the active site of this enzyme is found at the bottom of a deep and narrow gorge (Axelsen et al., 1994). We do not yet know whether this gorge provides a mechanism that somehow facilitates the rapid action of AChE. Selenium is an element that is essential for normal metabolic reactions in the body (Ani et al., 2007), and seems to reduce the toxicity of several metals probably by forming the selenide complexes (Ani et al., 2007; Moshtaghi et al., 2007). Selenium is also known to provide protection from reactive oxygen species (ROS) induced cell damages. Therefore, in this regards, the selenium has been shown to reverse some effects of ELF EMF on the AChE in vitro.

2. Materials and Methods

AChE [(EC 3.1.1.7 from *Electrophorus electricus* (electric eel)] was purchased from Sigma Chemical and Biochemical Company. Sodium selenate (Na_2SeO_4) was synthesized as explained earlier. All other materials and reagents were of analytical grade. The reported results in this paper are the outcome of triplicate measurements.

2.1 Enzyme Assay

AChE activity was determined at 25°C by the spectrophotometric method of Ellman (Ellman et al., 1961), based on the following colorimetric reactions:

Acetylthiocholine iodide $\xrightarrow{\text{AChE}}$ Thiocholine + Acetate

Thiocholine + Dithiobisnitrobenzoic acid (DTNB) $\longrightarrow$ 5-thio-2-nitro-benzoate (yellow)

The reaction mixture containing the 3 μM AChE with the 12.5×10^{-6} M acetylthiocholine iodide (ACh iodide) with different concentrations of Na_2SeO_4 (0, 390, 870, 1300 and 1800 μM) was kept in the electromagnetic fields 50, 100, 217 Hz, 300 μT for 30 min at 4°C and immediately after the DTNB was added to the reaction mixture the enzymatic activity was measured for 10 min in the absence of the field. The principle of the method is the measurement of the rate of production of thiocholine as acetylthiocholine iodide is hydrolyzed. This is accomplished by the continuous reaction of the thiol with DTNB (Ellman reagent) to produce the yellow anion of 5-thio-2-nitro-benzoate. The rate of color production was measured at 412 nm for 10 min with a UV-spectrophotometer Shimadzu, model UV-3100 with jacketed cell holders. All the experiments were run in phosphate buffer (0.1 M) at pH 7.4 in conventional thermostated quartz cell to maintain the temperature at 20 ± 0.1 °C. The initial velocity of enzyme was calculated using an absorption velocity of enzyme calculated using an absorption coefficient, $\epsilon = 1.36 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$ and one unit of enzymatic initial velocity is given as $\mu\text{mol}/\text{min}$ (Hekmat et al., 2008).

The rate calculated from the equations:

$$\text{Rate } (\mu\text{mol} / \text{min}) = \frac{\Delta \text{ absorbance} / \text{min}}{1.36 \times 10^4}$$

2.2 Circular Dichroism Studies

Circular Dichroism spectroscopy (CD) is a powerful tool for revealing any conformational change to the protein structure. The far-UV CD region (190-260 nm), which corresponds to peptide bond absorption, was analyzed by an AVIV Model 215 Spectropolarimeter (Lakewood, NJ, USA) to give the content of regular secondary structure of AChE. Protein solutions were prepared in the same buffer as used for the enzymatic assay. Protein solutions of 0.4 mg/mL were used to obtain the spectra after being incubated with different concentrations of sodium selenate (0, 390, 870, 1300 and 1800 μM) in the presence and absence of ELF-EMF at 4 °C. The CD spectrum of AChE, with different concentrations of sodium selenate was recorded under ELF- EMF at 200 to 250 nm using a quartz cell with the path length of 0.1 cm, 0.2 nm resolution, 20 nm/min scan speed, 2.0 s time constant, 10 nm band width and the sensitivity of 20 m° .

2.3 Intrinsic Fluorescence

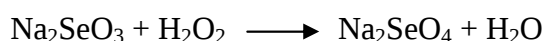
Intrinsic fluorescence intensity measurements were carried out using a Hitachi

spectrofluorometer (MPF-4 model), equipped with a thermostatically controlled cuvette compartment. The intrinsic emission of 3 μ M protein was seen at the excitation wavelength of 280 nm. The conditions of incubation were the same as those used for CD studies.

2.4 Preparation of Sodium Selenate

7.9 grams of so-called standard Se (99.9%) were dissolved in 13ml of warm 65%-nitric acid. The selenious acid, H_2SeO_3 , obtained, 1.2 grams, was then admixed with a stoichiometric amount of NaOH, 8 grams, as a 50%-solution of sodium hydroxide, there being obtained 17.3 grams of Sodium selenite and a further 3.6 grams of water. The pH was adjusted to 11, by adding a further minor amount of NaOH. Hydrogen peroxide (30%) was then stirred into the Sodium selenite solution in a stoichiometric excess, at a temperature of 70°C., the Sodium selenite being oxidized to Na_2SeO_4 . The resultant solution, contained Sodium selenite, and then we put down on a flat place and the supernatant solution was separated, then its concentration determined by Inductively Coupled Plasma (ICP, Varian VISTA-MPX).

The chemical reactions involved in the method are:



2.5 ELF-EMF

ELF-EMF was generated by a parallel set of Helmholtz solenoids with 500 turns of 0.7 mm coated copper wire. Each solenoid diameter was 27 cm. The coils were driven by 50, 100 and 217 HZ power through a variable transformer and generated a magnetic flux density of 0.3 mT. The samples were placed in the center half way between the plains of coils to receive a uniform field for 30 min. Attention was paid to eliminate all the metal elements around the field.

2.6 Statistical Analysis

Statistical analysis was performed by SPSS software using one way variance analysis ANOVA (Ver. 16, IBM Corporation, USA). Data were reported as mean $\pm$ standard deviation at significance level of $p < 0.05$.

3. Results

3.1 UV-Visible Absorption Spectra

The effect of sodium selenate on the activity of AChE can be determined by carrying out a series of velocity measurements in the presence of different concentrations of sodium selenate with and without ELF-EMF. As shown in Figure 1, AChE activity decreased by increasing the sodium selenate concentration. Figures 2a-2c, demonstrate a decrease in the enzyme activity in presence of increased concentrations of sodium selenate under ELF-EMF.

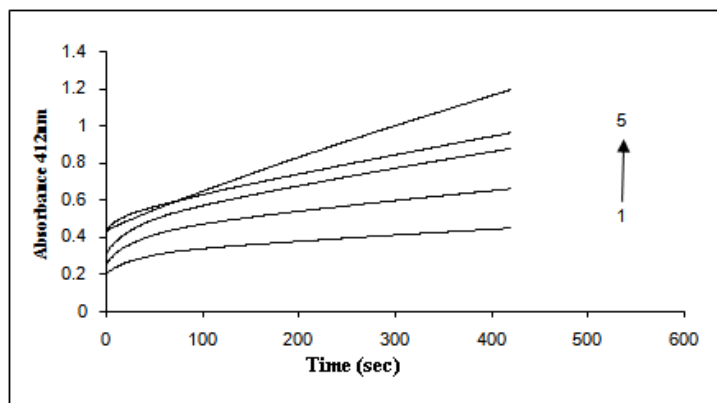
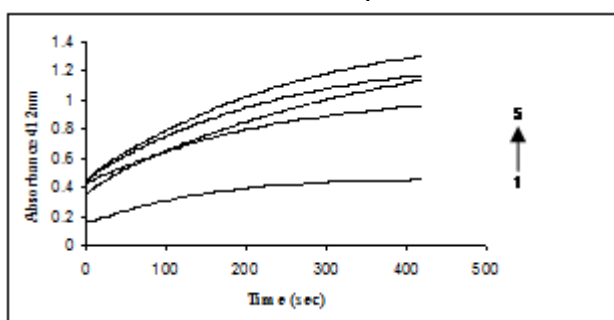
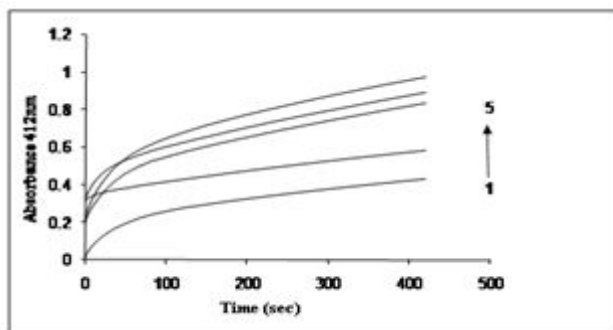


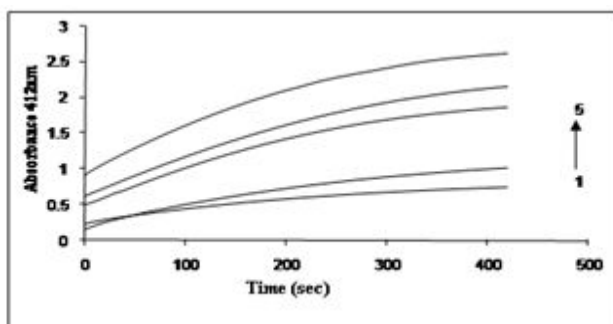
Figure 1. Absorption spectra of AChE in the presence of different concentrations of sodium selenate: 1: 1800, 2: 1300, 3: 870, 4: 390, 5: 0 μ M after incubation for 30 min at 4 °C.



a



b



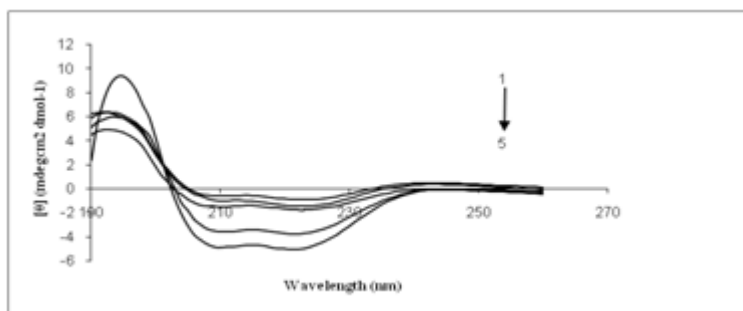
c

Figure 2. Absorption spectra of AChE at different concentrations of sodium selenate: 1: 1800,

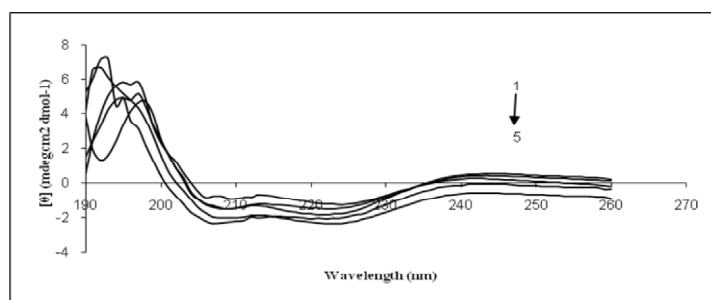
2: 1300, 3: 870, 4: 390, 5: 0 μ M after incubation for 30 min under the frequency of a:50 Hz ,
b: 100 Hz, c: 217 Hz and the intensity of 0.3 mT at 4 °C.

3.2 Circular Dichroism Studies

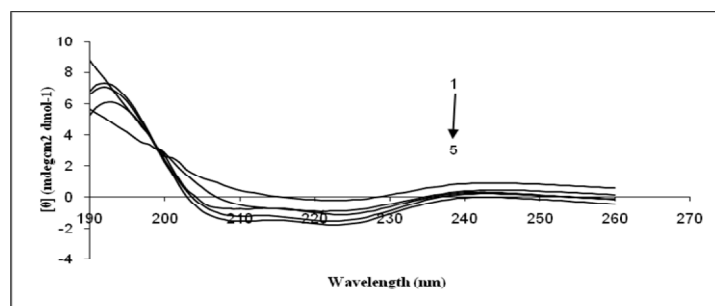
Far-UV CD spectra (190-260 nm) are used for the determination of a proteins' secondary structure. The peptide bond is the principal absorbing group. Studies in this region may provide information about the secondary structure of a protein (Sreerama and Woody, 2004; Saboury et al., 2004). During the present analysis, in order to consider the effect of different concentrations of sodium selenate, at different frequencies of ELF-EMF, on the conformational changes of AChE, Far-UV CD technique was used and the spectra can be observed in Figure 3. The CD spectra of AChE exhibited two negative minima at 208 nm and 222 nm, which is a typical characterization of the helical structure of this class of proteins (Hu et al., 2005). Figures 3b-d demonstrate the reduction of helicity of AChE in the presence of increasing concentrations of sodium selenate under 50, 100 and 217 Hz (0.3 mT). Within the wavelength region of 205–250 nm, the CD spectrum of a protein gives information about its conformation in relation to the secondary structure. The ELF-EMF decreases both of these bands, clearly indicating the decrease of helical content in the protein in the presence of 50, 100 and 217 Hz (0.3 mT).



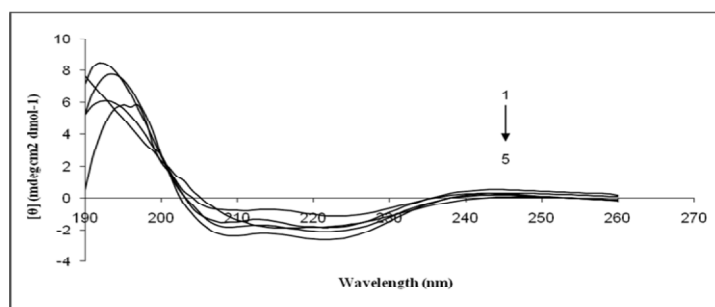
a



b



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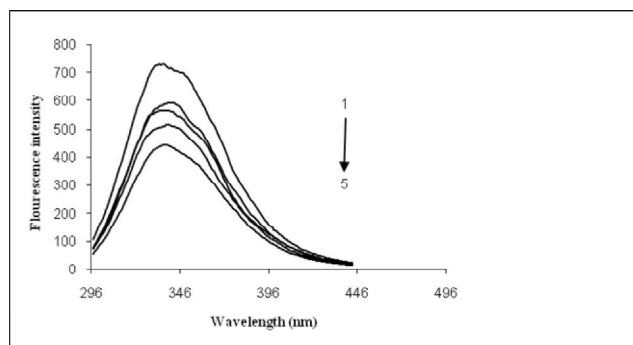
d

Figure 3. UV-CD spectra of AChE at different concentrations of sodium selenate: 1: 1800, 2: 1300, 3: 870, 4: 390, 5: 0 μ M after incubation for 30 min at 4 °C a: without ELF-EMF b: in the presence of 50 Hz, c: 100 Hz and d: 217 Hz .

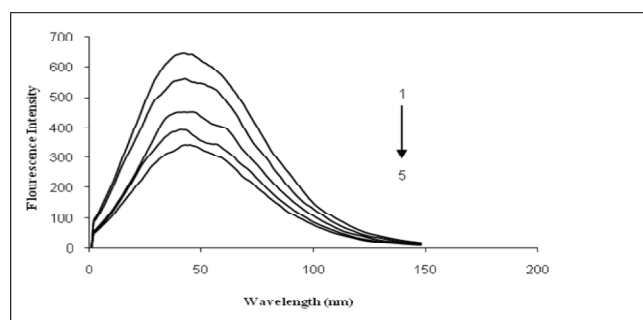
3.3 Fluorescence Studies

Intrinsic Trp fluorescence was used to monitor changes in the protein conformation in the presence of different concentrations of sodium selenate after incubation for 30 min. at 25 °C in the presence of 50, 100 and 217 Hz 0.3mT (Figure 4).

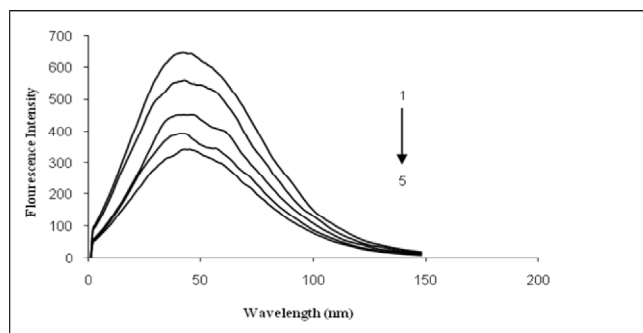
Figure 4a shows the intrinsic fluorescence emission spectra of AChE in the presence of sodium selenate and without ELF-EMF. Figures 4b-d illustrate the intrinsic fluorescence emission spectra of AChE in the presence of different concentrations of sodium selenate under 50, 100 and 217 Hz (0.3mT), respectively. Figures 4b, 4c and 4d demonstrate a decrease in the intrinsic fluorescence by increasing the sodium selenate concentrations. Figure 4a shows the plots of maximum emission intensity at 341 nm but figures 4b-4d show the plots of maximum emission intensity at 350 nm. Therefore, we have observed the wavelength shift during the present experiment. According to these figures, it is interesting to point out that in the presence of 50, 100 and 217 Hz (0.3 mT), the protein had more accessible hydrophobic patch than that without ELF-EMF, for sodium selenate.



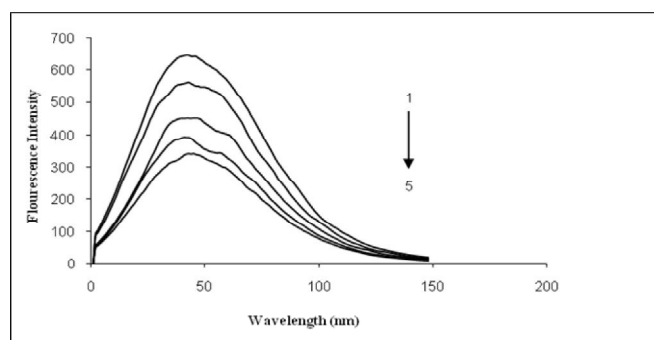
a



b



c



d

Figure 4. Chart of intrinsic emission of AChE at different concentrations of sodium selenate: 1:0, 2:390, 3:870, 4:1300, 5:1800 μM after incubation for 30 min at 4 °C, and different frequencies with 0.3 mT field intensity. 4a: without ELF-EMF, 4b: 50 Hz, 4c: 100 Hz and 4d: 217 Hz.

4. Discussion

Exposures to many EMF frequencies are increasing significantly as technology advances unabated and new applications are found. During the past 20 years the general public has become increasingly concerned about potential adverse health effects of exposure to electric and magnetic fields at ELF. The effects of electromagnetic fields on living microorganisms have been investigated for a long time. In one previous study, EMF affects some biochemical processes and result in changing some serum biochemical parameters and enzymes (Yaghmaei et al., 2009). This research as well as previous researchers strongly suggested that the field action is mediated by the structure which is crucial in determining the conditions of the enzyme inactivation (Ravera et al., 2004). Investigation on the role of selenium in biological systems is very important. Many studies have been done over the years to demonstrate the effects of selenium: helps promote a healthy liver, acts as an antioxidant against free radicals (Abubakar et al., 2004), may help prevent cancers (Fleet et al., 1997), protects against toxic metals in the body including mercury (Ani et al., 2007; Moshtaghi et al., 2007) and protects against heart disease (Cardoso et al., 2010). In the present study, UV-vis spectroscopy, CD and fluorescence techniques have been used to monitor the changes of structure of AChE under the frequency of 50, 100 and 217 Hz and the intensity of 0.3 mT in the presence of different concentrations of Na_2SeO_4 . Activity studies (Figure 1) showed that different concentrations of Na_2SeO_4 have an effect on the activity of AChE under ELF-EMF. ELF-EMF substantially alters the optimal temperature and optimal pH. However, there was a change in OD values when the samples were exposed to ELF-EMF at different frequencies which indicate there was alternation in enzyme activity. Previous studies revealed that application of ELF-EMF on enzymes such as Lipid peroxidase, Adenylate Kinase and Phosphatase under the field of 120 mT and 180-195 Hz, 250 mT and 75 Hz, 5 mT and 50 Hz caused to reduction on enzyme activity, respectively (Ravera et al., 2004). CD has proved to be an ideal technique to monitor conformational changes in proteins, which can occur as a result of changes in experimental parameters such as pH, temperature, binding of ligands and so on (Kelly and Price, 2000). Far-UV CD studies of AChE under ELF-EMF at different concentrations of Na_2SeO_4 showed significant affected of Na_2SeO_4 under ELF-EMF on the secondary structure of the protein. The protein has alpha/beta structure (Axelsen et al., 1994; Fathi and Farahzadi, 2012) while in the presence of 390, 870 μM concentrations of Na_2SeO_4 the content of α -helix structure of AChE was decreased and at a concentration of 1300 μM of Na_2SeO_4 a transition from α -helix to β -structure was appeared (Figure 3) then, it can be concluded that with increasing the concentrations of Na_2SeO_4 , β -sheet structure can induce in the secondary structure of AChE under ELF-EMF and consequently prevent the access of the substrate (ACh iodide) to the enzyme active site. Na_2SeO_4 induced changes in intrinsic fluorescence intensity of the Trp residues in AChE under ELF-EMF: with increasing the concentrations of Na_2SeO_4 , Trp fluorescence decreased. Combining the results from UV-vis, Far-UV CD and intrinsic fluorescence analysis, we conclude that the structure of AChE under ELF-EMF is altered by Na_2SeO_4 . Neuropathological studies have been reported that various antioxidants are decreased in different age-related degenerative diseases and thus, oxidative stress would have a central role in the pathogenesis of many disorders that involve neuronal

degeneration, including Alzheimer's disease (AD). AD was associated with deficiency in the brain neurotransmitter, ACh (Tabet, 2006). The inhibition of AChE enzyme, which catalyzes the breakdown of ACh, may be one of the most realistic approaches to the symptomatic treatment of AD (Pangestuti and Kim, 2010). Therefore, we suggest that the Na₂SeO₄ in the presence of ELF-EMF could be useful in treatment of AD disease.

5. Conclusion

In conclusion, our results suggested that ELF-EMF application affects the AChE activity, while increasing sodium selenate concentration had same effect. These effects might be related to structural changes of the AChE.

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