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Full Length Research Paper

Effect of electromagnetic field on acetylcholinesterase activity: *In vitro* study

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The *in vitro* effects of extremely low frequency (ELF)-electromagnetic field (EMF) (frequencies of 50, 100, 217 Hz, induction 300 µT) on the structure and activity of acetylcholinesterase (AChE) was investigated. AChE activity was measured with UV-vis spectroscopy. Circular dichroism spectroscopy and fluorescence spectroscopy were used for investigating the secondary and tertiary structure of enzyme. The results demonstrated that the ELF-EMF decreased enzyme activity and the main inactivation took place in 217 Hz ($p<0.05$). It can be concluded that increasing the frequencies of ELF-EMF caused an increase in the helicity of AChE; therefore, under ELF-EMF, the α-helix structure can induce the secondary structure of AChE. Also, the intrinsic fluorescence decreased by increasing the frequency of field. 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.

Key words: Acetylcholinestrase, circular dichroism spectroscopy, extremely low frequency electromagnetic field, fluorescence spectroscopy, UV-visible spectroscopy.

INTRODUCTION

The effects of extremely low frequency electromagnetic fields (ELF-EMFs) on biological systems like different enzymes, nucleic acids, cell proliferation etc. have been investigated (Volpe, 2003; Zwirska-Korczala, 2005). Small effects of ELF-EMFs on the activities of different enzymes such as antioxidative enzymes and acid phosphatase have been already reported (Zwirska-Korczala, 2005; Prashanth et al., 2009). As ELF-EMFs were found to affect the activity of membrane enzymes such as Na, K-ATPase or cytochrome oxidase and adenylate kinase (Ravera et al., 2004; Morelli et al., 2005), other studies have demonstrated that ELF-EMF with the frequency of 75 Hz and amplitudes above a 250 µT threshold decreased the activity of three membrane-bound blood cell- or synaptosome-enzymes: Alkaline phosphatase, phosphoglycerate kinase and acetylcholinesterase (AChE) up to 54 to 61% (Morelli et al., 2005). The effects of these fields on human body depend on amperage, frequency and exposure duration (Seyyedi et al., 2007). Therefore, this hypothesis could be checked that ELF-EMF affects the activity of AChE, which occurs with high specific activity in the brain and nervous tissues. AChE is one of the most important enzymes in nervous system and a little amount in the liver (Parveen et al., 2004), placenta, as well as the surface of erythrocytes membrane (Dvir et al., 2004).

AChE is found in multiple forms. The predominant forms of this enzyme are Globular (monomers, G1; dimmers, G2; and tetramers, G4) and asymmetric forms (one, A4; two, A8; or three, A12, tetramers linked to a collagen-like tail) (Massoulie and Bon; 1982). Mammalian brain AChE consists almost exclusively of globular forms, principally the tetramer (Grassi et al., 1982; Massoulie, 2002). This enzyme is a glycoprotein, a member of serine hydrolase enzymes and has four conserved amino acids

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including Serine, Histidin, Aspartate and Glutamate in its own active site and causes the termination of signal transduction in the somatic nervous systems with the degradation of neurotransmitter, acetylcholine (ACh) (Axelsen et al., 1994). 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). One study demonstrated that this gorge provides a mechanism that somehow facilitates the rapid action of AChE (Barteri et al., 2005).

The aim of this study was the most basic in this contex: to determine the influence of ELF-EMF on the activity and structure of acetylcholinesterase. The reason for this choice was both the availability of highly purified enzyme and tremendous increase in the use of electrical devices. Furthermore, AChE's enzymatic activity can easily be tested "*in vitro*" preparations, and thus, it provides a suitable model system to study the influence of EMF emission on cholinergic activity.

MATERIALS AND METHODS

In this study, acetylcholinesterase [(EC 3.1.1.7 from Electrophorus electricus (electric eel)] were supplied by sigma (St. Louis, MO, USA) and another chemicals obtained from Sigma and Merck chemical companies (Germany). All experimental procedures were repeated for three times.

Enzyme assay

AChE activity was determined at 25°C by the spectrophotometeric method of 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-benzoat (yellow)

The reaction mixture containing the 3 µM AChE with the 12.5×10^{-6} M acetylthiocholine iodide (ACh iodide) 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. Control sample were run in the same experimental condition as used previously but in the absence of 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-benzoat. The rate of color production was measured at 412 nm for 10 min with a Cary Spectrophotometer, 100 Bio-model, 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, $\varepsilon = 1.36\times10^{4}\ M^{-1}\ cm^{-1}$ and one unit of enzymatic initial velocity is given as µmol/min (Hekmat et al., 2008). The general schemes of enzymic reaction can be summarized as follows:

$$\text{Enz} + \text{AcCh} \underset{K_2}{\overset{K_1}{\rightleftharpoons}} (\text{EnzAcCh}) \xrightarrow{K_3} \text{EnzAc} + \text{Ch}$$

$$\text{EnzAc} + H_2O \xrightarrow{K_4} \text{Enz} + \text{AcOH}$$

Where: Enz is the enzyme, AcCh is the substrate (ACh), and Ch and AcOH are the products (choline and acetic acid, respectively). The rate is calculated from the equations as follows:

$$\text{Rate}\ (\mu\text{mol}/\text{min}) = \frac{\Delta\ \text{absorbance/min}}{1.36\times10^{4}}$$

Circular dichroism (CD) measurements

Circular dichroism spectroscopy is a powerful tool for revealing any conformational change to the protein structure (Sreerama and Woody, 2004; Hekmat et al., 2008). The secondary structure estimation of protein was studied using the Aviv circular dichroism spectrometer, Model 215 (Lakewood, NJ, USA). The spectro-photometer was equipped with a temperature programmer for controlling the rate of temperature changes. The CD spectrum of AChE was recorded from 200 to 250 nm using quartz cell, 0.1 cm path length, with a resolution of 0.2 nm, scan speed of 20 nm min^{-1}, time constant of 2.0 s, 10 nm band width and sensitivity of 20 m^{o}. 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 with incubation in the presence and absence of ELF-EMF at 4°C.

Intrinsic fluorescence measurements

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

Extremely low frequency electromagnetic fields (ELF-EMFs)

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 300 µT. 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.

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 ± standard deviation at significance level of $p < 0.05$.

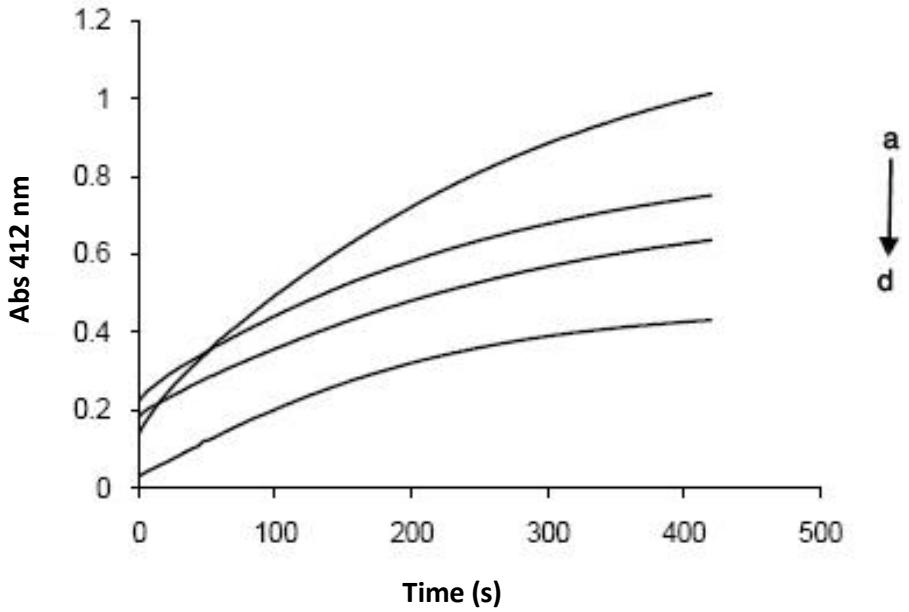

Figure 1. Kinetic plots of acetylcholinesterase after incubation for 30 min at 4°C, and different frequencies with 300 µT field intensity. a: without Electromagnetic field, b: 50 Hz, c: 100 Hz and d: 217 Hz. The mean±SD of three measurements for a, b, c and d were 0.37±0.03, 0.18±0.02, 0.11±0.01 and 0.05±0.01, respectively.

Table 1. Average rates of hydrolysis of ACh iodide.

Samples	Rate (µmol /min)
AChE without ELF-EMF	1.07 ± 0.34
AChE under 50 Hz, 300 µT	0.89 ± 0.06
AChE under 100 Hz, 300 µT	0.54 ± 0.04
AChE under 217 Hz, 300 µT	0.31 ± 0.02*

Each value represents the mean ± SD of three measurements. *Indicates $p<0.05$.

RESULTS

Enzyme activity measurements

Kinetic plot of AChE in the presence and absence of ELF-EMF were recorded, as described in Figure 1. When the enzymatic activity was measured after the exposure to the field, a dramatic decrease in enzymatic activity under the 217Hz was found ($p<0.05$). According to Table 1, with increasing the frequency of ELF-EMF the average rates of hydrolysis of ACh iodide was reduced, indicating decline in AChE activity.

Circular dichroism studies

Far-UV CD spectra (190 to 260 nm) are used for the determination of a protein secondary structure. The peptide bond is the main absorbing group. During the present analysis, in order to consider the effect of 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 2. Table 2 shows the content of the secondary structure of AChE in the presence and absence of ELF-EMF. Figure 2 demonstrates the increase of helicity of AChE under 50, 100 and 217 Hz (300 µT). Within the wavelength region of 205 to 250 nm, the CD spectrum of a protein gives information about its conformation in relation to the secondary structure. The ELF-EMF increase both of these bands, clearly indicating the increase of helical content in the protein in the presence of 50, 100 and 217 Hz (300 µT).

Fluorescence studies

Intrinsic Triptophan (Trp) fluorescence was used to

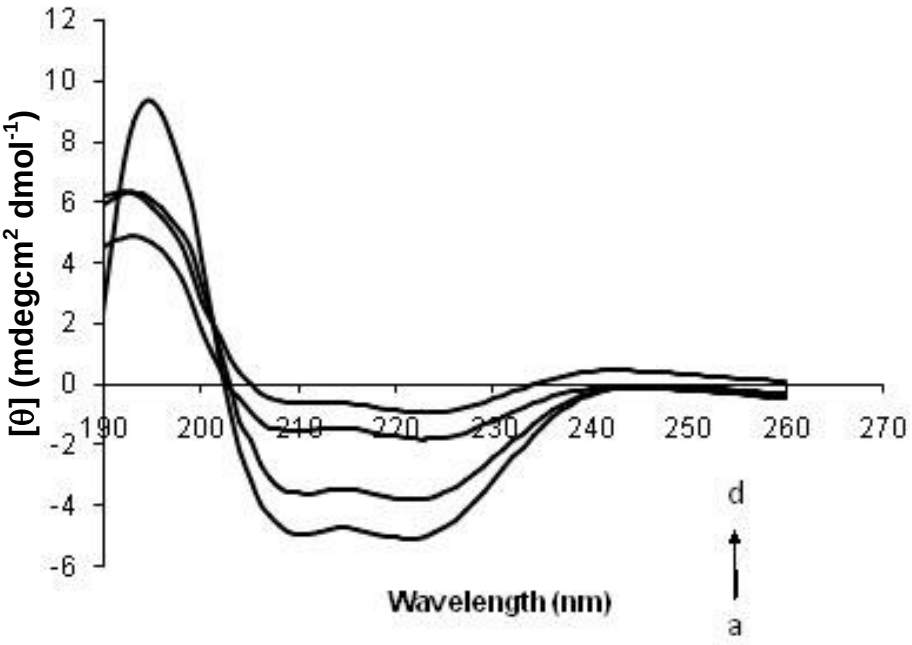

Figure 2. Far-UV-CD spectra of Acetylcholinesterase after incubation for 30 min at 4°C, and different frequencies with 300 µT field intensity.a: 217 Hz, b: 100 Hz, c: 50 Hz and d: without electromagnetic field.

Table 2. Content of the secondary structure of the AChE in the presence and absence of ELF-EMF using the Aviv program.

Samples	α-Helix (%)	β-Sheet (%)	Random coil (%)
AChE without ELF-EMF	49.8	14.3	33
AChE under 50 Hz, 300 µT	55.3	13.2	19.4
AChE under 100 Hz, 300 µT	77.8	11	11.2
AChE under 217 Hz, 300 µT	81.5	9.4	9.1

monitor changes in the protein conformation after incubation for 30 min at 4°C in the presence of 50, 100 and 217 Hz, 300 µT (Figure 3). Graphs a to d illustrate the intrinsic fluorescence emission spectra of AChE in the absence and presence of EMFs 50, 100 and 217 Hz (300 µT), respectively. These graphs demonstrate a decrease in the intrinsic fluorescence by increasing the frequency of field. This indicates that with increasing the frequencies of ELF-EMF, the tryptophan residues are gradually expose to more hydrophilic environment.

DISCUSSION

Exposures to many EMF frequencies are increasing significantly as technology advances unabated and new applications are found. While the enormous benefits of using electricity in everyday life and health care are unquestioned, 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 (Volpe, 2003). The effects of electromagnetic fields on living microorganisms have been investigated for a long time. The initial effect of electromagnetic field is to acute the key biochemical processes in different metabolic pathway (Ebadi et al., 2006). Various studies showed that electromagnetic fields affect some biochemical processes and result in changing some serum biochemical parameters and enzymes (Ames et al., 2005; Yaghmaei et al., 2009). Different mechanisms proposed for EMF action on biological systems include exchange of electrons, ions and dipoles (Ebadi et al., 2006). The effect of ELF-EMF on triton X-100 solublized acetylcholinesterase has been reported previously.

The full activity of enzyme is rapidly restored when the enzyme is removed from the field (Morelli et al., 2005), however, this study did not confirm such an effecting.

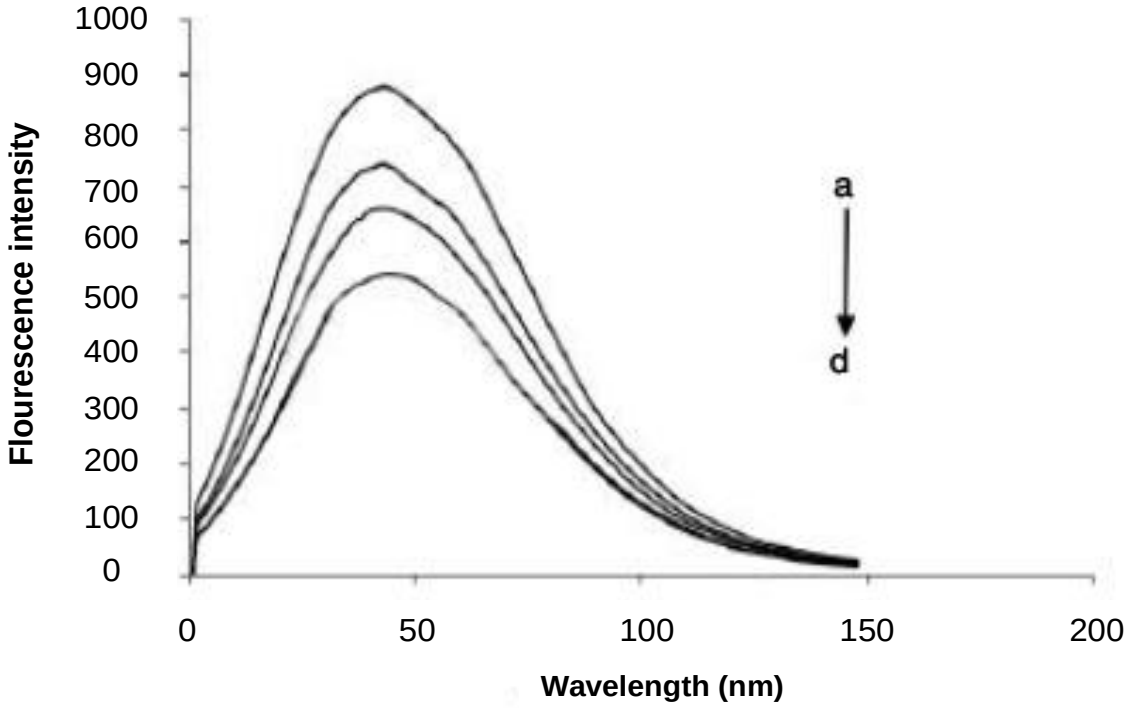

Figure 3. Intrinsic fluorescence spectra of acetylcholinesterase after incubation for 30 min at 4°C, and different frequencies with 300 µT field intensity. a: without electromagnetic field, b: 50 Hz, c: 100 Hz and d: 217 Hz.

This research and 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). The effects might be related to structural changes in the secondary and tertiary structure of the enzyme. Another previous study demonstrates that cellular phone emissions affect the structural and biochemical characteristics of acetylcholinesterase (Barteri et al., 2005). In the present study, UV-vis spectroscopy, CD and fluorescence techniques have been used to monitor the changes of structure of AChE induced by ELF-EMF. Activity studies (Figure 1) showed that different frequencies of EMF have an effect on the activity of AChE. Statistical evaluations elucidate significant differences in the 217 Hz, in which changes in the structure occurs leading to the enzyme inactivation ($p<0.05$; Table 1). CD has proved to be a 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 at different frequencies of ELF-EMF showed significant effect of EMF on the secondary structure of the protein. The native protein has alpha/beta structure (Axelsen et al., 1994) while under ELF-EMF the content of α-helix structure was increased (Figure 2 and Table 2) then, it can be concluded that with increasing the frequency of ELF-EMF, the α-helix structure can induce in the secondary structure of AChE and consequently prevent the access of the substrate (ACh iodide) to the enzyme active site. Taken together, the conclusion is reached that different frequencies of EMF can bring about changes in enzyme structure, which in turn can affect AChE interaction with the substrate (ACh iodide), and its activity. EMF-induced changes were observed in intrinsic fluorescence intensity of the Trp residues in AChE: with increasing the frequency of ELF-EMF, Trp fluorescence decreased. Combining the results from UV-vis, Far-UV CD and intrinsic fluorescence analysis, we conclude that the structure of AChE is altered by ELF-EMF.

Peculiar characteristic of acetylcholinesterase is the presence of a large negative potential near the active site (gorge), due to the presence of negatively charged residues located at the entrance, midway down and near the gorge base. This charged group distribution leads to an electrostatic field, whose potential was calculated solving the Poisson-Boltzman equation by means of the finite difference method (Warwicker and Watson, 1982) implemented in the Delphi algorithm (Gilson et al., 1988). Cholinesterase have a rather strong first moment of 800-1800 Debye roughly aligned along the gorge axis, so that a positive charged substrate will be drawn to the active site by its electrostatic field, creating a selective and efficient substrate-binding site interaction. Current theories and models on the effects of ELF-EMFs on

enzyme activities refer to changes of motion of ions such as calcium at the active site (Edmonds, 1993) or alterations in the binding parameters of ligand-receptor (Bianco and Chiabrera, 1992; Moggia et al., 1997).

However, the decrease in AChE activity in the prescence of ELF field, dose not seems to depend on changes of motion of ions such as calcium or magnesium. On the contrary, the effect of the field could be on the membrane organization and structure which would limit in some way the binding of the substrate to the active site of the enzyme. Neuropathological studies have been reported that Alzheimer's diseases (AD) were 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 ELF-EMF could be useful in treatment of AD disease.

Conclusions

In conclusion, our results suggested that ELF-EMF application affects the AchE activity. The effects might be related to structural changes in the secondary and tertiary structure of the enzyme.

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REFERENCES

Ames BD, Yamasak WE, Lee FD (2005). Dose generalized anxiety disorder predict coronary heart disease risk factors in dependently of major depressive disorder. Affect Disord., 88: 87-91.

Axelsen PH, Harel M, Silman I, Sussman JL (1994). Structure and dynamics of the active site gorge of acetylcholinesterase. Synergistic use of molecular dynamics simulation and X-ray crystallography. Protein Sci., 3: 188-197.

Barteri M, Pala A, Rotella S (2005). Structural and kinetic effects of mobile phone microwaves on acetylcholinesterase activity. Biophys. Chem., 113: 245-253.

Bianco B, Chiabrera A (1992). From the Langevin-Lorentz to the Zeeman model of electromagnetic effects on ligand-receptor binding. Bioelectrochem. Bioenerg., 28: 355-365.

Dvir H, Harel M, Bon S, Silman I (2004). The synaptic acetylcholinesterase tetramer assembles around a polyproline II helix. EMBO J., 23: 4394-4405.

Ebadi A, Sedghi GH, Zare S, Hayatgeibi H, Alivandi S (2006). Biological effect of power frequency magnetic fields on serum biochemical parameters in Guinea pigs. Pak. J. Biol. Sci., 6: 1083-1087.

Edmonds DT (1993). Larmor precession as a mechanism for the detection of static and alternating magnetic fields. Bioelectrochem. Bioenerg., 30: 3-12.

Ellman GL, Curtney KD, Andres V, Featherstone RM (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharm., 7: 88-95.

Gilson MK, Sharp KA, Homig BH (1988). Calculating electrostatic interactions in biomolecules: Method and error assessment. J. Comput. Chem., 9: 327-335.

Grassi J, Vigny M, Massoulie J (1982). Molecular forms of acetylcholinesterase in bovine caudate nucleus and superior cervical ganglion: solubility properties and hydrophobic character. J. Neurochem., 38: 457-469.

Hekmat A, Saboury AA, Divsalar A, Khanmohammadi M (2008). Conformational and structural changes of choline oxidase from Alcaligenes species by changing pH Values. Bull. Korean Chem. Soc., 29: 1510-1518.

Kelly SM, Price NC (2000). The use of circular dichroism in the investigation of protein structure and function, Curr. Prot. Pept. Sci., 1: 349-384.

Massoulie J, Bon S (1982). The molecular forms of cholinesterase in vertebrates. Annu. Rev. Neurosci., 5: 57-106.

Massoulie J (2002). The origin of the molecular diversity and functional anchoring of cholinesterase. Neurosignals. 11: 130-143.

Moggia E, Chiabrera A, Bianco B (1997). Fokker-Planck analysis of the Langevin-Lorentz equation: Application to ligand-receptor binding under electromagnetic exposure. J. Appl. Phys., 82: 4669-4677.

Morelli A, Ravera S, Panfoli I, Pepe IM (2005). Effects of extremely low frequency electromagnetic fields on membrane-associated enzymes. Arch. Biochem. Biophys., 441: 191-198.

Pangestuti R, Kim SK (2010). Neuroprotective properties of chitosan and its derivatives. Mar Drugs. 8: 2117-2128.

Parveen M, Kumar S, Singh P (2004). Kinetic Analysis of the in vivo Inhibition of Liver AchE in Air Breathing Fish Clarias batrachus (Linnaeus, 1758). J. Fish. Aquat. Sci., 21: 143-144.

Prashanth KS, Chouhan TRS , Nadiger S (2009). Effect of 50 Hz electromagnetic fields on acid phosphatase activity. Afr. J. Biochem. Res., 3: 060-065

Ravera S, Repaci E, Morelli A, Pepe IM, Botter R, Beruto D (2004). Electromagnetic field of extremely low frequency decreased adenylate kinase activity in retinal rod outer segment membranes. Bioelectrochemistry. 63(1-2): 317-320.

Seyyedi SS, Dadras MS, Tavirani MR, Mozdarani H, Toossi P, Zali AR (2007). Proteomic analysis in human fibroblasts by continuous exposure to extremely low frequency electromagnetic field. Pak. J. Biol. Sci., 22: 4108-4112.

Sreerama N, Woody RW (2004). On the analysis of membrane protein circular dichroism spectra. Protein Sci., 13: 100-112.

Tabet N (2006). Acetylcholinesterase inhibitors for Alzheimer's disease: Anti-inflammatories in acetylcholine clothing! Age and Ageing (AGE). 35: 336-338.

Volpe P (2003). Interactions of zero-frequency and oscillating magnetic fields with biostructures and biosystems, Photochem. Photobiol. Sci., 2: 637-648.

Warwicker J, Watson HC (1982). Calculation of the electric potential in the active site cleft due to alpha-helix dipoles. J. Mol. Biol., 157: 671-679.

Zwirska-Korczala K (2005). Effect of extremely low frequency electromagnetic fields on cell proliferation, antioxidative enzyme activities and lipid peroxidation in 3T3-L1 predipocytes- an *in vitro* study. J. Physiol. Pharmacol., 56: 101-108.

Yaghmaei P, Parivar k, Doranian D, Hashemi M, Torkaman F (2009). Study on the effect of extremely low frequency electromagnetic fields on some blood serum's lipoproteins, liver enzymes and P 448/P 450 cytochrome enzyme system in NMRI mice. Journal of Paramedical Sciences (JPS).1: 1735-7802.