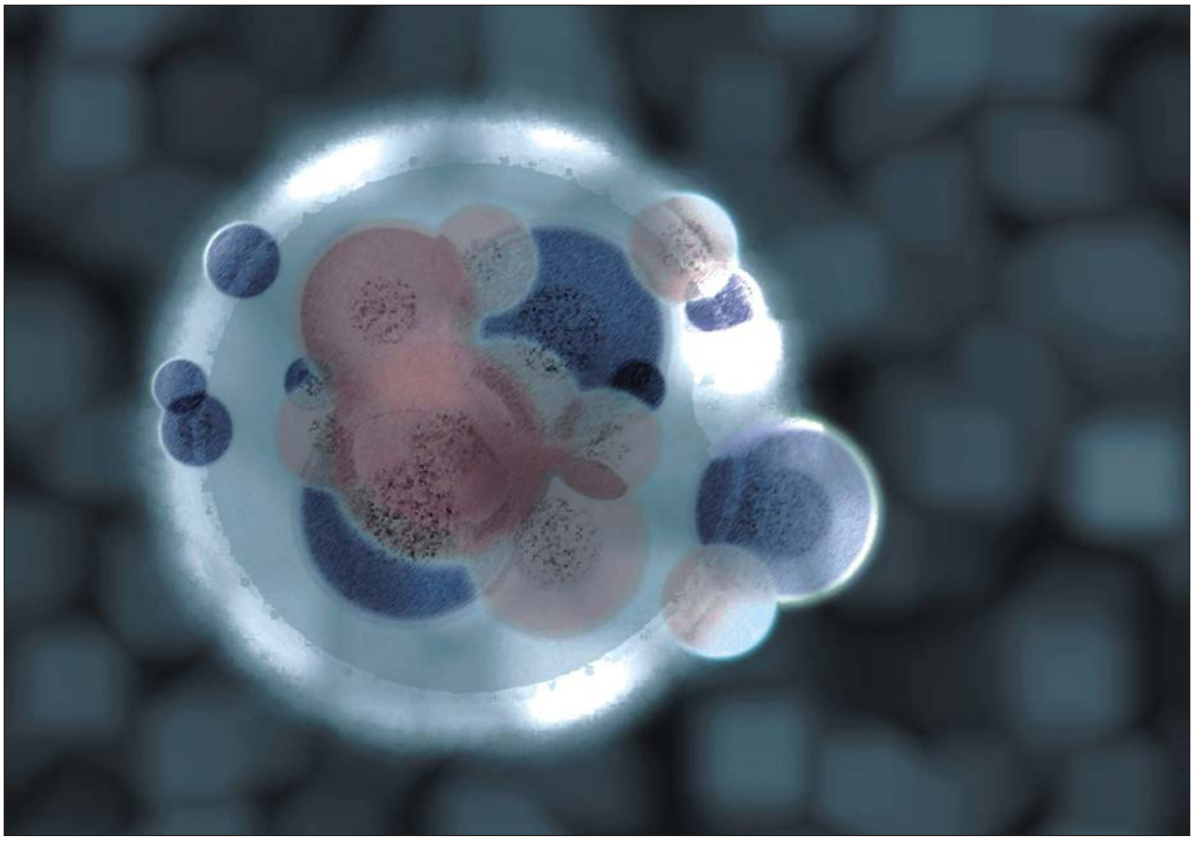

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Ultrastructural Change of Cerebellum in Exposed Rats to 3mT Electromagnetic Field

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Abstract: The aim of this study was to investigate ultrastructural changes of Cerebellum in 3mT electromagnetic field exposed rats. Total 30 adult female Wister rats with 3 months of age and weighing 210±10.6 g were used in this study. All female rats subdivided randomly to 2 groups: group 1, serve as untreated controls; group 2, was exposed to 3mT EMF for 4 months, 4 h day^{-1}. After 120 days all rats were killed and their tissue samples from Cerebellum were removed and prepared for electron microscopic studies. Present finding clearly demonstrated that number of purkinje cells in the cerebellum of EMF- exposed rats were decreased significantly ($p<0.01$) in comparison to control group. The other changes include: condensation of nuclei, dilatation of endoplasmic reticulum, breakdown and disappearance of crista in mitochondria and vacuolization of cytoplasm in the purkinje cells of cerebellum. The mean nuclear diameter in purkinje cells were 45.35±22.85 mm and 26.79±16.36 mm in control and experimental group respectively. The statistical analysis showed that the difference between two group was significant ($p = 0.03$). Axial ratio of nucleus of purkinje in control and experimental groups were 1.86±0.41 and 1.55±0.14 mm, respectively. The axial ratio of nucleus in purkinje of EMF-exposed cerebellum were decreased significantly in comparison to control group ($p = 0.02$). These findings indicate that long-term exposure to EMF has detrimental effects on central nervous system at cellular level.

Key words: Electron microscope, central nervous system, cellular level, Purkinje cells

INTRODUCTION

Human being, in the modern life, is exposed to Electromagnetic Field (EMF) from the generators and other electronic devices and this exposure is harmful for human being. Some harmful effects on biological systems are: teratogenic effects, mutagenic effects, immunological and hematological effects (Johansen, 2004). There are several studies which demonstrate that EMF has a harmful effect on central nervous system (BioInitiative Report, 2010). In the past two decades, many researches have shown that exposure to frequencies of 50-60 Hz and intensity of 2mT increases the risk of cancer (McCann *et al*., 1998; Zmyslony, 2007). However, there are controversial results regarding *in vivo* and *in vitro* the effect of EMF. Some studies have reported that weakness, headache and memory loss can be due to the effects of the EMF of mobile phone. Loscher and Liburdy (1998) reported that there are some relations between decrease of memory and learning in rudimentary after exposure to 2450 MHz of electromagnetic field. Humans are indeed, continuously exposed by home appliances diagnostic tools and industrial instruments with widely varying frequencies (Sul *et al*., 2006). The normal complex structure and functioning of the central nervous system are related to the prenatal and postnatal developments the Purkinje cells. Exposure to EMF during prenatal phase has been shown to cause the morphological change to the offspring and it is speculated that electromagnetic field may trigger alterations in CNS morphology too. The cerebellum is one of the best characterized regions of the brain with respect to the development. It undergoes dramatic developmental changes during the first 3 weeks of postnatal life in the mouse (Goldowitz and Hamre, 1998). Ragbetli *et al*. (2007) reported that purkinje cell development can be used to evaluate the effect of diclofenac sodium on the development of rat cerebellum; a reduced number of Purkinje cells may contribute to abnormal CNS development (Kudo *et al*., 2003; Sotelo, 2004). Rosli and Teoh (2009) recently reported that low EMF can induce irreversible destruction to the brain of adult mice, specifically the reduction of the purkinje cells with the sequel of thinning of the granular layer. The effect appears less remarkable in the offspring which were exposed to EMF during gestation period, as they were born with no obvious physical defects and the regeneration of the cerebellar granular layer in the developing mice masks the sinister Purkinje cells reduction. Previous work in our laboratory, using light microscope have shown that EMF could produce

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morphological changes in Cerebellum (Data not reported). Since ultrasructural studies of the effects of EMF are very rare, this study design to investigate ultrastructural changes of Cerebellum in EMF-exposed rats.

MATERIALS AND METHODS

Animals: This experimental study was performed in Department of anatomy at Tabriz University of Medical Sciences from March 2008 to August 2009. Total 30 adult female Wister rats with 3 months of age and weighing 210±10.6 g were used in this study. These animals were devided to into two groups randomly (15 rats as control group and 15 rats in experimental group). Total Animals housed in laboratory conditions with 28±2°C temperature and subjected to a natural photoperiod. Access to tap water and food was unlimited. All procedures were approved by international guidelines and by the Institute Research Ethics and Animal Care and Use Committee of Ahvaz Jundishapur University of Medical Sciences. Every effort was made to minimize the number of animals used and their suffering.

Electro-magnetic field irradiation and grouping: Irradiation was carried out a 3mT EMF for 4 months, 4 h day^{-1}, superconductive electromagnetic field. Rats in experimental groups were exposed in a perforated in 35×35 cm of the chamber to SMF in the core of electromagnetic field gantry at room temperature (24±1°C) for 30 min (Johansen, 2004; Saki *et al*., 2010).

Preparation of sample for electron microscopic study: For electron microscopy, the testicular specimens were fixed with 2.5% glutaraldehyde in 0.1 M sodium buffer phosphate (pH 7.2) for 3 h at 4°C, washed in the sanie buffer for 1 h at 4°C and post-fixed with 1% osmium tetroxide in sodium phosphate buffer for 1 h at 4°C. The tissues were then dehydrated in graded series of ethanol, starting at 50% each step for 10 min, after two changes in propylene oxide. The tissue specimens were embedded in araldite. After trimming blocks were cut on an ultranicrotome in 60 nm thick sections. These Sections were collected on copper grids separately and stained with uranyl acetate and lead citrate and examined in a LEO906 transitional electron microscope and used shit film for electron micrograph. Morphometric studies were carried out on electron micrographs using measurement and counting techniques (Razie *et al*., 2011).

Statistical analysis: In this study the number of cells in 10 fields from each section counted and then average number of cells in the control and experimental groups determined. The big (a) and small (b) nuclear diameter calculated and by using $d = \sqrt{a.b}$ and $a.b^{-1}$ the mean nuclear diameter (d) axial ratio was determined. The data were analyzed by software SPSS 13.0 and using student t-test. Differences between the means were considered to be significant when $p<0.05$ was achieved.

RESULTS

Present finding clearly demonstrated that number of purkinje cells in the cerebellum of EMF-exposed rats (Fig. 3-6) were decreased significantly ($p<0.01$) in comparison to control group (Fig. 1-3). The other changes

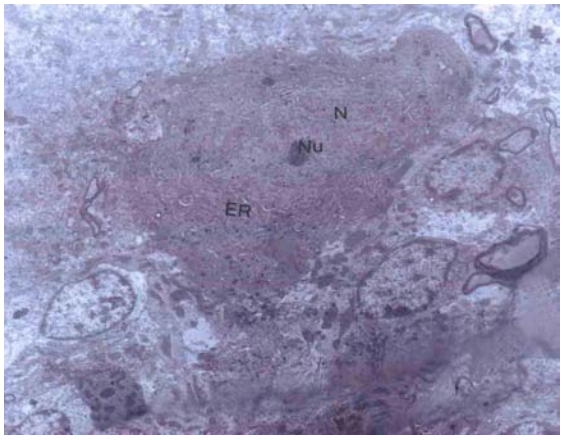

Fig. 1: Electro-micrograph of purkinje cell in the control group. Nuclei (N). Endoplasmic Reticulum (ER). Nucleus (Nu) (magnification x2904)

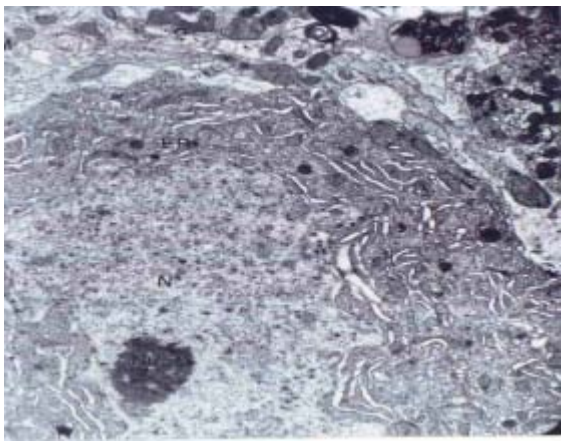

Fig. 2: Electro-micrograph of Purkinje cell in the control group. Nuclei (N). Endoplasmic Reticulum (ER). Nucleus (Nu). (Magnification x6034)

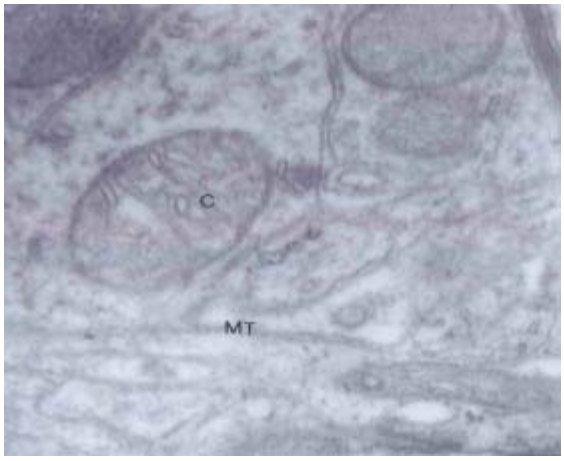

Fig. 3: Electro-micrography of Mitochondria of rat cerebellum in control group Microtubule (MT). crista mitochondria (C). (Magnification x2905)

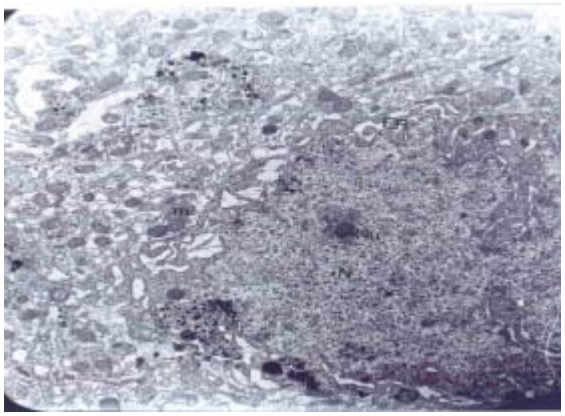

Fig. 5: Electro-micrograph of Purkinje cell in the experimental rat group. Nuclei (N), endoplasmic Reticulum (ER) Mitochondria (m) Nucleus (Nu), (Magnification x6000)

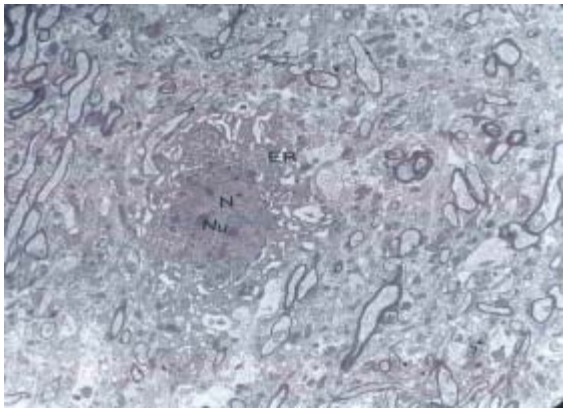

Fig. 4: Electro-micrograph of Purkinje cell in the experimental rat group. Nuclei (N). Endoplasmic Reticulum (ER). Nucleus (Nu). (Magnification x2704)

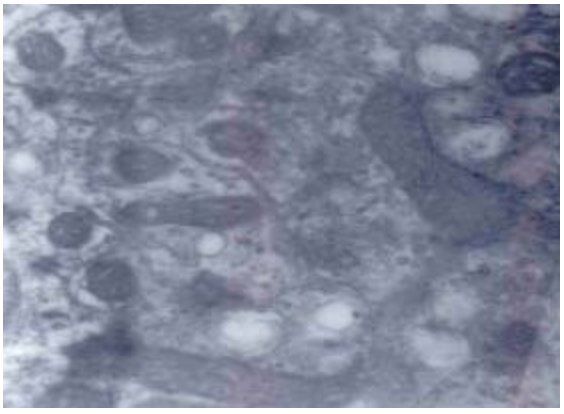

Fig. 6: Electro-micrograph of Mitochondria of rat Cerebellum in the experimental group (Magnification x2800)

morphological include: condensation of nuclei, dilatation of endoplasmic reticulum, breakdown and disappearance of crista in mitochondria and vacuolization of cytoplasm in the purkinje cells of Cerebellum (Fig. 4-6). The mean nuclear diameter in purkinje cells were 45.35±22.85 and 26.79±16.36 mm in control and experimental group, respectively. The statistical analysis showed that the difference between two groups was significant ($p = 0.03$). Axial ratio of nucleus of purkinje in control and experimental groups were 1.86±0.41 and 1.55±0.14 mm, respectively. The axial ratio of nucleus in purkinje of EMF-exposed cerebellum was decreased significantly in comparison to control group ($p = 0.02$).

DISCUSSION

Rapid advances in the Electromagnetic Field (EMF) technologies and communications have greatly increased the human populations' exposure to EMFs. The findings of this study show that cellular size of the Purkinje cells in cerebellum of 3mT electromagnetic field exposed rats significantly decreased in compare to control groups' cerebellum. This finding supported previous report of Rosli and Teoh (2009). The morphologic change can be because of decreased activity of nucleus and its result is decreasing activity of the cell. These findings reinforcement other studies which shown that EMF can decrease the DNA regeneration (Sul *et al*., 2006) and

affected the cellular genome (Manti and D'Arco, 2010; Lai and Singh, 2004; Maskey *et al*., 2009; Lisi *et al*., 2000). EMF can affect cell membrane integrity, glycoprotein and cellular actions, intra cellular enzymes, cytoskeleton and nucleus (Maskey *et al*., 2009; Lisi *et al*., 2000; Bordiushkov *et al*., 2000; Mausset *et al*., 2001; Goodman *et al*., 1995). This finding can be confirmed with this fact that neurotransmitters, for example, GABA in Purkinje cells decrease as a result of exposure to the EMF. (Mausset *et al*., 2001; Wang *et al*., 2005).

In present study, we observed that the number of Purkinje cells decreased in experimental group of study. The decrease in number of purkinje cells may due to harmful affects of EMF on scheduling death of cells and their apoptosis (Li and Wong, 2000; Fanelli *et al*., 1999). It has showed that EMF has irreversible effects on cellular immigration and their differentiation in cerebellar cortex. (Lisi *et al*., 2005). EMF has determinately effect in the suppression affect which my lead to increase the harmful effects of EMF by increase the free radical, whatever had destructive effects on the cells and tissues. The present study revealed the harmful effects of EMF on the cerebellum tissue in rats. At the molecular level EMF produces biological stress and free radical, which can make the susceptible animal population prone to congenital malformation, tissue and cell damage or death (Soeradi and Tadjudin, 1986; Wolf *et al*., 2005) and free radicals can cause oxidative stress at the cellular level, interfering with protein synthesis. These elements also play an important role in acute inflammation, endothelial destruction, resulting in tissue edema. It has been postulated that EMF-exposure produces high levels of oxidative stress as a result of its effect on the immune response (Zhitkevich *et al*., 2001) and long-term exposure to EMF may be linked to even higher levels of oxidative stress (Zmyslony, 2003). These findings indicate that long-term exposure to EMF has a detrimental effects on cerebellum at cellular level. These findings indicate that long-term exposure to EMF has a detrimental effect on central nervous system at cellular level.

In summary our findings showed that prolonged exposure to EMF with the power of 3mT can effect on cerebellum cells in this ways: hetrochromatisation of the nuclei in cells, dilation of membranous organelles (mitochondria, endoplasmic reticulum) and decreased number and size of Purkinje cells.

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