

Selenium supplementation ameliorates electromagnetic field-induced oxidative stress in the HEK293 cells[☆]

Nural Pastacı Özsoğacı^{a,*}, Dilek Düzgün Ergün^b, Sinem Durmuş^c, Matem Tunçdemir^d, Hafize Uzun^c, Remise Gelişgen^c, Derviş Özçelik^a

^a Biophysics Dept., Cerrahpaşa Medical Faculty, Istanbul University, Istanbul, Turkey

^b Biophysics Dept., Faculty of Medicine, Istanbul Aydın University, Istanbul, Turkey

^c Biochemistry Dept., Cerrahpaşa Medical Faculty, Istanbul University, Istanbul, Turkey

^d Medical Biology Dept., Cerrahpaşa Medical Faculty, Istanbul University, Istanbul, Turkey

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ABSTRACT

There is a widespread use of 2.4 GHz electromagnetic radiation emitting devices especially in communication and education. Recent studies show the adverse effects of electromagnetic fields (EMF) such as oxidative stress, cellular damage and apoptosis on tissues. Selenium (Se) has an antioxidant properties by inhibiting oxidative damage being within the structure of antioxidant enzymes like glutathione peroxidase (GSH-Px) and it has also regulatory function for cell cycle and apoptosis. The aim of this study was to investigate the effect of Se on 2.4 GHz frequency EMF exposed human embryonic kidney cells (HEK293) by means of alterations in apoptotic and oxidative stress parameters.

Our study was planned as control, EMF, 100 nM Se + EMF, 200 nM Se + EMF groups. EMF groups were exposed to 2.4 GHz EMF for 1 h, element groups were incubated with two different doses of Se added cell culture medium for 48 h before EMF exposure.

MDA levels were significantly higher whereas SOD and GSH-Px activities were significantly lower in EMF compared to control. 100 and 200 nM Se + EMF application decreased MDA levels, increased SOD and GSH-Px activities than EMF. Apoptosis and caspase-3 were statistically significantly higher but bcl-2 was lower in EMF than control. Apoptosis and caspase-3 were lower in 100 and 200 nM Se + EMF, although bcl-2 were higher than EMF.

In conclusion, Se has protective effects against 2.4 GHz EMF-induced oxidative stress by reducing lipid peroxidation, regulating SOD and GSH-Px activity. Also, Se has inhibitory effect on 2.4 GHz EMF induced apoptosis by increasing the expression of anti-apoptotic protein bcl-2 and suppressing apoptosis regulatory protein caspase-3.

1. Introduction

Wireless local area network (WLAN) emerged in late of 1990's and it is used in many areas especially in communication as parallel to technological progress. The communication is provided by electromagnetic radiation, thus societies are increasingly exposed to low frequency electromagnetic fields (EMF) [1–4].

In recent years, in vivo, in vitro and epidemiological researches have focused on the investigation of biological effects of EMF [1–3,5,6]. Studies have ascertained that EMF changes potentials of cells and

organelles via affecting enzymes, ion channels, membrane receptors and causes some diseases [3,7,8]. The adverse biological effects of EMF on tissues associated with oxidative stress which is generated reactive oxygen species (ROS) [3,9]. Thus it is indicated that EMF causes apoptosis by acting on (ROS) [2,9]. Although the positive correlation between ROS and EMF demonstrated with different experimental protocols, but the cellular and molecular mechanisms underlying this issue are under research [2,10,11].

In the human metabolism, there are enzymatic and nonenzymatic antioxidant systems that scavenge the harmful effects of ROS. Selenium

[☆] The study was carried out at cell culture laboratory at Biophysics Department of Cerrahpaşa Medical Faculty. Biochemical parameters and morphological analyzes were performed at laboratories of Biochemistry and Medical Biology Departments of Cerrahpaşa Medical Faculty.

* Corresponding author at: Cerrahpaşa Medical Faculty, Biophysics Dept., Fatih, 34098 Istanbul, Turkey.

E-mail addresses: nuralp@istanbul.edu.tr (N. Pastacı Özsoğacı), dilekergun@aydin.edu.tr (D. Düzgün Ergün), sinem.durmus@istanbul.edu.tr (S. Durmuş), tmatem@istanbul.edu.tr (M. Tunçdemir), uzun@istanbul.edu.tr (H. Uzun), remisa.gelisgen@istanbul.edu.tr (R. Gelişgen), dozcelik@istanbul.edu.tr (D. Özçelik).

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(Se) is an essential antioxidant element, which has a critical importance for cellular functions [12]. The antioxidant properties of Se associated with being the cofactor for glutathione peroxidase (GSH-Px). Se combines with proteins to create selenoproteins which prevent cellular damage from free radicals. Studies on different cells have shown that Se homeostasis modulates cell proliferation and apoptosis. The efficacy of Se is to be associated with its chemical formula and its dose [12–15].

The number of publications for investigating the effects of EMF on tissues are focused on brain and testicular tissues, but the number of literature investigating the effect in kidney tissue is very limited [1,16,17]. The aim of this study is to investigate the effect of selenium on 2.4 GHz frequency EMF exposed human embryonic kidney cells (HEK293) by means of alterations in apoptotic and oxidative stress parameters.

2. Materials and methods

2.1. Cells and chemicals

The human embryonic cell line (HEK293) was purchased from the American Type Culture Collection (ATCC®, Manassas, VA, USA). Dulbecco's Modified Eagle Medium (DMEM), Phosphate Buffered Saline (PBS), fetal bovine serum (FBS), dimethyl sulfoxide (DMSO) trypsin – EDTA, penicillin-streptomycin, proteinase K and tris base powder were obtained from Wisent, (Quebec, Canada). Phenylmethylsulfonyl fluoride (PMSF), thiobarbituric acid (TBA), trichloroacetic acid (TCA), protease inhibitor cocktail, ethylenediaminetetraacetic acid (EDTA), sodium selenite, xanthine oxidase, nitro blue tetrazolium (NBT), bovine serum albumin (BSA) were acquired from Sigma (Taufkirchen, Germany). Hydrogen peroxide (H₂O₂), paraformaldehyde, Triton X – 100, entellan, hematoxylin, zinc sulphate acquired from Merck (Darmstadt, Germany). Apoptag Plus In Situ Apoptosis Detection Peroxidase Kit and anti-caspase 3, active form were obtained from Merck Millipore (Darmstadt, Germany). AEC substrate kit and histostain-plus bulk kit were obtained from Invitrogen by Life Technologies (California, USA). Bcl-2 antibody was purchased from Novus (Abingdon, United Kingdom) and Glutathione Peroxidase Activity Kit was obtained from Enzo (New York, USA).

2.2. Cell culture

Cells were proliferated in DMEM supplemented with 10% (v/v) heat-inactivated FBS, and 1% (v/v) penicillin/streptomycin at 37 °C under a humidified atmosphere of 5% CO₂. In all experiments, HEK293 cells were grown in plastic tissue culture flasks and tissue culture plates. Cell passages were maintained between 5 and 15 for all experiments, and medium was changed every 2 days. Cells were monitored using light microscopy during test.

2.3. Exposure system and design

A voltage-controlled oscillator (Feedback, England, 532/VCO/0138) and monopole antenna is used for 2.4 GHz frequency EMF exposure system. The voltage controlled oscillator (Feedback, England, 532/VCO/0138) was connected with two direct voltage sources (Yıldırım, AC/DC Switching Power Supply, CYS 152) which output 15 V as constant voltage and 3.3 V as variable voltage. The output signal was controlled by a spectrum analyzer (Rohde&Schwarz). The oscillator output power was measured as 2.6 dB m and electric field intensity was measured as 8 V/m at the bottom of the antenna, – 40 dBm and 2 V/m was measured within cell culture flasks. Distance was ordered as 18 cm between the culture flasks and antenna of the exposure system. The exposure system created with plexiglass equipment to prevent possible reflections of radiation (Fig. 1). This system put in a shielded cage which exterior surface coated with aluminum and interior surface coated with EMF absorber material (Fig. 2).

2.4. Experimental procedure

The cells were equally divided into four main groups; control, EMF, 100 nM Se + EMF, 200 nM Se + EMF. At the EMF groups, the cells were exposed to 2.4 GHz frequency EMF for 1 h and at the Se groups, the cells were incubated with a complete medium which prepared by adding 100 nM and 200 nM sodium selenite for 48 h before EMF exposure.

Cells were seeded in flasks at a density of 1×10^6 cells/T25 flask for measurement of oxidative stress parameters. For measurement of biochemical markers, cell lysates were prepared after the experimental procedure. On the other hand, cells were seeded on to coverslips which placed in to the wells of cell culture plate at a density of 15×10^4 cells/well. After the experimental application cells were fixed with 4% paraformaldehyde for performing morphological assessment.

2.5. Measurement of malondialdehyde (MDA) levels

MDA levels were measured by thiobarbituric acid method of Buege et al. [18]. This spectrophotometric measurement is based on the generated color by the reaction between MDA and thiobarbituric acid (TBA) at 90–95 °C. The levels of MDA were determined by using the absorbance coefficient of the MDA–TBA complex at 535 nm by spectrophotometer. The MDA levels of the cells were expressed as nmol/ml.

2.6. Measurement of superoxide dismutase (SOD) activity

SOD enzyme activity was determined by the method of Sun et al. [19]. This method is based on the inhibition of nitroblue tetrazolium (NBT) reduction by superoxide radicals which produced by xanthine/xanthine oxidase systems. The resultant color was measured at 560 nm on a spectrophotometer and SOD activities were expressed as U/ml.

2.7. Measurement of glutathione peroxidase (GSH-Px) activity

GSH-Px activity of HEK293 cells was measured with commercial Glutathione Peroxidase Activity Kit. According to the manufacturer's instruction, GSH-Px activity was assessed by reading the decrease in absorbance value of NADPH at 340 nm for 15 min at intervals of 1 min on a spectrophotometer. The results were obtained in U/ml.

2.8. Detection of apoptosis

The apoptosis assay was performed by the terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) method based on in situ detection of DNA fragments with a commercial kit (Apoptag Plus *in situ* Apoptosis Detection Peroxidase Kit-Millipore, USA). The manufacturer's instruction was optimized and applied some changes for the measurement. TUNEL sections were examined under light microscope (Leica DM 2500) interfaced with a camera (Leica, DFC 280). Ten different fields on each slide were randomly selected at $\times 20$ magnification on a light microscope. The apoptotic index (APOI) was calculated by counting stained apoptotic (brown stain) and normal (green stain) cells.

2.9. Immunohistochemistry for active caspase-3 and Bcl-2 staining

HEK293 cells fixed on to the coverslips with 4% paraformaldehyde for immunohistochemical staining of active caspase-3 and bcl-2. Immunohistochemical staining was performed with Streptavidin-biotin-peroxidase method by using active anti-caspase-3 (polyclonal antibody, AB3623, Millipore, 1:50 dilution) and bcl-2 (monoclonal antibody, NB110-55551, Novus Biotechnology, 1:50 dilution) antibodies. Immunostaining procedures were carried out in the Histostain-Plus Bulk Kits (Invitrogen 2nd Generation LAB-Sa Detection System) with modified according to the antibodies. The cell cytoplasm stained red by

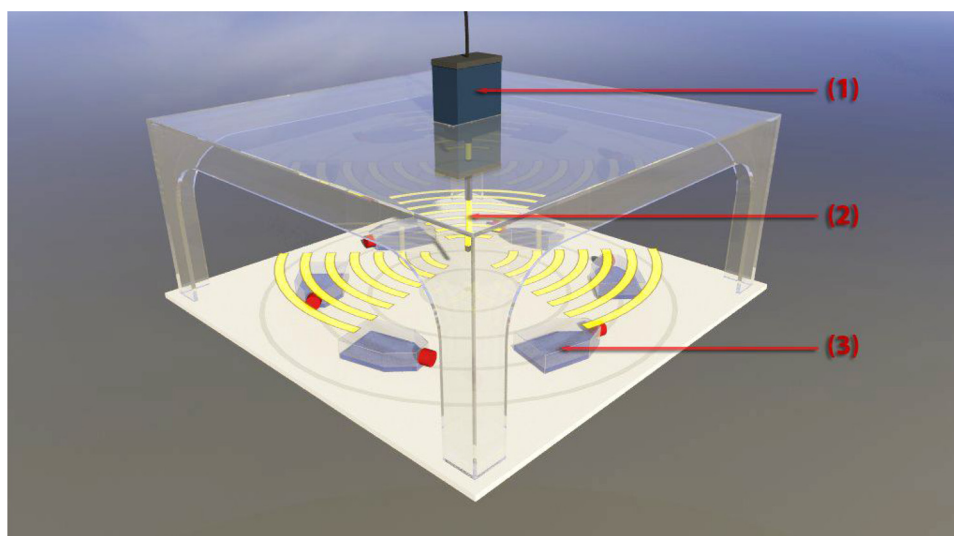


Fig. 1. Schematic diagram of 2.4 GHz EMF exposure device.
(1) GHz EMF oscillator; (2) Monopole antenna; (3) Cell culture flasks.

active caspase-3 and bcl-2 antibody in immunohistochemical staining, these cells were regarded as caspase-3 and bcl-2 immunopositive cells. Statistical evaluation was performed by counting immunopositive cells in randomly selected 10 fields at $\times 20$ magnification on a light microscope.

2.10. Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows version 20.1 (SPSS Inc. Chicago, IL, USA). Data were assessed by one-way ANOVA following appropriate transformation to normalized data and equalized variance where necessary. All the analyses were assumed statistically significant at $p < 0.05$. Data were expressed as the mean (M) $\pm$ standard deviation (SD).

3. Results

3.1. Biochemical parameters

The mean levels of oxidative stress parameters in control, EMF, 100 nM and 200 nM Se + EMF groups were given in Table 1. The

results showed that 2.4 GHz frequency EMF exposure significantly increase MDA levels of HEK293 cells than control group ($p < 0.001$). However, MDA levels of 100 nM and 200 nM Se + EMF groups were significantly higher than EMF group ($p < 0.05$; $p < 0.001$; respectively).

In the present research, when we compared the values of SOD activity, there was a significant decrease between EMF group and control group ($p < 0.05$). Besides, Se (100 nM and 200 nM) + EMF groups were significantly higher values of SOD activity than EMF group ($p < 0.05$).

Similarly, GSH-Px activity of the control group was significantly lower versus EMF group ($p < 0.05$). Significant increment was also determined between 100–200 nM Se + EMF groups and EMF group at GSH-Px activity ($p < 0.05$).

3.2. TUNEL and immunohistochemistry assay

APOI values and immunopositivity of bcl-2 and caspase-3 were given in Table 2. The number of apoptotic cells were significantly increased in EMF group according to control group ($p < 0.001$). The apoptotic cell number at the different doses of Se (100–200 nM) incubated groups were significantly decreased than EMF group

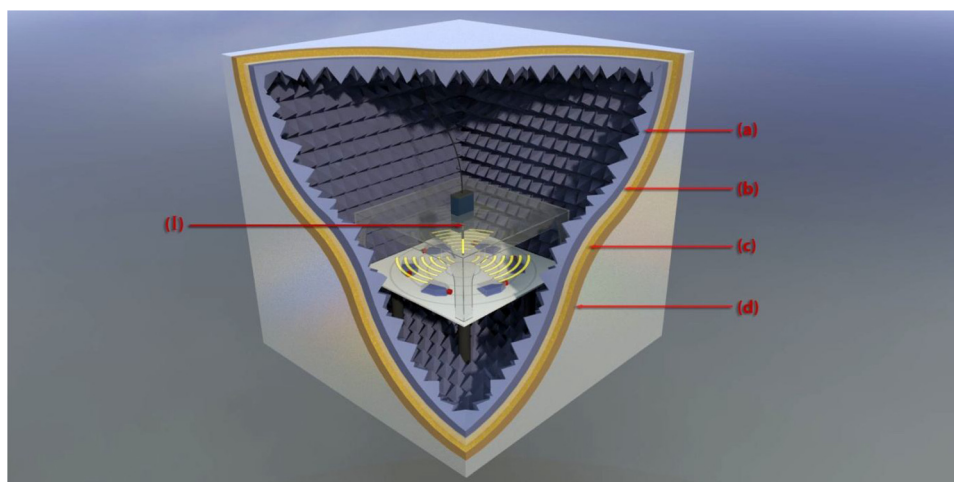


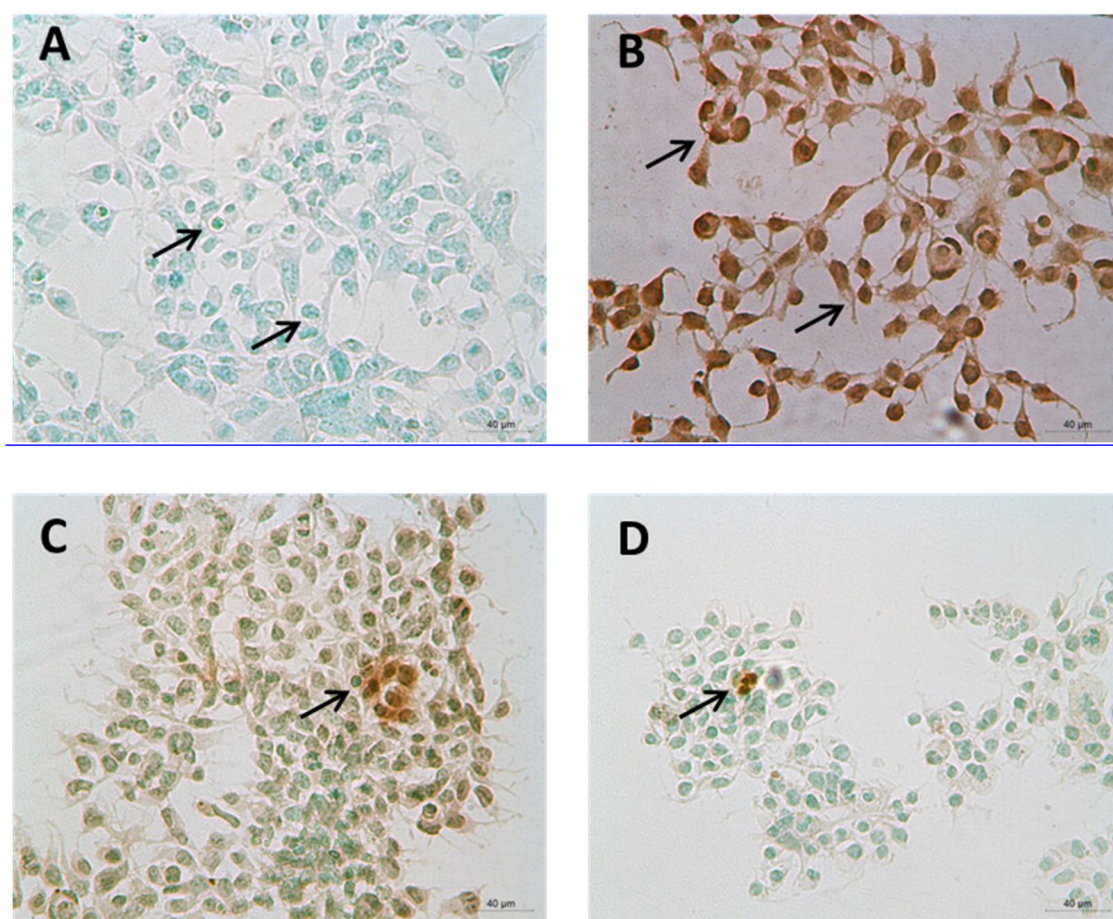
Fig. 2. Schematic view of EMF exposure system.
(1) GHz EMF oscillator; (a) EMF absorber surface (b,d) Aluminum surface (c) Wooden cabine.

Table 1Biochemical parameters of control and EMF exposed HEK293 cells. (Mean $\pm$ SD).

Parameter	Control	EMF	100 nM Se + EMF	200 nM Se + EMF
MDA (nmol/ml)	0.78 $\pm$ 0.1	2.16 $\pm$ 0.0 ^{a,***}	1.97 $\pm$ 0.1 ^{b,*}	1.58 $\pm$ 0.08 ^{b,***}
SOD (U/ml)	0.27 $\pm$ 0.04	0.14 $\pm$ 0.0 ^{a,*}	0.38 $\pm$ 0.07 ^{b,*}	0.32 $\pm$ 0.05 ^{b,*}
GSH-Px (U/ml)	21.7 $\pm$ 4.1	14.4 $\pm$ 0.4 ^{a,*}	21.3 $\pm$ 3.3 ^{b,*}	22.8 $\pm$ 3.8 ^{b,*}

^a Statistical comparison of control with EMF, 100 nM Se + EMF and 200 nM Se + EMF.^b Statistical comparison of EMF with 100 nM Se + EMF, 200 nM Se + EMF.* $p < 0.05$.*** $p < 0.001$.**Table 2**Immunohistochemical effects of different doses of Se on EMF exposed HEK293 cells. (Mean $\pm$ SD).

Parameter	Control	EMF	100 nM Se + EMF	200 nM Se + EMF
APOI	4.51 $\pm$ 1.4	77.5 $\pm$ 22 ^{b,***}	22.5 $\pm$ 2.9 ^{b,***}	8.10 $\pm$ 4.6 ^{b,***}
Bcl-2 (+)	21.2 $\pm$ 5.3	5.2 $\pm$ 2.8 ^{a,***}	26 $\pm$ 9.3 ^{b,***}	18 $\pm$ 5.1 ^{b,***}
Caspase-3 (+)	7.6 $\pm$ 3.6	17.4 $\pm$ 1.2 ^{b,*}	3.8 $\pm$ 1.5 ^{b,***}	1.4 $\pm$ 1.4 ^{b,***}

^a Statistical comparison of control with EMF, 100 nM Se + EMF and 200 nM Se + EMF.^b Statistical comparison of EMF with 100 nM Se + EMF, 200 nM Se + EMF.* $p < 0.05$.** $p < 0.01$.*** $p < 0.001$.**Fig. 3.** TUNEL assay of apoptotic HEK 293 cells.(A) Control group, (B) EMF group, (C) 100 nM Se + EMF group, (D) 200 nM Se + EMF group. Contrast staining: Methyl Green (Scale bar 40 μ m), TUNEL immunopositive cells ($\rightarrow$).

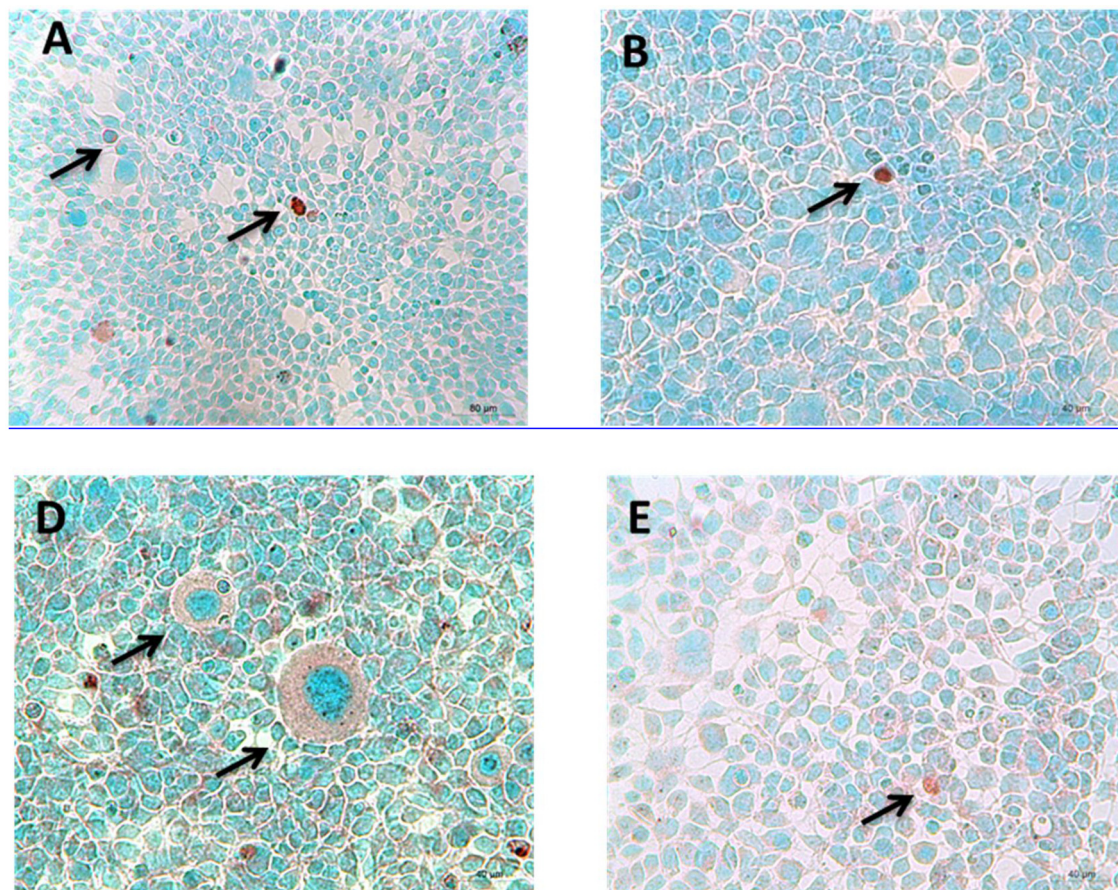


Fig. 4. Bcl-2 immunohistochemical staining of HEK 293 cells.

(A) Control group, (B) EMF group, (C) 100 nM Se + EMF group, (D) 200 nM Se + EMF group. Contrast staining: Methyl Green (Scale bar 40 μ m). Bcl-2 immunopositive cells ($\rightarrow$).

($p < 0.001$) (Fig. 3, Table 2).

Bcl-2 immunopositivity was observed to be statistically lower in 2.4 GHz frequency EMF group than control group ($p < 0.001$). On the other hand, 100 nM Se + EMF and 200 nM Se + EMF groups bcl-2 immunopositivity was significantly higher than EMF group (respectively; $p < 0.001$, $p < 0.01$) (Fig. 4, Table 2).

The number of caspase-3 (+) cells was significantly higher in EMF group than control group ($p < 0.05$). In 100–200 nM Se + EMF groups the caspase-3 immunopositivity were statistically significantly lower versus EMF group ($p < 0.001$) (Fig. 5, Table 2).

4. Discussion and conclusion

Electromagnetic wave emitting devices with 2.4 GHz frequency are widely used for communication in many public areas and the level of 2.4 GHz frequency EMF in our environment increase day by day [20]. So that people are exposed to EMF at a harmful level due to the power densities caused by the devices. In their study of oxidative stress and metabolic impairments induced with 2.45 frequency Wi-Fi signal, Salah et al. [3] determined that EMF decreased GSH-Px, SOD and CAT activity levels and increased MDA levels in renal and liver tissues in rats. Shahn et al. [20] stated in their study on mice that 2 h/day for 45 days EMF increased ROS and decreased SOD, CAT and GSH-Px activities. Another a similar study, Ozguner et al. [21] determined that MDA and nitric oxide increased and SOD, CAT and GSH-Px activity decreased in 900 MHz EMF exposed rat kidney tissue. Many studies were pointed out that electromagnetic radiation (EMR) induce free radicals, followed by decrement of mitochondrial energy, increment of proteolytic enzymes and DNA fragmentation resulted from cellular apoptosis

[3,9,10]. Children and pregnant women may be also damage by these adverse effects of EMF. Studies clearly showed that EMF applied in the prenatal period was effective on various organs [5].

Since people could not control their environmental exposure to EMF, researchers are focused on activate our defense system for protecting our body to environmental stresses. For this reason, in this study we aimed to evaluate the effects of EMF on human kidney embryonic cells and protectively of Se against 2.4 GHz EMF exposure. Turker et al. [10] and Gumral et al. [22] analyzed the protective effect of sodium selenite on oxidative stress in rats induced with 1 h for 28 days 2.45 GHz frequency EMR on heart tissue, plasma and erythrocytes. In consistent with our study, the same frequency EMF was shown to increase lipid peroxidation, to decrease GSH-Px activity; and Se exposure was shown to decrease lipid peroxidation and to increase GSH-Px activity.

In our study, the efficacy of Se on 2.4 GHz frequency EMF exposed HEK293 cells was investigated biochemically and morphologically. The significant increase in MDA levels by 2.4 GHz frequency EMF was found to be significantly reduced by 100 nM Se application and when 200 nM Se was applied the significance was found to be higher. In the case of SOD and GSH-Px enzyme activities, EMF decreased significantly SOD and GSH-Px activities, whereas incubation with Se before the application of EMF, increased significantly this reduction.

Merola et al. [23] determined that some cellular phones had higher values compared with border values of International Commission of Non-Ionizing Radiation Protection and this value affected over apoptosis in neuroblastoma cells. Esmekaya et al. [24] who observed the effect of 900 MHz frequency EMF on rat thyroid tissue morphologically, stated that 20 min/day for 3 weeks EMF exposure increased caspase-3

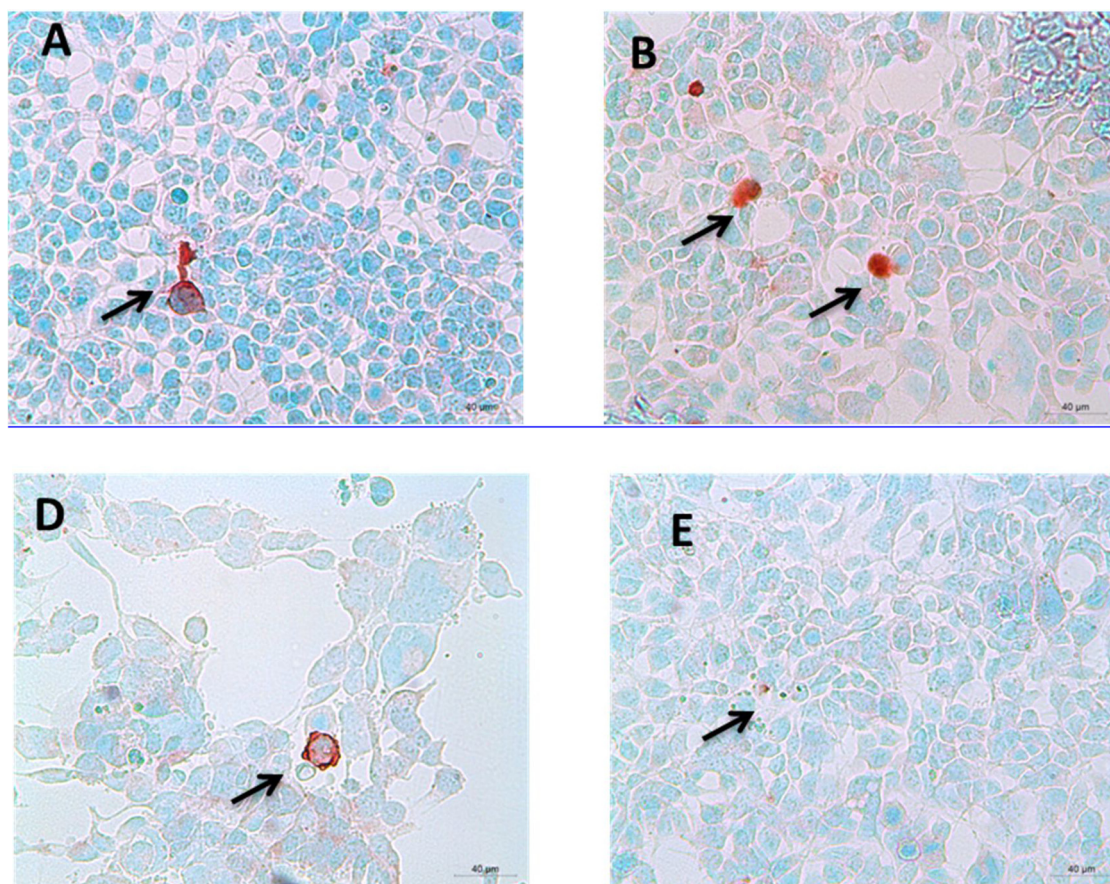


Fig. 5. Active caspase-3 immunohistochemical staining of HEK293 cell.

(A) Control group, (B) EMF, (C) 100 nM Se + EMF group, (D) 200 nM Se + EMF group. Contrast staining: Methyl Green (Scale bar 40 µm). Active caspase-3 immunopositive cells (→).

and caspase-9 activity and apoptotic bodies formation. Oral et al. [11] propounded in their study that 900 MHz EMF increased lipid peroxidation in rat endometrium tissue. The increment of caspase-3, caspase-8 and bax was observed in the immunohistochemical evaluation of this study. Guler et al. [2] exposed 1800 MHz frequency EMF on pregnant and non-pregnant rabbits, apoptotic cells were determined with TUNEL staining only in brain tissue, anterior epithelial cells of the eye and lens of the eye in pregnant, non-pregnants and infants. Ait-Aissa [17] exposed pregnant rats to Wi-Fi signals to produce 0, 0.08, 0.4 and 4 W/kg SAR, which were the critical values accepted by ICNIRP. When apoptosis was analyzed with TUNEL method in rat brain, there was no difference in rat brain which exposed to EMF in pre- and post-natal period.

Besides the analysis of biochemical parameters, the effects of EMF exposure on HEK293 cells and Se incubated cells before EMF, apoptotic activities were also analyzed. Furthermore, anti-apoptotic protein bcl-2 and apoptosis regulatory protein caspase-3 assay was held utilizing immunohistochemical staining on HEK293 cells in order to investigate apoptosis pathways. It has been observed that exposure to 2.4 GHz frequency EMF in HEK293 cells induced the increase of apoptotic cells significantly. Bcl-2 immunopositivity decreased significantly and caspase-3 immunopositivity increased statistically after exposure of 2.4 GHz frequency EMF in HEK293 cells.

Se is a major antioxidant with being a cofactor of GSH-Px. Beside that, in experimental studies, apoptosis induction or inhibition was related with dose of Se. Low doses of Se decreases apoptosis induced by oxidative stress, however high doses increase the radical formation resulting with apoptosis [25–27]. Studies were showed that high concentrations of Se increase apoptosis in cells parallel to the increase in lipid peroxidation, thereby preventing cancer cells from growing.

Besides, low concentration of Se decreases lipid peroxidation so that cell counts increase [26,28,29].

We also studied the application of different Se concentrations related with the prevention of oxidative stress dependent apoptosis in EMF exposed HEK293 cells, which we did not encounter in the literature. Apoptotic cell count increased significantly with 2.4 GHz frequency EMF, but Se incubation before 2.4 GHz frequency EMF decreased apoptotic cell count significantly. It was determined that exposure of 2.4 GHz frequency EMF significantly decreased bcl-2 and increased caspase-3 immunopositivity. 100–200 nM Se incubation before EMF exposure increased bcl-2 immunopositivity significantly. Beside that, the increased caspase-3 immunopositivity in the EMF-exposed group was significantly reduced in the 100–200 nM Se-treated before EMF exposed groups.

Uguz et al. [14] determined that Se incubation regulated oxidative stress induced apoptosis over caspase-3 in HL-60 cells. Kahya et al. [13] observed the protective effect of Se on metastatic mammary cancer cells which exposed to 900 MHz frequency EMF for 1 h. In the study, cell vitality, ROS production, mitochondrial membrane depolarization, apoptosis, caspase-3 and caspase-9 were evaluated. Se inhibited EMF induced oxidative damage and increased cell vitality by diminishing oxidative stress and normalizing mitochondrial potentials.

According to the result of our study, incubation with Se may be stated as a protective agent for HEK293 cells that decreases caspase-3 and increases bcl-2 against apoptosis induced by 2.4 GHz frequency EMF exposure.

Limited data about the harmful effects of EMF on fetal and postnatal renal tissue, have revealed the possible developmental anomalies such as cystic formations located in tubulus, glomerulus and renal

parenchyma [30,31].

Different results were reported in studies that analyzed EMF effects on renal tissues. These various results may be related with different factors like gender, exposure methods and exposure time. Studies investigating possible side effects of EMF on pregnant women and also fetus are increasingly continuing today. Few articles have been published reporting the effects of EMF on fetus [32–34].

Increase of oxidative stress in cells causes oxidation of macromolecules and these are associated with DNA mutations, aging and cell death. Moreover, oxygen radical formations play a significant role in the release of apoptosis-regulating proteins such as caspases [35]. The critical balance between apoptotic and anti-apoptotic factors is crucial for embryologic development of renal diseases and cancers [36–38].

An experimental study reported that low frequency EMF exposure caused DNA double-strand breaks and increased bcl-2 expression [39]. Thus, the balance between cell proliferation and cell death was suggested to deteriorate after EMF exposure. Previous studies determined that exposure of 50–60 Hz frequency EMF had toxic effects over brain embryonic cells by increasing apoptotic cell count [40].

As a result, in our study the HEK 293 cells exposure to EMF at the frequency of 2.4 GHz was investigated by determining the alterations on oxidative stress, apoptotic index and immunohistochemistry. 2.4 GHz frequency EMF exposure revealed that increase of caspase-3 immunopositivity and decrease of bcl-2 immunopositivity as apoptosis markers are consistent with previous results on different embryonic cells.

However, incubation with different doses of Se on HEK293 cells before EMF exposure increase of bcl-2 immunopositivity, decrease of caspase-3 immunopositivity and thereby decrease in apoptotic index are important results of our study.

The protective effect of Se against apoptosis related with EMF induced oxidative stress, may be because of anti-oxidant features of Se and its cofactor situation in antioxidant enzymes. According to our results, the exposure of 2.4 GHz EMF on HEK293 cells caused oxidative stress by increasing lipid peroxidation and decreasing antioxidant enzymes SOD and GSH-Px activity. Moreover, Se provide protective effects against 2.4 GHz EMF-induced oxidative stress by reducing oxidative stress and lipid peroxidation, regulating SOD and GSH-Px activity. We can also say that Se have inhibitory effect on 2.4 GHz EMF-induced apoptosis by increasing the expression of apoptotic regulatory protein bcl-2 and suppressing pro-apoptotic protein caspase-3. Se incubation before EMF exposure was concluded to be protective against deterioration of caspase-3 and bcl-2 regulation.

Conflict of interest

None.

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