



Protective Effects of Zinc on 2.45 GHz Electromagnetic Radiation-Induced Oxidative Stress and Apoptosis in HEK293 Cells

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Abstract

Several epidemiological studies have shown that exposure to electromagnetic radiation (EMR) can be harmful to human health. The purpose of this study was to examine oxidative parameters and apoptosis induced by EMR in human kidney embryonic cells (HEK293) and to investigate whether zinc (Zn) has protective effect on EMR-induced apoptosis in HEK293 cells. For our experiment, HEK293 cells were divided into four main groups, control, EMR, 50 μ M Zn + EMR, and 100 μ M Zn + EMR. HEK293 cells of EMR groups were exposed to 2.45 GHz EMR for 1 h. In Zn groups, HEK293 cells were incubated with different concentrations of Zn for 48 h before EMR exposure. Oxidative stress parameters were determined by spectrophotometric method; bcl-2 and caspase-3 were assessed immunohistochemically and TUNEL method was performed for apoptotic activity. EMR group had higher malondialdehyde (MDA) level and lower superoxide dismutase (SOD) activity compared with control group. In Zn-applied groups, MDA was decreased and SOD activity was increased compared with EMR group. The number of the apoptotic cells and caspase-3 immunopositive cells at EMR group was increased significantly compared with the control group, whereas bcl-2 was decreased. Besides, Zn-treated groups showed a significant reduction in the number of apoptotic cells and caspase-3 from that of EMR group, whereas there was an increase in bcl-2 immunopositivity. Our findings show that EMR caused oxidative stress and apoptotic activation in HEK293 cells. Zn seems to have protective effects on the EMR by increasing SOD activity and bcl-2 immunopositivity, decreasing lipid peroxidation and caspase-3 immunopositivity.

Keywords Zinc · Electromagnetic radiation · Apoptosis · Oxidative stress · HEK293

Introduction

Humans are increasingly exposed to electromagnetic radiation (EMR) which is generated from high power lines, TV and radio transformers, radars, base stations, mobile communication, and personal computers. Especially the widespread usage of

wireless technologies (Wi-Fi) has increased environmental EMR levels over the last years. As a result of this, EMR exposure has become more common in houses, workplaces, schools, and public areas without our awareness. However, wireless communication provides big comfort in our daily life, and there is much concern about its possible health effects [1–3].

Most of the recent epidemiological and experimental (-in vivo/in vitro) studies have indicated that acute or chronic exposure of EMR in different frequency ranges may increase some diseases such as cancer, immunological disorders, blood brain barrier damage, headache, neuropsychiatric effects, and endocrine changes [3, 4]. Especially repeated studies reported the hazardous effects of Wi-Fi on kidney and testicular tissues due to usage of wireless devices like laptop computers and cell phones, near kidney and reproductive organs [3, 5]. The adverse biological effects of EMR are related with reactive oxygen species (ROS) that involve superoxide anions, hydrogen peroxide, and hydroxyl radicals. EMR-induced ROS generate oxidative stress and lipid peroxidation; thus, exposure to EMR is associated with enhanced ROS production which in turn induces apoptosis. The signal for apoptosis is generated

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possibly via lipid peroxidation when radiation acts on cell membranes [4, 6–8]. Some researches demonstrated these results with different experimental protocols [6, 9].

Zinc (Zn) is an essential trace element and it has structural and catalytic functions in living cells. Zn is also critical for nucleic acid and protein metabolism, cell growth, proliferation, differentiation, and death. Therefore, the concentration of Zn is strictly regulated by many molecules involved in the uptake and excretion in the organism [10, 11]. Although the role of zinc as either an antioxidant or prooxidant varies by diversity and complexity of Zn activity, it is known that Zn has different roles for protecting biological structures against free radical damages, such as regulating metallothionein levels, being an important component of superoxide dismutase (SOD), being a protective agent for thiol groups, suppressing lipid peroxidation on mitochondria, and stabilizing cell membrane [12, 13]. Thus, several researches reported that the changes of Zn homeostasis may generate apoptotic cell death [10, 11].

Effects of EMR generally have been analyzed on brain and testicular tissues, as we could reach the literature there have been limited data about kidney tissue exposed to EMR [8, 12–14]. Thus, we aimed to study the oxidative parameters and apoptosis in HEK293 induced by 2.45 GHz frequency EMR and to determine the possible protective effects of Zn apoptosis in HEK293 cells induced by EMR.

Materials and Methods

Cells and Reagents

HEK293 cell line was purchased from the American Type Culture Collection (ATCC®, Manassas, VA, USA). Dulbecco's modified eagle medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, trypsin-EDTA, and phosphate buffered saline were acquired from Wisent, (Quebec, Canada). Thiobarbituric acid (TBA), trichloroacetic acid, sodium selenite, xanthine oxidase, and nitro blue tetrazolium (NBT) were obtained from Sigma (Taufkirchen, Germany). Hydrogen peroxide, paraformaldehyde, Triton X-100, and zinc sulphate (ZnSO_4) were obtained from Merck (Darmstadt, Germany). Apoptag Plus In Situ Apoptosis Detection Peroxidase Kit and anti-caspase 3, active form, were purchased from Merck Millipore (Darmstadt, Germany). AEC substrate kit and Histostain-Plus Bulk Kit were obtained from Invitrogen by Life Technologies (CA, USA). Bcl-2 antibody was purchased from Novus (Abingdon, UK).

Cell Culture

HEK293 cells were cultured and maintained at 37 °C in a humidified atmosphere of 5% CO_2 supplemented with DMEM medium containing 10% (v/v) inactivated FBS and 1% (v/v)

penicillin/streptomycin. HEK293 cells were cultured in tissue culture flasks and tissue culture plates. The passage number used was on average between 5 and 15 in this study. The cell culture media were changed every 2 days. Light microscopy was used for monitoring cells during the test procedure.

Exposure System and Design

HEK293 cells were exposed to 2.45 GHz frequency EMR by a voltage-controlled oscillator (Feedback, England, 532/VCO/0138) and monopole antenna. Constant voltage source (15 V) and variable voltage source (3.3 V) (Yıldırım, AC/DC Switching Power Supply, CYS 152) were connected to the voltage-controlled oscillator to get 2.45 GHz frequency EMR. The output signal of the voltage controlled oscillator was controlled by spectrum analyzer (Rohde & Schwarz). At the bottom of the antenna, the oscillator output power was 2.6 dBm and electric field intensity was 8 V/m. Cell culture flasks were placed under the antenna, where the oscillator output power was –40 dBm and electric field intensity was 2 V/m. Cell culture flasks were located 18 cm far from antenna of the exposure system. Plexiglass material was used in exposure system to reduce unwanted reflections of radiation (Fig. 1). This system is placed in a shielded cage which exterior surface coated with aluminum and interior surface coated with EMR absorber material (Fig. 2).

Study Groups

HEK293 cells were equally divided into four main groups: control, 2.45 GHz EMR, 50 μM Zn + 2.45 GHz EMR, and 100 μM Zn + 2.45 GHz EMR. ZnSO_4 was used as a chemical agent in Zn groups. The cells of both Zn-applied groups were incubated with a medium prepared with 50 μM and 100 μM ZnSO_4 for 48 h before 2.45 GHz EMR exposure [15]. The cells of three EMR-exposed groups were exposed to 2.45 GHz EMR for 1 h in the shielded cage.

HEK293 cells were seeded in T25 flasks at a density of 1×10^6 cells per flask and after each experiment cell lysates were prepared for measurement of biochemical markers. The cells were seeded onto coverslips which were placed in to the wells of cell culture plate and after the experimental application samples were fixed with 4% paraformaldehyde for performing morphological assessment. All experiments were repeated six times.

Measurement of Malondialdehyde Levels

Malondialdehyde (MDA) levels were measured according to the method of Buege et al. based on spectrophotometric measurement of the reaction between TBA and MDA [16]. The levels of MDA were determined by using a spectrophotometer (UV-1800 Shimadzu UV-VIS Spectrophotometer, Tokyo, Japan) and assessed by the absorbance coefficient of the

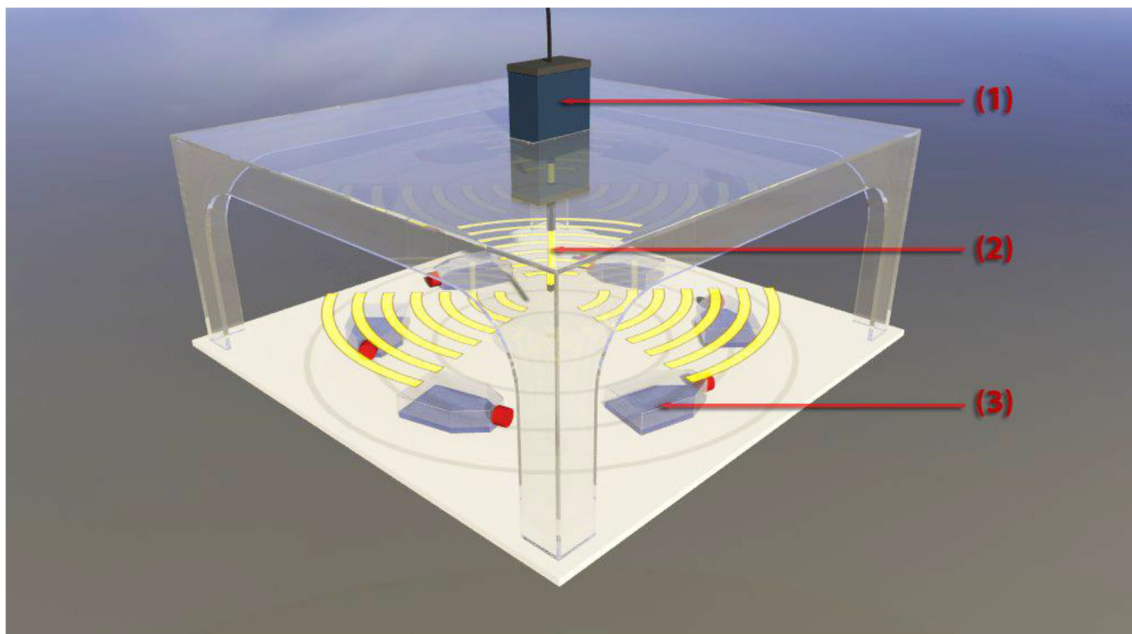


Fig. 1 The experimental set-up of 2.45 GHz EMR exposure device. (1) 2.45 GHz EMR oscillator. (2) Monopole antenna. (3) Cell culture flasks

MDA–TBA complex at 535 nm. MDA concentrations of the cells were expressed as nM/ml.

Measurement of Superoxide Dismutase Activity

Superoxide dismutase (SOD) activity was analyzed utilizing the method of Sun et al. with a principle of NBT reduction by the xanthine/xanthine oxidase systems as a superoxide generator [17]. One unit of SOD was defined as the enzyme amount causing 50% inhibition in the NBT reduction rate. SOD activity was expressed as U/ml.

Detection of Apoptosis

Terminal deoxynucleotidyl transferase-mediated dUTP nick end-labeling (TUNEL) staining method was used for detecting apoptotic cells by a commercial kit (Apoptag Plus in situ Apoptosis Detection Peroxidase Kit–Millipore, USA). The stainings were carried out according to the manufacturer's instruction. TUNEL sections were examined under light microscope (Leica DM 2500) interfaced with a camera (Leica, DFC 280). Ten different areas on each slide were examined at high

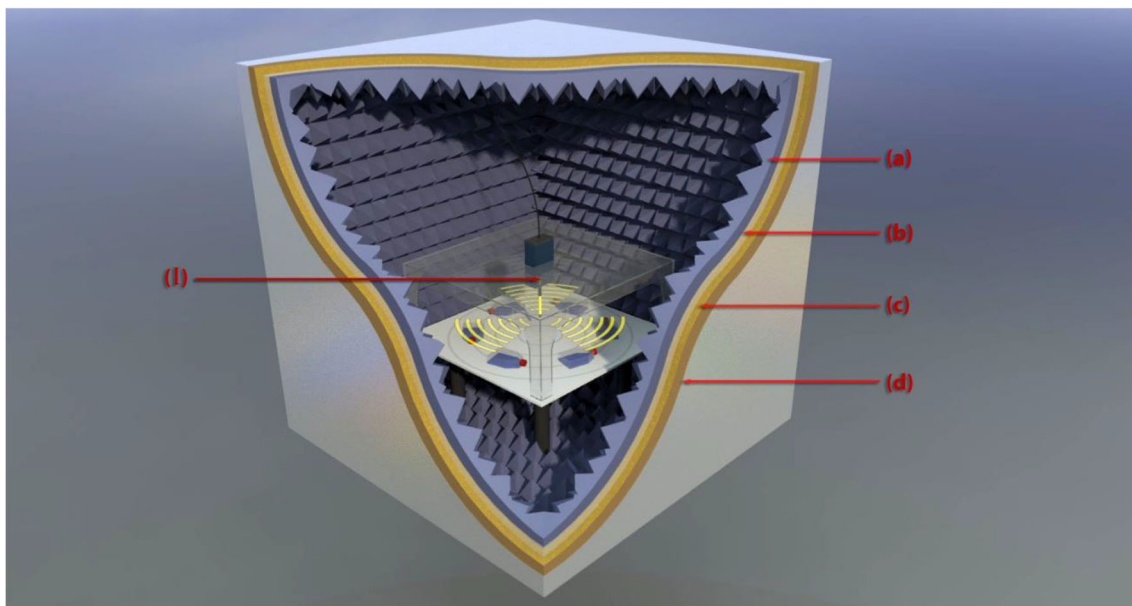


Fig. 2 The experimental set-up of EMR exposure system. (1) 2.45 GHz EMR oscillator. (a) EMR absorber surface. (b and d) Aluminum surface. (c) Wooden cabinet

magnification. The apoptotic index was calculated by counting stained cells.

Immunohistochemistry for Active Caspase-3 and Bcl-2 Staining

HEK293 cells were 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 with modified according to the antibodies. Caspase-3 and bcl-2 immunopositive cells were counted at ten different areas of the slides for statistical analysis.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) 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. $p < 0.05$ values were considered to indicate significance. Data were expressed as the mean (M) $\pm$ standard deviation (SD).

Results

Oxidative Stress Results

The mean MDA levels and SOD activities of four groups are shown in Figs. 3 and 4, respectively. MDA levels of 2.45 GHz EMR group were significantly higher than control group ($p < 0.001$). 50 μM + 2.45 GHz EMR and 100 μM Zn +

2.45 GHz EMR groups' MDA levels were significantly lower than 2.45 GHz EMR group ($p < 0.05$) (Fig. 3).

The mean values of SOD activities were significantly lower in 2.45 GHz EMR group than control group ($p < 0.05$). Incubation of 50 μM and 100 μM before EMR exposure was concluded to decrease SOD activity ($p < 0.05$) (Fig. 4) in EMR group.

Apoptosis and Apoptosis-Related Protein Results

TUNEL-positive apoptotic HEK293 cells are shown in Fig. 5 and apoptotic index values are given in Fig. 6. The number of apoptotic cells was significantly increased in 2.45 GHz EMR group (Fig. 5b) compared with control group (Fig. 5a) ($p < 0.001$). Incubation with different doses of Zn (50–100 μM) significantly increased the number of apoptotic cells (Fig. 5c, d) compared with 2.45 GHz EMR group ($p < 0.001$).

Bcl-2 immunopositive HEK293 cells are shown in Fig. 7 and cell counts of the immunopositive cells are given in Fig. 8. It is observed that bcl-2 immunopositive cells reduced in 2.45 GHz EMR group (Fig. 7b) versus control group (Fig. 7a) ($p < 0.001$). There was no significant increment at the number of bcl-2 immunopositive cells between 50 μM Zn + 2.45 GHz EMR group (Fig. 7c) and 2.45 GHz EMR group whereas incubation with 100 μM Zn before the EMR exposure (Fig. 7d) significantly increased bcl-2 immunopositivity of HEK293 cells compared with 2.45 GHz EMR group ($p < 0.01$).

Active caspase-3 immunopositive HEK293 cells are shown in Fig. 9 and the cell counts are given in Fig. 10. The number of caspase-3 immunopositive cells in the 2.45 GHz EMR group (Fig. 9b) increased significantly compared with control group (Fig. 9a) ($p < 0.05$). Before EMR exposure, incubating the cells with 50–100 μM Zn (Fig. 9c, d) significantly decreased the caspase-3 immunopositivity compared with 2.45 GHz EMR group ($p < 0.001$).

Fig. 3 Effects of 2.45 GHz EMR exposure on MDA levels of control, 2.45 GHz, 50 μM Zn + 2.45 GHz, and 100 μM + 2.45 GHz groups. HEK293 cells were incubated with or without 50 μM or 100 μM Zn supplemented media for 48 h; then, 2.45 GHz groups were exposed to EMR for 1 h. MDA measurements were performed at cell lysates after EMR exposure. The results are expressed as the mean $\pm$ S.D. ($n = 6$) (* $p < 0.05$, ** $p < 0.001$)

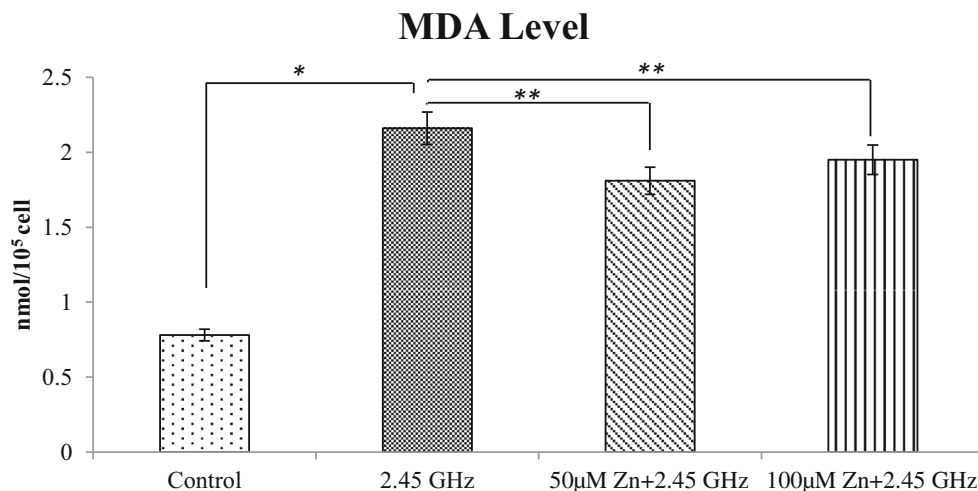
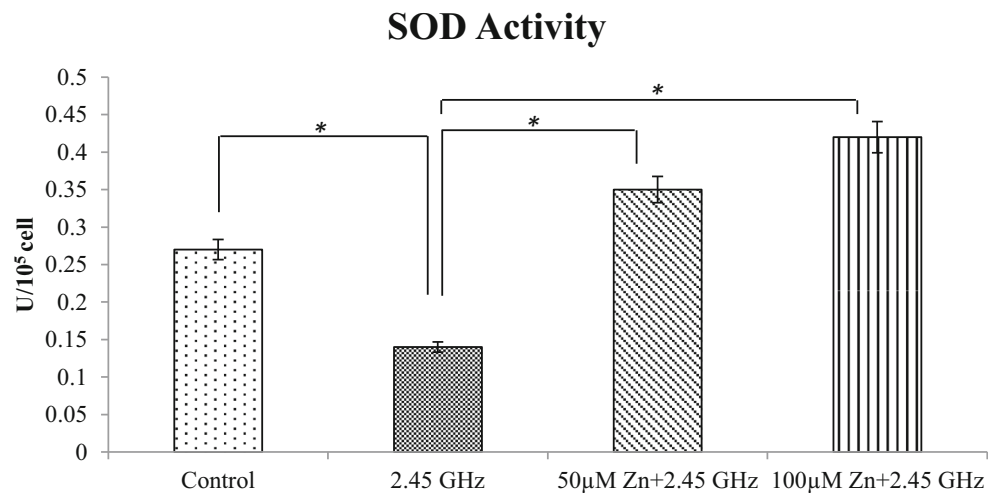


Fig. 4 Effects of 2.45 GHz EMR exposure on SOD activities of control, 2.45 GHz, 50 μ M Zn + 2.45 GHz, and 100 μ M Zn + 2.45 GHz groups. HEK293 cells were incubated with or without 50 μ M or 100 μ M Zn-supplemented media for 48 h, and then were exposed to EMR for 1 h in 2.45 GHz groups. SOD measurements were performed at cell lysates after EMR exposure. The results are expressed as the mean $\pm$ S.D. ($n = 6$) ($*p < 0.05$)



Discussion

Wireless local area network technology emerged in the beginning of 1990s and the usage of it has been augmenting fastly in many different areas such as industry, military, scientific, and medical sectors. Wi-Fi network involves at least one Wi-Fi

antenna connected to the internet and a series of computers, laptops, or other wireless devices. Wi-Fi communication is provided by electromagnetic waves; thus, people are intensively exposed to 2.45 GHz EMR. Exposure to EMR causes a variety of health effects as reported in various studies. The positive correlation between ROS and EMR is widely known in

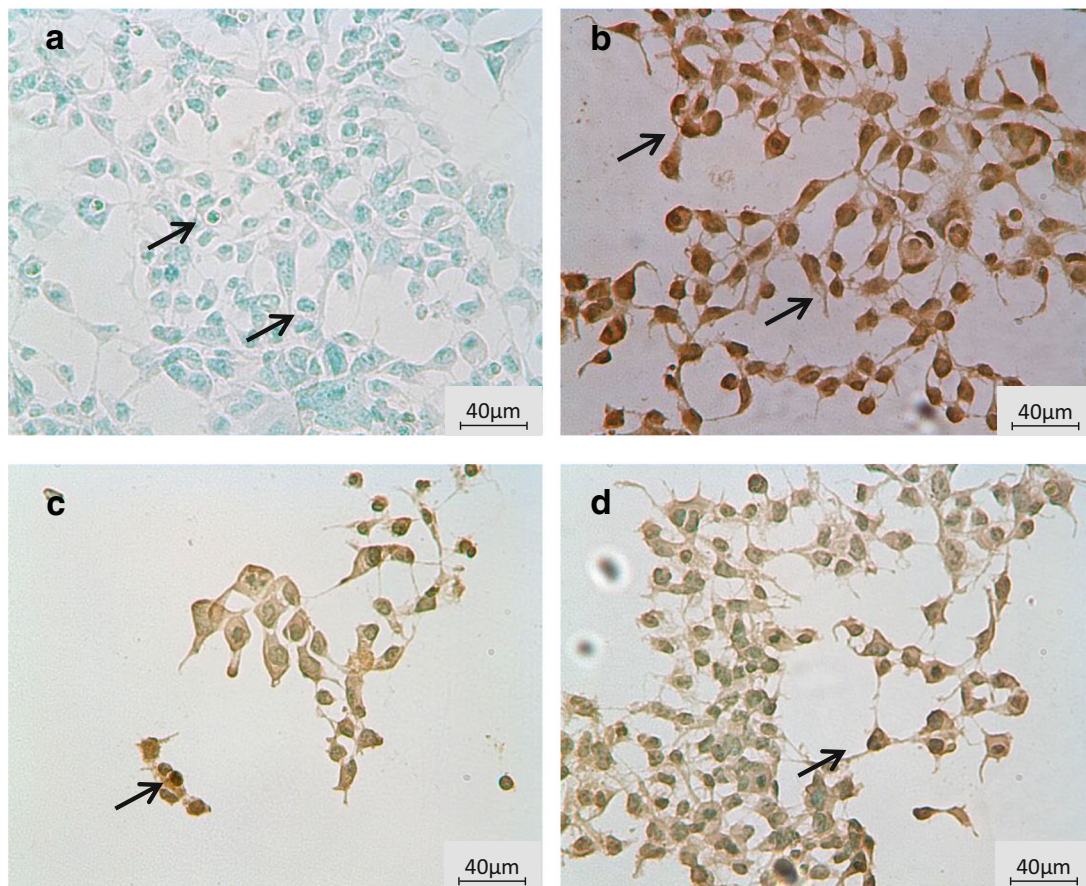


Fig. 5 TUNEL assay of apoptotic HEK293 cells. Dark brown-stained cells were considered to be TUNEL positive ($\rightarrow$). Control group (a), 2.45 GHz group (b), 50 μ M Zn + 2.45 GHz group (c), and 100 μ M Zn + 2.45 GHz group (d). Contrast stain: methyl green (scale bar 40 μ m)

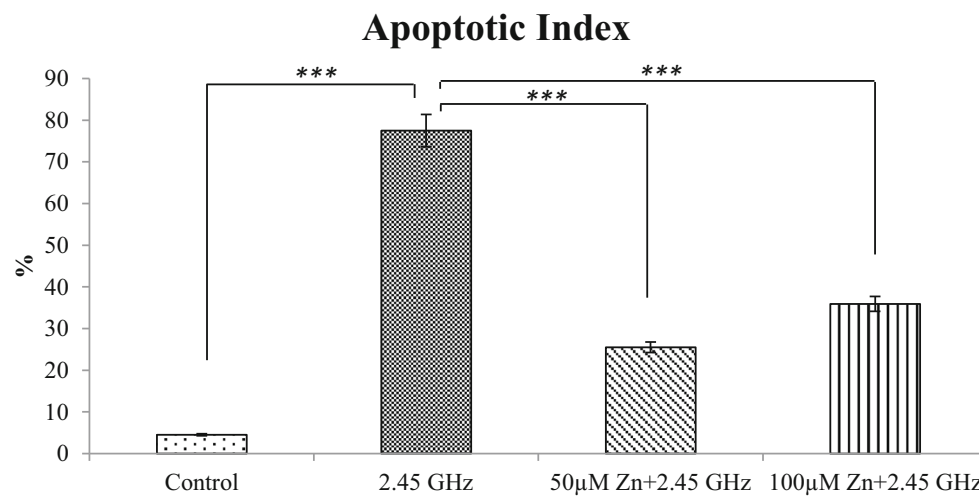


Fig. 6 Apoptotic index values of control, 2.45 GHz, 50 µM Zn + 2.45 GHz and 100 µM Zn + 2.45 GHz groups of HEK293 cells. HEK293 cells were incubated with or without 50 µM or 100 µM Zn-supplemented

media for 48 h, and then were exposed to EMR for 1 h in 2.45 GHz groups. The results are expressed as the mean $\pm$ S.D. ($n = 10$). (***) $p < 0.001$

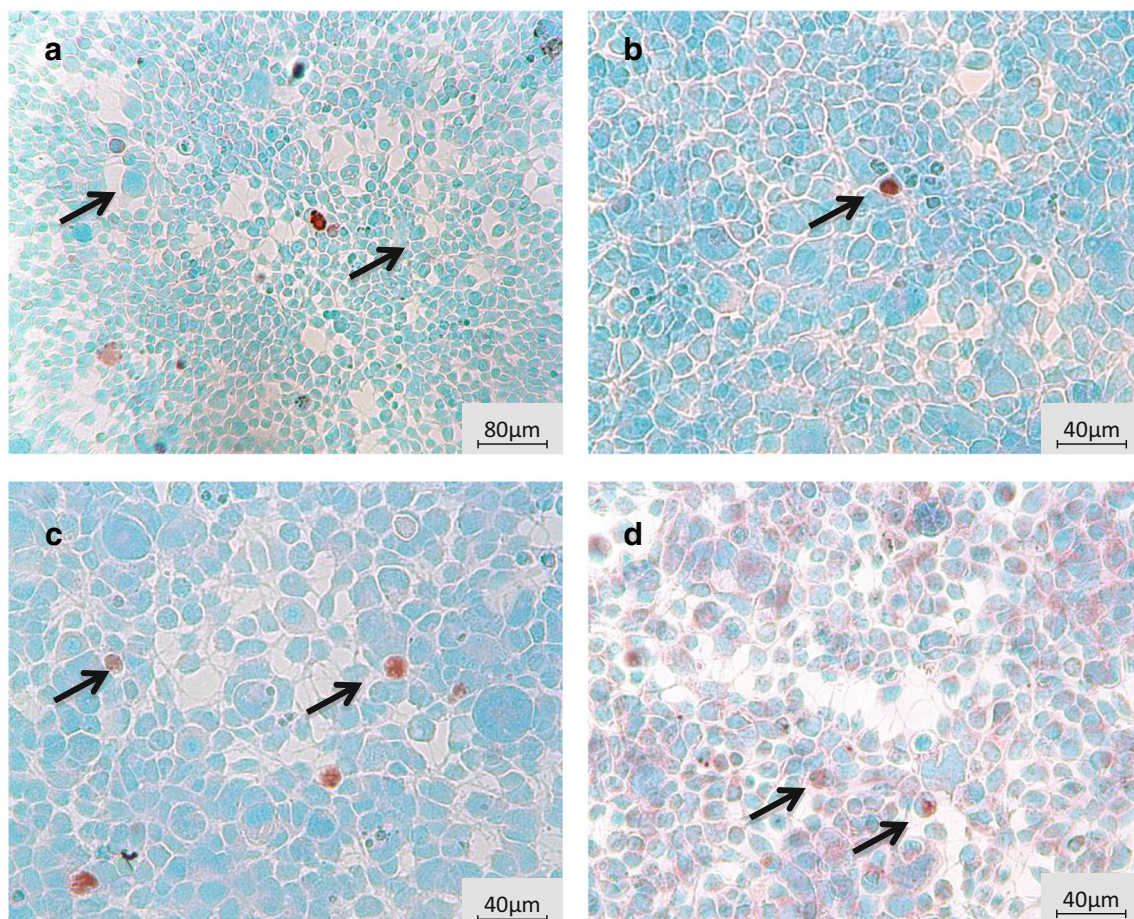


Fig. 7 Bcl-2 immunohistochemical staining of HEK293 cells. Bcl-2 immunopositive cells ($\rightarrow$). Control group (a), 2.45 GHz group (b), 50 µM Zn + 2.45 GHz group (c), and 100 µM Zn + 2.45 GHz group (d). Contrast stain: methyl green (scale bar 40 µm)

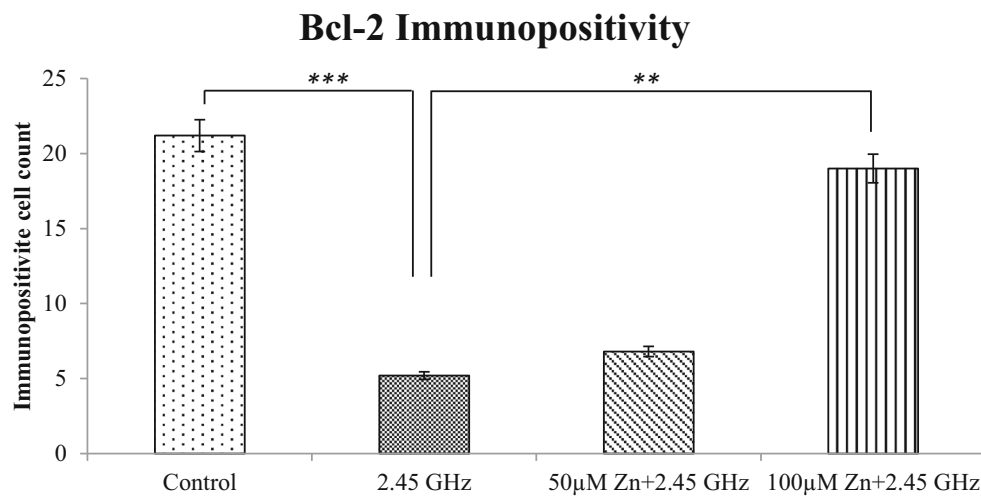


Fig. 8 Bcl-2 immunopositive cell counts of control, 2.45 GHz, 50 µM Zn + 2.45 GHz, and 100 µM Zn + 2.45 GHz groups of HEK293 cells. HEK293 cells were incubated with or without 50 µM or 100 µM Zn-

supplemented media for 48 h, and then were exposed to EMR for 1 h in 2.45 GHz groups. The results are expressed as the mean ± S.D. ($n = 10$) (** $p < 0.01$, *** $p < 0.001$)

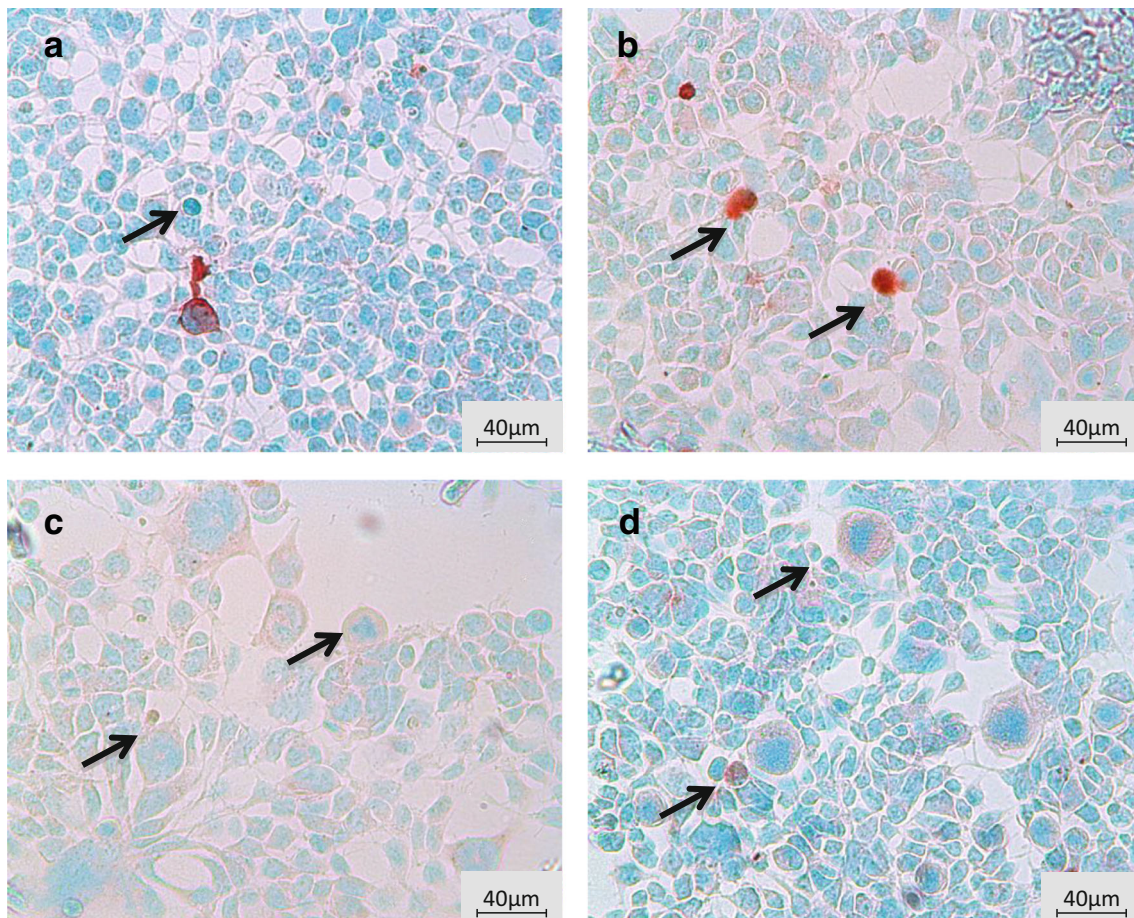


Fig. 9 Active caspase-3 immunohistochemically staining of HEK293 cell. Active caspase-3 immunopositive cells (→). Control group (a), 2.45 GHz group (b), 50 µM Zn + 2.45 GHz group (c), and 100 µM Zn + 2.45 GHz group (d). Contrast stain: methyl green (scale bar 40 µm)

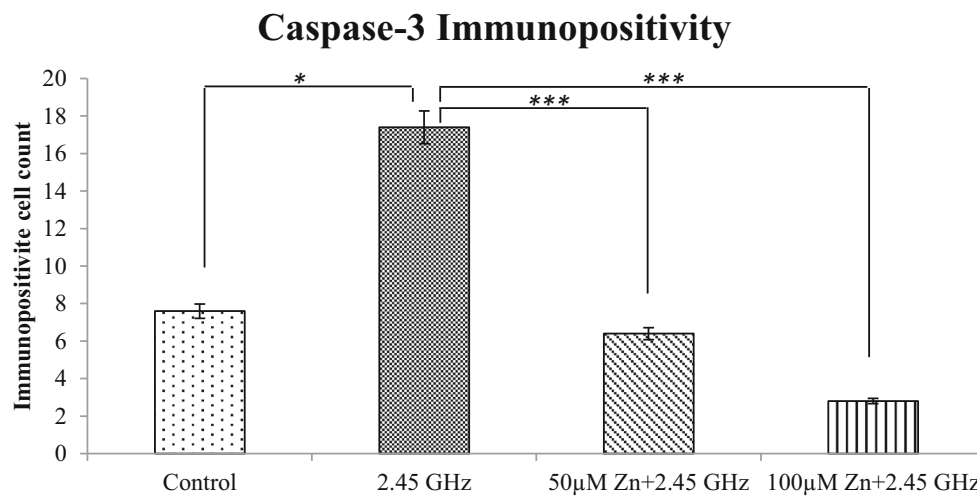


Fig. 10 Active caspase-3 immunopositive cell counts of control, 2.45 GHz, 50 µM Zn + 2.45 GHz, and 100 µM + 2.45 GHz groups of HEK293 cells. HEK293 cells were incubated with or without 50 µM or

100 µM Zn-supplemented media for 48 h, and then were exposed to EMR for 1 h in 2.45 GHz groups. The results are expressed as the mean $\pm$ S.D. ($n = 10$) (* $p < 0.05$, *** $p < 0.001$)

biological systems; however, the cellular and molecular mechanisms underlying this issue are still under research [3, 18, 19].

Living organisms have enzymatic and non-enzymatic antioxidants which scavenge the harmful effects of ROS. It is widely accepted that various diseases are the consequence of the imbalance of oxidants and antioxidants status [7]. Salah et al. [4] researched oxidative stress and metabolic impairments induced with 2.45 GHz frequency Wi-Fi signal and determined that EMR decreased SOD level as an enzymatic antioxidant and increased MDA levels at rats' renal and liver tissues. Morphological studies pointed out that oxidative stress resulted from EMR induces cellular apoptosis by consumption of mitochondrial energy, increase of proteolytic enzymes, and fragmentation of DNA [4, 7].

Zn is an essential trace metal required for many metabolic pathways in human health with its critical level. Zn level is crucial—whether its deficiency or its excess—as Zn deficiency may cause especially neuronal and immune system defects or Zn excess may cause cellular oxidative stress. One of the versatile functions of Zn is related with its cofactor specialty for enzymes that carry importance for vitality, cell division, apoptosis, and intracellular signaling [8, 10, 13]. The effective role of Zn against oxidative stress is related with being in the structure of protective proteins such as Cu/Zn SOD and metallothioneins. Researches on humans, experimental animals, and tissue culture support the relationship between Zn level and regulation of oxidative stress [13, 20].

The present study investigated the efficacy of Zn on biochemical and morphological parameters on 2.45 GHz frequency EMR-exposed HEK293 cells. As a marker for lipid peroxidation, we observed statistically significant increment in MDA level at 2.45 GHz EMR group than control group,

beside that the enzymatic antioxidant SOD significantly decreased at 2.45 GHz EMR group than control group. Incubating HEK293 cells with 50 µM and 100 µM Zn significantly prevents the increment of lipid peroxidation and reduction of SOD activity. As a result, we determined that Zn incubation significantly affects MDA levels and SOD activity on EMR-exposed HEK293 cells.

Shahin et al. [21] studied on mice exposed to EMR 2 h/day for 45 days, and observed the increment of reactive oxygen species and the reduction of SOD activity. In another similar study, it was determined that 900 MHz EMR exposure increased MDA and decreased SOD activity in renal tissue of rat [22]. Bediz et al. [8] reported the effect of Zn on lipid peroxidation induced by 50 Hz frequency EMR. The exposure of low-frequency EMR for a long time interval increased lipid peroxidation, which was low in EMR + Zn group both for plasma and brain tissue. Despite the differences in exposure methods in these studies, the protective effect of Zn against lipid peroxidation on EMR induction was consistent with our study.

On the other hand, researchers found a relation between EMR exposure and apoptotic cell death [6]. For instance EMR, which has higher specific absorption rate (SAR) values than border values of the International Commission of Non-ionizing Radiation (ICNIRP), caused apoptotic cell death in neuroblastoma cells [23]. Also, increment of caspase-3 activity and apoptotic bodies was seen at rat thyroid tissue after the 3-week 20 min/day 900 MHz frequency EMR exposure [24]. Oral et al. [9] reported that 900 MHz EMR increased lipid peroxidation in rat endometrium tissue, and besides, they detected the increase of caspase-3 immunohistochemically at the same tissue. In their study related with pregnant and non-pregnant rabbits exposed to 1800 MHz frequency EMR,

Guler et al. [6] determined apoptotic cells in brain tissue, anterior epithelial cells, and lens of the eye in pregnant and non-pregnant rabbits, and infants with TUNEL staining method. In another study, pregnant rats were daily exposed to Wi-Fi signal at 0, 0.08, 0.4, and 4 W/kg SAR levels, which were the critical levels accepted by ICNIRP. Apoptosis was analyzed with TUNEL method, and there was no difference in rat brain after pre- and post-natal EMR exposure [14].

The alteration of Zn homeostasis affects the sensitivity to apoptosis of cells [25–27]. Previous studies have shown that Zn deficiency increases cell death via apoptosis; however, Zn supplementation inhibits apoptosis in different organs and cultured cells. Zn incubation indicated to suppress apoptosis induced by several agents [28–31]. Supplementation of 10 μ M/L Zn to mesenchymal stem cells of the bone marrow reduced apoptosis, sustained cell viability, and increased vascular endothelial growth factor [32]. Besides, Zn blocked both apoptosis caused by dexamethasone and the generation of reactive oxygen species in HCT-116 colon epithelial cells [33].

Caspase-3 is known to be a pro-apoptotic protein; thus, the existence of caspase-3 is evaluated as a marker of the initiation of apoptosis. Recent studies have introduced that Zn prevented the cells from apoptosis by inhibiting caspase-3 activity. Schrantz et al. [10] determined that specific concentrations of Zn-regulated apoptosis by activating caspase-3 in human B lymphoma. It was reported that caspase-3 expression and apoptosis decreased with 8 μ M Zn supplementation in cultured Sertoli cells [31]. Zinc curtailed apoptosis by inhibiting caspases in the eosinophils [34]. In vivo studies stated that the increase of apoptosis was directly or indirectly a consequence of intracellular decrease of Zn. Zn is indicated to balance between bcl-2 and bax by increasing the expression of anti-apoptotic protein bcl-2 and decreasing the expression of pro-apoptotic protein bax [35–37].

It has been observed in our study that exposure to 2.45 GHz frequency EMR significantly induced apoptosis in HEK293 cells. Before the 2.45 GHz frequency EMR exposure, incubation with 50 μ M and 100 μ M Zn significantly decreased apoptotic cell count. The alteration of anti-apoptotic protein bcl-2 and pro-apoptotic protein caspase-3 was investigated with immunohistochemical staining. Bcl-2 immunopositivity significantly decreased and caspase-3 immunopositivity significantly increased after the exposure of 2.45 GHz frequency EMR on HEK293 cells. Incubated with 50 μ M Zn before 2.45 GHz frequency EMR did not alter bcl-2 immunopositivity, though caspase-3 immunopositivity significantly decreased. Incubation with 100 μ M Zn significantly increased bcl-2 and decreased caspase-3 immunopositivity. Our results were consistent with the studies that analyzed the anti-apoptotic effect of Zn incubation in various cells and tissues as follows: caspase-3 immunopositivity decreased and bcl-2 immunopositivity increased with Zn incubation.

Researchers have reported different results about EMR effects on renal tissue. The different results may be altered with different factors like exposure methods, application time and concentration, and distance to EMR source. There has been growing interest in harmful effects of EMR on fetus in connection with EMR-exposed pregnant rats, and besides, the adverse effects of EMR on fetus have been reported in a few studies. The possible developmental anomalies such as cystic formations located in tubulus, glomerulus, and renal parenchyma on fetal and postnatal renal tissue caused from EMR have revealed in limited studies [38, 39].

Apoptosis has a crucial role for development and tissue homeostasis in living things and it is carefully regulated and balanced with pro-apoptotic and anti-apoptotic proteins. It is also known that cellular stresses, which cause oxidation of macromolecules, have role in apoptosis regulation with increase of the activation of caspases. Caspases are the main pro-apoptotic protein family, that their activation is a hallmark for apoptosis [40]. Failure at the regulation of apoptotic and anti-apoptotic factors resulted in pathological conditions such as renal diseases and embryologic development of cancers [41–43]. Researchers reported that EMR exposure deteriorates the balance of apoptotic and anti-apoptotic proteins. The toxic effects of 50–60 Hz EMR on embryonic development of brain were related with increased apoptotic cells [44, 45].

We pointed out the effects of 2.45 GHz frequency EMR on HEK293 cells and we determined that exposure to EMR enhances apoptotic cell death via increase of caspase-3 immunopositivity and reduces bcl-2 immunopositivity, as markers of apoptosis. These results are consistent with previous studies on different cells. We also observed the protective role of Zn incubation against EMR-induced apoptotic cell death by increasing bcl-2 immunopositivity and decreasing caspase-3 immunopositivity. The preventive features of Zn against apoptosis and oxidative stress induced by EMR may be related with being within the structure of antioxidant enzymes.

In conclusion, our data revealed out that Zn maintains protective effects against 2.45 GHz EMR-induced oxidative stress by reducing lipid peroxidation and increasing SOD activity. Our study also demonstrates that Zn has an inhibitory effect on 2.45 GHz EMR-induced apoptosis by increasing the expression of anti-apoptotic protein bcl-2 and by suppressing the proapoptotic protein caspase-3. It was concluded that Zn supplementation prior to EMR exposure was protective against deterioration of caspase-3 and bcl-2 regulation in cells HEK293. However, further studies are warranted to ascertain the potential usefulness of zinc to correcting the dysregulation of apoptosis in cells HEK293 before EMR exposure.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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