Oxidative Effects of Electromagnetic Radiation on Liver, Lung, Kidney and Heart Tissues of Diabetic and Normal Rats

Elektromanyetik Radyasyonun Diyabetik ve Normal Sıçanların Karaciğer, Akciğer, Böbrek ve Kalp Dokuları Üzerindeki Oksidatif Etkileri

Bahriye Sirav[1]*, Dilek Kuzay[2*], Kevser Delen[1], Sinem Oruc[1], Cigdem Ozer[3]

[1] Department of Biophysics, Gazi University, Faculty of Medicine, Ankara, Turkey
[2] Department of Physiology, Ahi Evran University, Faculty of Medicine, Kırşehir, Turkey
[3] Department of Physiology, Gazi University, Faculty of Medicine, Ankara, Turkey
[*] Both of these authors contributed equally to this study.

ABSTRACT

Purpose: The possible health risks of Radiofrequency Radiation (RFR) and Extremely Low-Frequency Magnetic Fields (ELF-MF) are becoming popular subjects for scientific interest. The objective of this study was to observe the possible effects of 50 Hz ELF-MF and 2100 MHz RFR on diabetic and normal rat liver, lung, kidney and heart tissues using oxidant parameters after one-month exposure.
Material and Methods: 60 adult wistar albino rats were used for the present study. 2100 MHz RFR exposure (SAR: 0.23 W/kg) was performed for one month (30 min/day, 5 days/week). ELF-MF (50 Hz) were used with 8 Gauss for 30 min/day, 5 days per week for one month. There were 10 groups (n=6): Control (C), Control Diabetic (C-D), Sham (S), Sham Diabetic (S-D), ELF magnetic field exposed (ELF) and ELF magnetic field exposed Diabetic (ELF-D), RFR exposed (RFR), RFR exposed Diabetic (RFR-D), ELF-MF and RFR exposed (ELF-RFR) and ELF-MF-RFR exposed Diabetic (ELF-RFR-D). Tissue glutathione (GSH), malondialdehyde (MDA), and total nitric oxide (NO) levels were determined.
Results: ELF and RFR exposures increased the NO and MDA levels ($p<0.05$), and decreased the GSH levels ($p<0.05$) in both diabetic and non-diabetic rats, yet more significantly in diabetic animals. The most marked effect was observed in ELF-RFR-D group ($p<0.05$).
Conclusion: Both radiation exposures caused oxidative stress in tissues while decreasing the antioxidant level more distinctively in diabetic rats.

Keywords: Oxidative Stress; Extremely Low Frequency Magnetic Fields (ELF-MF); Radio-Frequency Radiation (RFR)

Received: 01.13.2020 **Accepted:** 03.12.2021

ÖZET

Amaç: Radyofrekans Radyasyonunun (RFR) ve Çok Düşük Frekanslı Manyetik Alanların (ÇDF-MF) olası sağlık riskleri, son yıllarda popüler araştırma konusu haline gelmektedir. Bu çalışmanın amacı, diyabetik ve normal sıçanlarda bir ay boyunca 50 Hz ELF-MF ve 2100 MHz RFR maruziyetinin karaciğer, akciğer, böbrek ve kalp dokularında oksidan parametreler üzerindeki olası etkilerini incelemektir.
Yöntem: Bu çalışmada 60 erişkin wistar albino cinsi sıçan kullanıldı. Bir ay boyunca 2100 MHz RFR (30 dakika / gün, 5 gün / hafta, SAR: 0.23 W / kg) maruziyeti uygulandı. ELF-MF maruziyeti (50 Hz), 8 Gauss ile 30 dakika / gün, haftada 5 gün, bir ay süreyle uygulandı. 10 grup (n = 6) oluşturuldu: Kontrol (C), Kontrol Diyabetik (CD), Sham (S), Sham Diyabetik (SD), ELF uygulanan (ELF) ve ELF uygulanan Diyabetik (ELF-D), RFR uygulanan (RFR), RFR uygulanan Diyabetik (RFR-D), ELF-MF ve RFR uygulanan (ELF-RFR) ve ELF-MF-RFR'ye maruz kalan Diyabetik (ELF-RFR-D) grup olarak gruplar belirlendi. Doku glutatyon (GSH), malondialdehit (MDA) ve toplam nitrik oksit (NO) seviyeleri incelendi.
Bulgular: ELF ve RFR maruziyeti, hem diyabetik hem de diyabetik olmayan sıçanlarda NO ve MDA seviyelerini arttırdı ($p <0.05$) ve GSH seviyelerini düşürdü ($p <0.05$), diyabetik gruplarda bu fark daha anlamlı olarak gözlendi. En belirgin etki ELF-RFR-D grubunda görüldü ($p <0.05$).
Sonuç: Her iki radyasyona maruz kalma, dokularda oksidatif strese neden olurken, diyabetik sıçanlarda antioksidan seviyesini daha belirgin bir şekilde düşürmüştür.

Anahtar Sözcükler: Oksidatif Stres; Çok Düşük Frekanslı Manyetik Alanlar (ÇDF-MF); Radyo Frekansı Radyasyonu (RFR)

Geliş Tarihi: 13.01.2020 **Kabul Tarihi:** 12.03.2021

ORCID IDs: B.S.0000-0001-6003-6556, D.K. 0000-0002-1460-9883, K.D.0000-0001-5678-9088, S.O.0000-0001-9124-1245, C.O. 0000-0001-9679-4260

Address for Correspondence / Yazışma Adresi: Assoc. Prof. Dr. Bahriye SIRAV Department of Biophysics Faculty of Medicine, Gazi University, 06510 Ankara, TURKEY. E-mail: bahriyes@gazi.edu.tr

doi:http://dx.doi.org/10.12996/gmj.2021.90

INTRODUCTION

Daily exposure to extremely low-frequency magnetic fields (ELF-MF) and Radio-Frequency Radiation (RFR) might be harmful to human health. The debate about biological effects of this fields has become a public health concern in the last 30 years. This has resulted in the classification of ELF-MF and RFR into category 2B in 2002 and in 2011 respectively, ie, agents that are "possibly carcinogenic to humans" by the International Agency for Research on Cancer (IARC) (1-2). Many studies have declared an association between ELF-MF and RFR exposures and human health, with a variety of clinical diseases, including: cancer (brain, colon, breast), neurodegenerative diseases, acute childhood leukemia, genotoxicity, increased risk of miscarriage, infertility, and others (3-7). Su et al. (8) reported that paternal exposure to ELF-MF can be risk factor for childhood leukaemia. Greenland et al. (9) have published a pooled analysis study about ELF-MF and childhood leukaemia. Together, these analyses from a number of well-designed studies show a fairly consistent association between ELF-MF and childhood leukaemia strengths above 0.4 μT, with an approximately two-fold increase in risk. The WHO/IARC has classified RFR as possibly carcinogenic to humans (Group 2B), based on an increased risk for brain cancer, associated with wireless phone use (2).

In general population, normal and sick people expose RFR due to increasing demand of usage of household and office instruments and mobile phones and related base stations, and one of the most common disease is diabetes in modern life. Diabetes, called 'diabetes mellitus' in clinical terminology, is a very serious and growing health care problem worldwide and is associated with severe acute and chronic complications. Diabetes is characterized by hyperglycemia and vascular complications. The primary cause of these complications is chronic hyperglycemia. Hyperglycemia can increase production of free radicals and subsequent oxidative damage. Excessive production of the free radicals cause oxidative stress (10). It is thougt that diabetes is a disease that can exacerbate the oxidative damage. So diabetes can make the person more prone to the oxidative damage (11). Streptozotocin (STZ) is an agent used to induced diabetes in animals via its toxic effects on pancreatic beta cells. STZ behaves as a potential source of oxidative stress, which induces genotoxicity (12).

Oxidative stress can effect many physiological and cellular processes such as cell growth, gene expression, and cell death. Free radicals are reactive charged particles with unpaired electrons (13). Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) have an essential role in many biochemical events when they are at low concentrations (14). However, high levels of the free radicals lead to oxidative and nitrosative stress (15). Manikonda et al (16) investigated the influence of long-term exposure of rats to ELF-MF, focusing on oxidative effects of this fields on different regions of rat's brain. They found that ELF-MF increased oxidative stress and altered glutathione (GSH/GSSG) levels in all the regions of the brain tissue. Also oxidative stress caused by ELF-MF exposure was more significantly at 100 μT than at 50 μT. Deng et al (17) studied the effect of aluminum and ELF-MF (2 mT, 4 h/day, 8 weeks) on oxidative stress and the memory of mice. Memory impairment was assessed with the Morris water maze (MWM) task. Pathologic abnormalities such as overexpression of phosphorylated tau protein and neuronal cell loss in the hippocampus and cerebral cortex was also investigated. The results showed a significant increase in the levels of malondialdehyde (MDA) and statistically decrease in superoxide dismutase (SOD) activity in the ELF-MF group, ELF-MF + Al (aluminum) group, and load Al group in brain and serum. However, ELF-MF + Al treatment caused no more hazardous than aluminum and ELF-MF did, respectively. Akdag et al (18) showed that exposure to 100 μT and 500 μT ELF-MF had no affect on lipid peroxidation, oxidative or antioxidative processes, or reproductive system such as testes morphology and sperm count of rats. However, ELF-MF exposure (500 μT) affected active-caspase-3 activity in long term exposure cases, which is a well-known apoptotic indicator. Wyszkowska et al. (19) studied the effects of 50 Hz, 7 mT MF on male Wistar rats by using two different time period, i.e 24 hour for 1 day or 1 hour for 7 days exposures. Results showed that while 24 hour for 1 day ELF-MF exposure lead to increase in inflammatory cytokines, 1 hour for 7 days exposure did not cause to any changes. In a study from Duan et al. (20), mice were exposed to ELF-MF (28 day, 4 h/day, 50 Hz, 8 mT). After exposure, cognitive functions were tested by Morris Water Maze test and hippocampus tissues were analysed for markers of oxidative stress. They observed that ELF-MF exposure caused memory and learning deficit and oxidative damage in hippocampus tissues of the mice.

Recently, many experiments has been carried out to analyze possible oxidative effects of RFR exposure. A number of studies reported several apparently pathophysiological effects of mobile phone exposure on various tissues, including reproductive organs (21-24). It has also been found that electromagnetic field exposure induced reversible or irreversible functional and structural changes at the cellular level (25-26). In recent years, many studies observed that mobile telephones could increase free radical formation in tissues because of the EMF they produce, which finally results in oxidant damage (27-28). In the current study, it was aimed to compare the effects of each exposures and wanted to see if there is an extra effect of both exposures taken at the same animals. The aim of present study was to examine whether exposure to the ELF-MF and 2.1 GHz RFR leads to oxidative damage to the liver, lung, kidney and heart tissues of diabetic and normal rats.

MATERIALS and METHODS

Experimental setup and treatments

Sixty adult male *Wistar albino rats* (220-270 g) were used in the experiment. The rats were randomly separated into 10 groups equally (n=6 for each group): Control (C), Control Diabetic (C-D), Sham (S), Sham Diabetic (S-D), ELF magnetic field exposed (ELF) and ELF magnetic field exposed Diabetic (ELF-D), RFR exposed (RFR), RFR exposed Diabetic (RFR-D), ELF-MF and RFR exposed (ELF-RFR) and ELF-MF and RFR exposed Diabetic (ELF-RFR-D). All rats were placed in plexiglass cages in a room with humidity (50 - 55 %), temperature (22 °C), and a 12-hours light dark cycle. All procedures of the study were carried out with the confirmation of the Gazi University Experimental Animals Local Ethics Committee. The rats were fed laboratory pellet chow and water was given *ad libitum*. After several days of stabilization period of the animals the experiment was performed. The ELF group animals were exposed to 50 Hz magnetic fields and RFR group animals were exposed to 2100 MHz RFR for 30 min/day, 5 days/week for one month. No experimental manipulation was performed to control group animals. By a single intraperitoneal injection of STZ (65 mg/kg, Sigma-Aldrich, St. Louis, MO) diabetes was induced. Blood glucose was measured after 72 hours following the injection using a glucose meter (Life Scan Inc., USA). Animals with blood glucose levels over 250 mg/dl were considered as diabetic. After exposure period (one-month), the rats were decapitated following anesthesia with intramuscular ketamine (50 mg/kg) and xylazine (5 mg/kg) injection.

Exposure system

Details of the ELF-MF exposure system *(Figure 1)* has been described before (29). Briefly, by using circular Helmholtz coils with winding embedded in an open wooden circular frame a 50 Hz MF of 8 Gauss was generated. The system consisted of two parallel horizontal flat circular coils (21.375 cm apart and 42.75 cm in diameter with a common axis). Each coil was constructed of insulated copper wire and had 154 turns. 50 Hz sinusoidal current was created at the output of the circuit driven by a specially designed variable transformer, 2.7 kVA in power (Navelsan, Ankara, Turkey) that allowed modulation of the intensity of the MF. The MF value at the center of the exposure system was measured by Electromagnetic Field Analyzer (EFA 300, Narda, Germany). The background geomagnetic field value was recorded as 20 microT. Rats were placed in plastic cage and the cage (polymethyl methacrylate, 15 x 20 x 20 cm) was placed symmetrically throughout the center axis and perpendicular to the mid-line of the coils. In order to avoid the temperature increase inside the cage, the cage was constantly aerated.

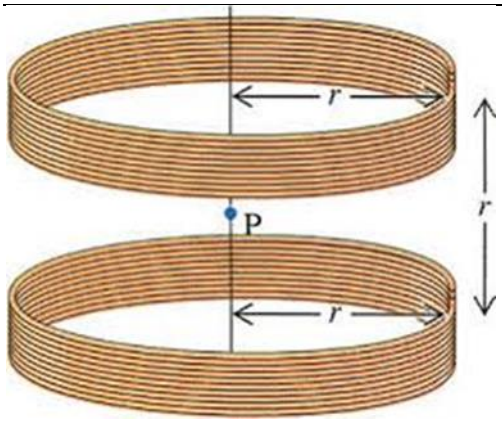

Figure 1. ELF magnetic fields exposure system

RFR exposure performed by using vector signal generator (Rohde & Schwartz, model SMBV100A, Germany) and horn antenna (ETS Lindgren, Model 3164-03, USA) (Figure 2). Rats were housed in plastic cage (polymethyl methacrylate plastic cage). The cage was placed symmetrically along the axis of the horn antenna.

To prevent the temperature increase inside the cage, the cage was constantly aerated. In order to get sufficient field intensity the cage was placed in the near field area of the antenna. RFR electric field was measurement was performed with Broadband RF Survey Meter (Narda EMR 300, Germany) via the appropriate electric field probe type 8.3. Electric field value of was measured as 17.25 V/m. The background electric field level was measured as 0.1 - 0.21 V/m. Power density average value was measured at the mid-point of the bottom of the cage. The maximum value of the power density was found throughout the axis of the antenna and uniformly decreased with the distance from the antenna.

The specific absorption rate (SAR, W/kg) is a fundamental dosimetric quantity of RFR (30). The SAR value of 4 W/kg is known as the threshold for the induction of biological thermal effects of RFR (31). SAR value was calculated using the following equation: SAR = $\sigma/\rho[E_{RMS}^2]$ [W/kg]. σ is the mean electrical conductivity of the tissue Siemens/meter (S/m), ρ is the mass density (kg/m^3) and E_{RMS} is the root mean square value of the electric field (V/m) inside the tissue (32-33). Based on the average of the dielectric properties the rat body was considered as an equivalent tissue of the 36 tissues in the rat segmented at Brook Air Force Base. Mass density (1105 kg/m^3) and conductivity (0.87 S/m) were derived from the equivalent tissue by using dielectric properties and mass densities of these tissues. Whole body average SAR value was calculated as 0.23 W/kg.

Prior and after exposure session body temperature (rectal) of rats was recorded. The RFR exposure did not cause any changes in rectal temperature values of the rats.

Electromagnetic field applications of the ELF-MF and RFR groups were done to the animal one by one separetely.

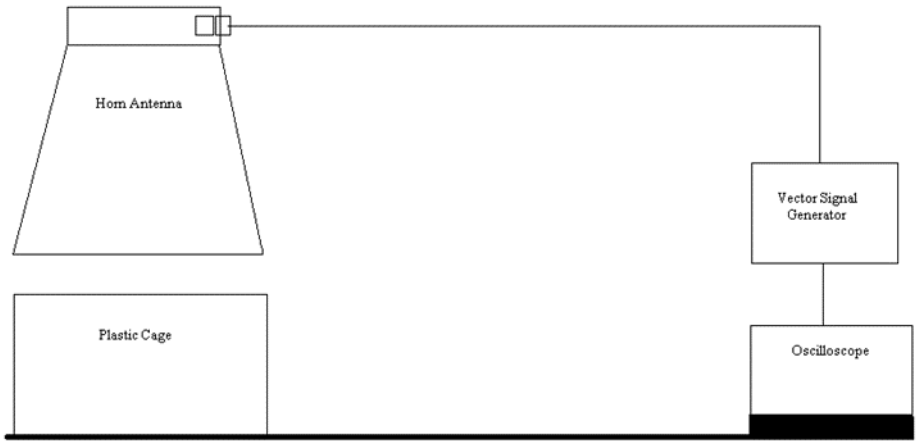

Figure 2. RFR exposure system

Measurement of tissue lipid peroxidation and GSH levels

Lesion areas were measured and tissue samples were kept in –80°C until the assay. By measuring the formation of thiobarbituric acid reactive substances (TBARS) lipid peroxidation was quantified. Homogenization of the samples were done in a tissue homogenizer (Heideloph Diax 900, Germany) in ice-cold trichloroacetic acid (TCA, 1 g tissue in 10 ml 10% TCA). Homogenate was centrifugated for 10 min at 3000 × *g* (Hermle Z 323 K, Germany). After this step 750 µl of supernatant was added to an equal volume of 0.67% (m/v) thiobarbituric acid (TBA) and heated at 100°C for 15 min. Samples absorbance was measured at 535 nm. Lipid peroxide levels are expressed in terms of MDA equivalents by using an extinction coefficient of 1.56 × 105 l·mol–1·cm–1.

By Ellman method with some modification the GSH levels were determined. Briefly, centrifugation (3 000 × *g* for 10 min) of the homogenates were performed. 0.5 ml of supernatant was added to 2 ml of 0.3 M $Na_2HPO_4{\cdot}2H_2O$ solution. After this, dithiobisnitrobenzoate solution (2 ml, 0.4 mg/ml 1% sodium citrate) was added. The absorbance of the samples at 412 nm was measured with a spectrophotometer (UV 1208, Shimadsu, Japan). The GSH levels were calculated with an extinction coefficient of 13 600 l·mol–1·cm^{-1}.

Determination of NO (Griess assay)

NO levels of the tissues were obtained from Elisa reader by vanadium chloride (VCl_3)/Griess assay. Tissue homogenization was performed in phosphate buffer saline (pH = 7) and samples were centrifuged for 5 min at 2000 × *g*. After centrifugation step, 0.25 ml of 0.3 M NaOH was added to 0.5 ml supernatant. The samples incubated for 5 min at room temperature. 0.25 ml of 5% (w/v) ZnSO4 was added following the incubation. The mixture was then centrifuged (3000 × *g* for 20 min). After centrifugation supernatants were used for the assays. Serial dilution of the Nitrate standard solution was performed and the samples loaded to the plates (100 µl). Griess reagents sulphanilamide (SULF) (50 µl) and Vanadium III chloride (VCl_3) (100 µl) and N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) (50 µl) were added to each well. Samples were incubated in 37°C for 45 min, and measurements were done at 540 nm using ELISA reader (34).

Statistical analysis

All data were presented as mean values ± SD. Kruskal Wallis and Mann-Whitney U test was used. $p < 0.05$ considered as statistically significant. Data were analyzed by using SPSS version 13.0.

Ethical Statement

All experimental procedures were performed according to Eyropean Community (EU Directive 2010/63/EU for animal experiments) and National Institutes of Heath guidelines for the care and use of laboratory animals. The study approved by the local ethical committee of the Gazi University, Ankara.
There is no conflict of interest including any financial, personal or other relationships with other people or organizations that could inappropriately influence, or perceived to influence this work ("nothing to declare"). All authors have materially participated in the research or in the article preparation. This study supported by grants from Gazi University Research Foundation, No: 01/2011-18 and No: 2002-07.

RESULTS

RFR exposure decreased GSH levels in heart, kidney, lung and liver tissues of diabetic and nondiabetic rats. ELF exposure decreased GSH levels in lung and heart tissues. When RFR and ELF exposure applied together, it was observed that GSH levels decreased in all tissues. RFR exposure increased MDA levels in all tissues. Different results were observed in different tissues. No statistically significant differences were observed between control and sham groups. Results were presented in tables (Table 1-4).

Table 1. Mean ± SD values of MDA, NO and GSH levels in liver tissues

Group	MDA (nmol/g)	NO (μmol/g)	GSH (nmol/g)
Control	2,74 ± 0,29	9,51 ± 0,91	10,47 ± 0,88
Sham	2,66 ± 0,09	9,72 ± 0,81	10,90 ± 0,89
RFR exposed	3,51 ± 0,47*	11,46 ± 1,72	9,22 ± 0,88*
ELF exposed	3,19 ± 0,38*	11,77 ± 1,95*	10,21 ± 1,50
RFR + ELF exposed	4,99 ± 0,88*	11,44 ± 2,40	8,74 ± 0,83*
Control diabetic	4,86 ± 0,70	13,56 ± 1,80	8,95 ± 0,21
Sham diabetic	4,83 ± 0,86	13,27 ± 1,25	8,88 ± 0,28
RFR exposed diabetic	5,86 ± 0,71*	13,99 ± 1,24	8,10 ± 0,62*
ELF exposed diabetic	5,30 ± 0,33	14,09 ± 1,04	8,90 ± 0,59
RFR + ELF exposed diabetic	6,56 ± 0,78*	15,96 ± 1,56*	6,40 ± 1,03*

The values are means ± SD; $n = 6$. * shows statistically important results ($p < 0.05$) with respect to their own sham or control groups.

Table 2. Mean ± SD values of MDA, NO and GSH levels in lung tissues

Group	MDA (nmol/g)	NO (μmol/g)	GSH (nmol/g)
Control	4,34 ± 0,63	9,21 ± 1,14	3,77 ± 0,20
Sham	4,38 ± 0,44	9,25 ± 1,15	3,78 ± 0,27
RFR exposed	4,96 ± 0,40*	10,27 ± 1,13	3,50 ± 0,24
ELF exposed	4,68 ± 0,26	11,13 ± 1,72*	3,33 ± 0,31*
RFR + ELF exposed	4,75 ± 0,49	12,85 ± 0,97*	3,17 ± 0,32*
Control diabetic	5,24 ± 0,53	14,23 ± 1,26	3,17 ± 0,42
Sham diabetic	5,15 ± 0,66	13,96 ± 1,71	2,96 ± 0,24
RFR exposed diabetic	6,41 ± 0,65*	14,68 ± 1,72	2,45 ± 0,39*
ELF exposed diabetic	6,07 ± 0,25*	15,10 ± 1,51	2,70 ± 0,17*
RFR + ELF exposed diabetic	7,09 ± 0,67*	19,10 ± 1,71*	2,60 ± 0,32*

The values are means ± SD; $n = 6$. * shows statistically important results ($p < 0.05$) with respect to their own sham or control groups.

Table 3. Mean ± SD values of MDA, NO and GSH levels in kidney tissues

Group	MDA (nmol/g)	NO (μmol/g)	GSH (nmol/g)
Control	4,15 ± 0,35	10,72 ± 1,60	2,17 ± 0,19
Sham	4,04 ± 0,24	10,00 ± 1,30	2,13 ± 0,14
RFR exposed	5,15 ± 0,51*	11,47 ± 1,87	1,85 ± 0,20*
ELF exposed	5,00 ± 0,54*	12,19 ± 1,30*	1,97 ± 0,15
RFR + ELF exposed	5,45 ± 0,45*	13,50 ± 1,30*	1,87 ± 0,21*
Control diabetic	5,96 ± 0,46	15,64 ± 1,65	1,73 ± 0,20
Sham diabetic	4,96 ± 0,26	14,76 ± 1,97	1,73 ± 0,18
RFR exposed diabetic	6,69 ± 0,32*	14,24 ± 0,79	1,49 ± 0,09*
ELFexposed diabetic	5,96 ± 0,75	15,44 ± 1,83	1,72 ± 0,22
RFR + ELF exposed diabetic	8,91 ± 0,90*	17,65 ± 2,79	1,42 ± 0,20*

The values are means ± SD; $n = 6$. * shows statistically important results ($p < 0.05$) with respect to their own sham or control groups.

Table 4. Mean ± SD values of MDA, NO and GSH levels in heart tissues

Group	MDA (nmol/g)	NO (μmol/g)	GSH (nmol/g)
Control	3,62 ±0,38	10,29±1,40	3,57 ± 0,29
Sham	3,42 ± 0,30	10,21±1,56	3,51 ± 0,26
RFR exposed	4,51 ± 0,07*	13,26±1,27*	3,19 ± 0,17*
ELF exposed	3,85 ± 0,55	12,05±1,65	3,14 ± 0,16*
RFR + ELF exposed	4,64 ± 0,41*	13,70±1,32*	3,03 ± 0,14*
Control diabetic	5,00 ±0,48	15,75±2,17	2,59 ± 0,18
Sham diabetic	4,90 ± 0,36	14,88±2,42	2,47 ± 0,15
RFR exposed diabetic	5,98 ± 0,66*	16,45±1,88	1,81 ± 0,26*
ELFexposed diabetic	5,28 ± 0,47	16,78±1,48	1,96 ± 0,23*
RFR + ELF exposed diabetic	6,86 ± 0,72*	22,77±3,14*	1,78 ± 0,29*

The values are means ± SD; $n = 6$. * shows statistically important results ($p < 0.05$) with respect to their own sham or control groups.

DISCUSSION

In the present study, oxidative effects of ELF-MF and RFR on the diabetic and normal rats heart, liver, lung and kidney tissues were examined. There were different groups to see the possible effects of alone RFR exposure, alone ELF exposure and both frequency applications. The statistically most important change in the oxidative parameters were seen in the diabetic groups which received both exposures. Diabetic anormalities alone produce oxidative changes of tissues. However, if there is an extra electromagnetic exposure, this changes get exacerbated and could be reason to many critical health problems, such as cancer. A number of epidemiological studies indicate that there is a correlation between ELF electromagnetic field exposure and human health effects such as cancer (35), in particular childhood leukemia (36-37), or between wire code and cancer (38). Almost all of these epidemiological studies have considered residential and/or occupational ELF magnetic field exposure. ELF magnetic fields interact with living systems by two well established mechanisms: induced electric fields produced in accord with Faraday's law of magnetic induction, and direct magnetic field effects on magnetic particles such as the crystals of magnetite (Fe_3O_4) that have been found in a number of organisms (39). A large number of other possible interaction mechanisms, including resonance effects, have been discussed. One of possible causal mechanism to explain ELF magnetic fields & cancer association; magnetic fields can reduce melatonin levels in both animals and humans (40-41). While reduced melatonin levels may increase the risk of cancer, the role of magnetic fields in mediating the reduction of melatonin affecting cancer is unproven. There are many oxidative effect studies about ELF-MF exposures.

The daily use of most of the communication devices i.e. mobile phones, results in chronic exposure to RFR. The interaction mechanism between the biological system and radio frequency radiation is not clear. RFR has thermal or/and non-thermal effects on organisms. Both in vivo and in vitro studies found that tissue heating as a result of thermal effect by high level RFR exposure is the most commonly accepted mechanism of RFR for biological systems. Non-thermal effect mechanism means no increase in temperature observed, is another popular mechanism in recent years. There are a few studies carried out to examine the effects of RFR similar to the 3 G mobile phone on rats focusing on the oxidative stress.

Alkis et al. (25) investigated oxidative effects of mobile phones in brain tissue of rats. They showed increased MDA, NO and TOS level and decreased TAS level. They determided that the mechanisms of oxidative effects of mobile phones in the brain tissue might be associated with ROS. Pandey and Giri (21) investigated the influence of RFR on SOD and GSH-P_x activities in testes tissues of mice. They found that RFR decreased the activities of antioxidant enzymes. Sabban et al. (42) observed that RFR exposure has oxidative effects in brain and liver tissues of fetal and maternal rats. Gulati et al. (43) showed RFR has no oxidative or apoptotic effect on human lymphocytes cell culture. They also found that in vitro RFR exposure caused DNA damage. Devrim et al (44) investigated effects of RFR and vitamin C on antioxidant and oxidant parameters in different tissues (heart, ovary, liver and, kidney) and erythrocytes of rats. MDA, NO, and GSH-P_x levels in the erythrocytes increased significantly in the RFR group compared to the control group. It was observed that CAT activity and MDA level increased significantly, whereas ADA activities and NO levels decreased in the RFR group in the kidney tissues. However, in the heart tissues ADA, MDA, and NO activities significantly decreased in the exposed group. They concluded that RFR from mobile phones caused oxidative stress and peroxidation in the kidney tissues and erythrocytes of the rats. Vitamin C seems to have partial protective role against the oxidative stress in the erythrocytes.

In the recent study, we proved that ELF-MF, 2100 MHz RFR and both exposures lead to oxidative stress especially on diabetic rats. This induction mediated by increase of lipid peroxidation and the reduction of GSH. The study also showed that RFR could enhance NO production. Based on the findings of this study, we could say that ELF-MF and RFR are one of the environmental stressors and this fields can cause oxidative and nitrosative stress in lung, liver, kidney and heart tissues. Oxidative stress is a condition associated with many human diseases including myocardial infarction, neurodegenerative diseases, atherosclerosis, heart failure, chronic liver and lung diseases, etc. So, mobile phone usage should be limited, human being's electromagnetic exposures should be controlled. The constant exposure of modern society to electromagnetic fields has raised considerable concerns about the potential risks to especially people who have chronic problems such as diabetes.

Conflict of interest
No conflict of interest was declared by the authors.

REFERENCES

1. IARC Non-Ionizing Radiation. Part 1, *Static and Extremely Low Frequency (ELF) Electric and Magnetic Fields*. 2000,Vol 80, Lyon.
2. Non-Ionizing Radiation, Part 2: Radiofrequency Electromagnetic Fields. IARC MONOGRAPHS ON THE EVALUATION OF CARCINOGENIC RISKS TO HUMANS. 2013; Volume 102.
3. Ghahremani S, Shiroudbakhshi K, Kordasiabi AH et al. Exposure to Magnetic Fields and Childhood Leukemia: An Overview of Meta-Analysis. Int J Pediatr. 2020; Vol.8, N.5, Serial No.77.
4. Hardell L and Carlberg M. Mobile phones, cordless phones and rates of brain tumors in different age groups in the Swedish National Inpatient Register and the Swedish Cancer Register during 1998-2015. Plos One. 2017; 12(10), e0185461.
5. Guerra MF, Lacoste MG, Anzulovich AC et al. Magnetic fields, cancer and circadian rhythms: hypotheses on the relevance of intermittence and cycling. Proceedings of the Royal Society B, 2019, 286.1916: 20192337.
6. Moon JH. Health effects of electromagnetic fields on children. Clinical and Experimental Pediatrics 2020, 63.11: 422.
7. Chen Q, Lang L, Wu W, et al. A meta-analysis on the relationship between exposure to ELF-EMFs and the risk of female breast cancer. PLoS One. 2013; 8(7), e69272.
8. Su L, Zhao C, Jin Y, et al. Association between parental occupational exposure to extremely low frequency magnetic fields and childhood nervous system tumors risk: A meta-analysis. Science of the Total Environment 2018, 642, 1406-1414.
9. Greenland S, Sheppard AR, Kaune WT, et al. A pooled analysis of magnetic fields, wire codes and childhood leukaemia. Epidemiology 2000; 11: 624–634.
10. Abbasihormozi S, Babapour V. Stress hormone and oxidative stress biomarkers link obesity and diabetes with reduced fertility potential. Cell Journal (Yakhteh) 2019, 21(3), 307.
11. Ighodaro OM. Molecular pathways associated with oxidative stress in diabetes mellitus. Biomedicine & Pharmacotherapy 2018, 108, 656-662.
12. Wang F, Haili L, Hang Z et al. Antidiabetic activity and chemical composition of Sanbai melon seed oil. Evidence-Based Complementary and Alternative Medicine. 2018; 5434156.
13. Alkadi, H. A review on free radicals and antioxidants. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders) 2020, 20(1), 16-26.
14. Abrahams S, Haylett WL, Johnson, G, et al. Antioxidant effects of curcumin in models of neurodegeneration, aging, oxidative and nitrosative stress: A review. Neuroscience. 2019;406, 1-21.
15. Zhang L, Wang X, Cueto R et al. Biochemical basis and metabolic interplay of redox regulation.Redox biology. 2019; 26, 101284.
16. Manikonda PK, Rajendra P, Devendranath D, et al. Extremely low frequency magnetic fields induce oxidative stress in rat brain. Gen Physiol Biophys. 2014, 33(1):81-90.
17. Deng Y, Zhang Y, Jia S, et al. Effects of aluminum and extremely low frequency electromagnetic radiation on oxidative stress and memory in brain of mice. Biol Trace Elem Res. 2013 Dec;156(1-3):243-52.
18. Akdag MZ, Dasdag S, Uzunlar AK, et al. Can safe and long-term exposure to extremely low frequency (50 Hz) magnetic fields affect apoptosis, reproduction, and oxidative stress? Int J Radiat Biol. 2013 Dec;89(12):1053-60.
19. Wyszkowska J, JeRdrzejewski T, Piotrowski J et al. Evaluation of the influence of in vivo exposure to extremely low-frequency magnetic fields on the plasma levels of pro-inflammatory cytokines in rats. International journal of radiation biology. 2018; 94.10: 909-917.
20. Jelenkovic A, Janac B, Pesic V, et al. Effects of extremely low-frequency magnetic field in the brain of rats. Brain Res. Bull. 2006, 68, 355–360.
21. Pandey N, and Sarbani G. Melatonin attenuates radiofrequency radiation (900 MHz)-induced oxidative stress, DNA damage and cell cycle arrest in germ cells of male Swiss albino mice. Toxicology and industrial health. 2018; 34.5: 315-327.

22. Yakymenko I, Tsybulin O, Sidorik E, et al. Oxidative mechanisms of biological activity of low-intensity radiofrequency radiation. Electromagnetic biology and medicine. 2016; 35.2: 186-202.
23. Alkis ME, Akdag MZ, Dasdag S, et al. Single-strand DNA breaks and oxidative changes in rat testes exposed to radiofrequency radiation emitted from cellular phones. Biotechnology & Biotechnological Equipment, 2019; 33.1: 1733-1740.
24. Nassar SA, Algazeery A, Ahmed G, et al. Histological, Immunohistochemical and Molecular Alterations in Immature Mice Testes Due to Chronic Exposure to Mobile Phone Radiofrequency Radiation. The Egyptian Journal of Hospital Medicine 2020, 78(1), 128-135.
25. Alkis ME, Bilgin HM, Akpolat V, et al. Effect of 900-, 1800-, and 2100-MHz radiofrequency radiation on DNA and oxidative stress in brain. Electromagnetic Biology and Medicine. 2019; 38.1: 32-47.
26. Somosy Z. Radiation response of cell organelles. Micron. 2000 Apr; 31(2):165-81.
27. Dasdag S, and Akdag MZ. The link between radiofrequencies emitted from wireless technologies and oxidative stress. Journal of chemical neuroanatomy. 2016; 75: 85-93.
28. Yakymenko I, Burlaka A, Tsybulin A, et al. Oxidative and mutagenic effects of low intensity GSM 1800 MHz microwave radiation. 2018; 40, 282–287.
29. Canseven AG, Seyhan N. Design, installation and standardization of homogeneous magnetic field systems for experimental animals. IFMBE Proceedings, 2005, Vol. 11. Prague: IFMBE, 2005. ISSN 1727-1983. In: Hozman J, Kneppo P, editors. Proceedings of the 3rd European Medical & Biological Engineering Conference (EMBEC 2005). Prague, Czech Republic, 20 – 25 November 2005. pp 2333 – 2338.
30. Melnick, R. L. Commentary on the utility of the National Toxicology Program study on cell phone radiofrequency radiation data for assessing human health risks despite unfounded criticisms aimed at minimizing the findings of adverse health effects. Environmental research 2019, 168, 1-6.
31. Bernardi P, Cavagnaro M, Pisa S, et al. Specific absorption rate and temperature elevation in a subject exposed in the far-field of radio-frequency sources operating in the 10-900-MHz range. IEEE Trans Biomed Eng 2003; 50:295–304.
32. Busygina AV, Komnatnov ME. Estimation and simulation of SAR distribution in biological objects excited by various types of electromagnetic signals. In 2017 International Multi-Conference on Engineering, Computer and Information Sciences (SIBIRCON) 2017, (pp. 386-389). IEEE.
33. Ahma L, Ibrani M, Hamiti E. Computation of SAR distribution in a human exposed to mobile phone electromagnetic fields. PIERS (Progress in electromagnetic research) Proceedings 2010, pp. 1580 – 1582.
34. Miranda KM, Espey MG, Wink DA. A rapid, simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide 2005, 5, 62–71.
35. Shahbazi-Gahrouei D, Razavi S, Koosha F et al. Exposure of Extremely-Low Frequency (ELF) magnetic field may cause human cancer. Acta Medica International 2017, 4(1), 32.
36. Núñez-Enríquez JC, Correa-Correa V, Flores-Lujano J et al. Extremely Low-Frequency Magnetic Fields and the Risk of Childhood B-Lineage Acute Lymphoblastic Leukemia in a City With High Incidence of Leukemia and Elevated Exposure to ELF Magnetic Fields. Bioelectromagnetics 2020, 41(8), 581-597.
37. Kroll ME, Swanson J, Vincent TJ et al. Childhood cancer and magnetic fields from high-voltage power lines in England and Wales: a case–control study. British journal of cancer 2010, 103(7), 1122-1127.
38. Wertheimer N, Leeper E. Electrical wiring configurations and childhood cancer. Am J Epidemiol 1979, 109:273-384.
39. Polk C, Postow E. Handbook of Biological Effects of Electromagnetic Fields, 1996, pp 1-23, 337-345, 435-533, Second Edition, CRC Press, Florida, USA.
40. Kato M, Honna K, Shigemitsu T, et al. Effects of exposure to a circularly polarised 50-Hz magnetic field on plasma and pineal melatonin levels in rats.Bioelectromagnetics 1993; 14: 97–106.
41. Davis S, Kaune WT, Mirick DK, et al, Residential magnetic fields, light-at-night, and nocturnal urinary 6-Sulfatoxymelatonin concentration in women. Am J Epidemiol 2001; 154: 591–600.
42. Sabban IF, Pangesti G, Saragih HT. Effects of exposure to electromagnetic waves from 3G mobile phones on oxidative stress in fetal rats. Pakistan Veterinary Journal 2018, 38(4).
43. Gulati S, Kosik P, Durdik M et al. Effects of different mobile phone UMTS signals on DNA, apoptosis and oxidative stress in human lymphocytes. Environmental Pollution 2020, 267, 115632.
44. Devrim E, Ergüder IB, Kılıçoğlu B, et al. Effects of Electromagnetic Radiation Use on Oxidant/Antioxidant Status and DNA Turn-over Enzyme Activities in Erythrocytes and Heart, Kidney, Liver, and Ovary Tissues From Rats: Possible Protective Role of Vitamin C.Toxicol Mech Methods. 2008 Jan;18(9):679-83.