DOI: 10.4149/BLL_2018_088

Bratisl Med J 2018; 119 (8)
481 – 489

EXPERIMENTAL STUDY

The physiopathological effects of quercetin on oxidative stress in radiation of 4.5 g mobile phone exposed liver tissue of rat

Postaci I[1], Coskun O[2], Senol N[3], Aslankoc R[4], Comlekci S[2]

Department of Bioengineering, Institute of Science, Suleyman Demirel University,Isparta, Turkey.
irempostaci@gmail.com

ABSTRACT
OBJECTIVE: The study was aimed to evaluate the physiopathological consideration of the effects of electromagnetic field (EMF) from the radiation of 4.5 G mobile phones on the liver tissue of rats and quercetin (Qu) applied as an antioxidant for reducing these effects.
METHODS: Male Wistar-Albino rats were divided into four groups with 8 rats in each group. Group 1 (control group), Group 2 (sham group), Group 3 (EMF group) and Group 4 (EMF + Qu). From the animals sacrificed at the end of the 30th day; liver tissues were taken for histopathological and immunohistochemical examinations.
RESULTS: In the liver tissue of the electromagnetic field group; dilatation of sinusitis was determined to be higher than in the sham group. It was concluded that the concentration of caspase-3 and TNF-α immunopositive cells was in the EMF group (+3) level and also the immunostaining was stronger, it caused an increase in malondialdehyde level, the difference between the groups was statistically significant, in terms of superoxide dismutase, catalase activities, the difference was not significant.
CONCLUSION: It was determined that 2600 MHz EMF exposure caused damage to the liver, 100 mg/kg/day quercetin was not sufficient to prevent this damage *(Tab. 5, Fig. 15, Ref. 27)*. Text in PDF *www.elis.sk.*
KEY WORDS: 4.5 G, liver, mobile phone, quercetin, rat.

Introduction

If we consider that mobile phone users use their mobile phones and access internet for a few hours per day, it can be thought that process will be the case with 4.5 G operating frequency and conditions. 4.5 G-type communication will be available with a wider bandwidth in digital modulation from 2600 MHz center frequency.

However, the high rate of downloading and video viewing suggests that the exposure time will be reduced, but it can be said that the service will not change when the effects of personal communication are taken into account in terms of habits in modern life. A higher frequency broadcast, which will increase bandwidth, makes it a reality that people will be exposed to higher SAR (Specific Absorption Rate) values. As is known, even at the same electric field values in the environment, the conductivities of living tissues increase with frequency. Therefore, the amount of energy absorbed in the tissue is also increasing.

Mobile phones are often placed in front or side pockets (near the liver). When carried in the waist region, the antenna of the device is located close to the abdominal organs, causing concern about the biological interactions between electromagnetic radiation and internal organs. The electromagnetic field of mobile phones can be absorbed by various body organs according to the places they are carried. The liver is an easier target for the effects of the electromagnetic field due to its high iron content (1). This increases the importance of the examination of the liver.

EMF's affect cells and tissues by thermal and non-thermal pathways, depending on their different frequencies and powers. Biological effects such as headaches, eye burns, dizziness, fatigue, malaise, which are caused by very low frequency EMFs, are formed by non-thermal routes. The long-term effects on living molecular and chemical bonds, genetic material (DNA), cell morphology, proliferation, apoptosis, membrane structure and function and body defense systems are the effects of nonthermal pathways created by high frequency EMFs. The occurrence of biological effects depends on the intensity of EMF, frequency, body measurements, electrical properties of the body, the distance of EMF and the duration of action. Biological effects such as nervous system diseases such as Alzheimer's, Parkinson's and multiple sclerosis, alteration of hormone and enzyme systems, teratogenic effect and some anomalies in spermatocyte chromosomes observe in the research conducted on this topic (2, 3).

[1]Department of Bioengineering, Institute of Science Suleyman Demirel University Isparta, Turkey, [2]Department of Electronics and Communication Engineering, Suleyman Demirel University Isparta, Turkey, [3]Department of Nutrition and Dietetics, Faculty of Health Sciences, Suleyman Demirel University Isparta, Turkey, and [4]Department of Physiology, Faculty of Health Sciences, Suleyman Demirel University Isparta, Turkey

Address for correspondence: I. Postaci, Bioengineering. Suleyman Demirel University 32260 Isparta, Turkey.
Phone: +902462111373, Fax: +902462370859

Acknowledgment: This study has been supported by Suleyman Demirel University Scientific Research Project Council (Project Code 4741-YL 2-16).

The research shows that the magnetic field is more effective than the electric field. That is, in some of the components of a device that measures waves in the brain (EEG) in mechanisms exposed to severe magnetism, short-term changes observe. However, no significant effect has been seen in studies conducted on humans. In another study on mice, the mice were first trained to be rewarded and then let under the influence of electric and magnetic fields. Compared to those who were not affected by the effects of these fields, a decrease in response rate is observed (4, 5).

Even Exremely Low Frequency (ELF) fields are low energy fields; in some cases, it is known that cell membranes are highly sensitive to externally applied low frequency fields. Very low signal changes can lead to important biochemical responses in cell function (6, 7).

In a study of Khitrov et al, which they investigate the effect of microwave radiation on potassium ion transport and oxygen consumption in perfused rat livers, they report that the perfused rat livers which are exposed to microwave irradiation (2450 MHz, 0.1–5 W/g) are not different from rat livers the inducted with heating effects. This finding discloses that the mechanism of microwave radiation is a "thermal" effect (8).

D'Andrea et al, investigate the physiological and behavioral effects of chronic exposure to 2450 MHz microwave in their study. Long-Evans Male adult rats were exposed to an average power density of mW/cm^2 for sixteen weeks for 2450 MHz CW microwaves. The resulting dose rate was 1.23 (+/– 0.25 SEM) mW/g. Measurements of 17-ketosteroid levels in urine during 1, 5, 9, 12 weeks of exposure and mass of adrenals, heart and liver show no evidence of stress during the 16 weeks post-exposure period (9).

Çinar et al, investigated the positive effects of 10 kV/m, 1.9 kV/m and 0.9 kV/m electric field application on wound healing. They determined the differences in wound healing process between groups connected to collagen fiber density, inflammation infiltration density, capillary proliferation and exudate presence parameters. As a result, it has been observed that the application of 0.9 kV/m–1.9 kV/m direct current (DC) electric field with 30 µs repetition improves collagen synthesis and wound healing positively. Despite its density, it has been concluded that the 0.9–1.9 kV/m pulsed electric field accelerated the recovery, but 10 kV/m field slowed down this recovery process (10).

The imbalance between the free oxygen radicals and the antioxidant defense system leads to the resulting oxidative stress. Oxidative stress affects certain cellular events such as inflammation, cell proliferation and cell survival and death. Free radicals cause oxidative stress formation and can lead to cell death. Antioxidant enzymes like superoxide dismutase (SOD), catalase, reactive oxygen species convert water and oxygen. These antioxidants are known to protect cells and tissues against oxidative damage (11).

Yürekli investigated the effects of base station radiation at 945 MHz in a repeatable experimental setup according to the conditions in free space on oxidative stresses in young adult male Wistar albino rats. At the conclusion of the study, it has been observed that MDA levels increased significantly ($p < 0.0001$) and reduced glutathione (GSH) levels were decreased significantly ($p < 0.0001$). when an electromagnetic field was applied with a power level of 3.67 W/m^2 (SAR = 11.3 mW/kg). Also a significant increase in SOD level ($p = 0.0190$) is observed (12).

Kuzay D et al applied radio frequency-RF radiation to both healthy and diabetic rats at 20 min/day, 5 days/week for one month at 2100 MHz. As a result of exposure; both healthy and diabetic rats had increased levels of MDA in the testes and decreased glutathione (GSH) levels, one of the powerful antioxidants produced by the body. It is stated in the literature that exposure to RF radiation increases oxidative stress and decreases antioxidant capacity, depending on the duration of exposure (13).

Quercetin will be used as an antioxidant to prevent damage to the tissues caused by the electromagnetic field exposure. Quercetin (3,5,7,30,40-pentahydroxyfl avone), is a member of the flavonoid family of polyphenols. Quercetin is found in a variety of food products and plants such as fruits, vegetables, tea, coffee, onions, apples and blueberries. Quercetin is a natural antioxidant and its anticancer properties have been demonstrated in *in vivo* and *in vitro* experiments. It is known as a potential anti-cancer agent. Several studies have shown that quercetin has an important role in the inhibition of breast, colon, prostate, ovarian, lung tumor cancer cells and protection of various diseases such as pulmonary diseases, cardiovascular diseases and neurodegenerative disorders (14, 15).

The lack of literature on the use of quercetin (3, 3', 4', 5,7-pentahydroxy flavone) in order to reduce the damage and the damage caused by the electromagnetic field generated by the 4.5 G mobile telephone transmission, increases the importance of the chosen topic. In addition, control of whether applied 2600 MHz electromagnetic field caused apoptosis and necrosis by staining induced by caspase 3 with TNF-α antibodies and MDA measurement which is an oxidative stress indicator, tissue protein determination and measurement of SOD, CAT enzyme activities, also made the subject original. This planned study is not only up-to-date; it is also very important because of the methods used and animal study.

So, the study aims to evaluate the physiopathological consideration of the effects of EMF from the radiation of 4.5 G mobile phones on the liver tissue of rats and quercetin to be applied as an antioxidant for reducing these effects.

Material and methods

Chemicals

Quercetin (95 %) was purchased from Sigma-Aldrich, America. Ketamine was purchased from Richter Pharma AG, Wels, Austria and Xylazine was purchased from Alfasan International B.V., Woerden, Holland.

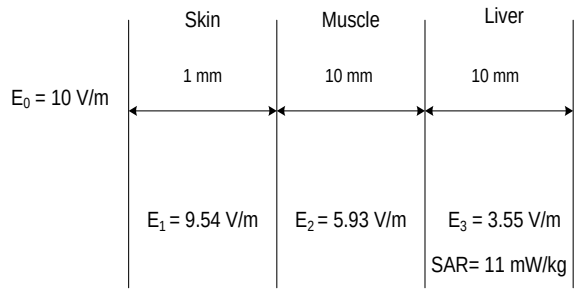
Working groups

The experiment was carried out in 32 male Wistar rats with average weight 418g and 30–34 weeks old, obtained from Experimental Animal Research Center of Suleyman Demirel University. This study was approved by the ethics committee of the same center (05.05.2016–06/04). All the animals were housed under temperature and light-controlled conditions (12:12-hour light-dark cycle, 22 ± 2 °C), with food and water available adlibitum.

Tab. 1. Dielectric properties of body organs according to IFAC (Applied Physics Institute) at 2600 MHz.

	Conductivity (S/m)	Relative Permittivity (F/m)
Skin	1.5357	37.845
Muscle	1.8429	52.546
Liver	1.7879	42.79

Tab. 2. SAR value account on the liver.



All rats were randomly divided into four groups as EMF, EMF + Quercetin (Qu), Sham and Control. EMF and EMF + Qu groups were exposed 1 hour/day EMF for 30 days. During this period EMF is applied to EMF and EMF+ Qu groups and in addition Quercetin (Qu) gavage method is applied to EMF+Qu group. The working groups were formed as follows:

I. group: Control group. Rats without EMF exposure and gavage application (n = 8),
II. group: Sham group. Rats kept in EMF apparatus (n = 8),
III. group: EMF group. Rats exposed to EMF and administered 1 ml of water through gavage method (n = 8),
IV. group: EMF + Qu group. Rats exposed to EMF and applied 100 mg/kg/day of antioxidant with gavage method (16). (Quercetin (3,3 ‘, 4’, 5,7-pentahydroxy flavone) (n = 8).

Experimental design

We used a test transmitter (SET Electronics Communication, Sakarya, Turkey) capable of supplying 1 W RMS power to a 50 Ohm output load with 2600 MHz center frequency (2550–2650 MHz tuned) as an EMF source. The monopole antenna of this device has 50 ohm impedance and was located in the middle of the Carousel experimental setup.

Through test transmitter also the waveform of the electromagnetic wave fed to the antenna can be adjusted as pulsed/continuous and the power van is adjusted between 0.1 W–1 W. Thus, the intensity of the desired electromagnetic field can be generated in the close or far area of the antenna. By using the Electromagnetic Field Meter (EXTECH Instruments Corporation, U.S.A), the transmitter output power was fixed at that point up to a value of 10 V/m of the nearest electric field to the target tissue (rat liver) in the experimental setup. Thus, the SAR value in the target tissue during exposure can be calculated. A literature search was conducted for the electrical properties of the target tissue at 2600 MHz frequency. These values were used to calculate the mean

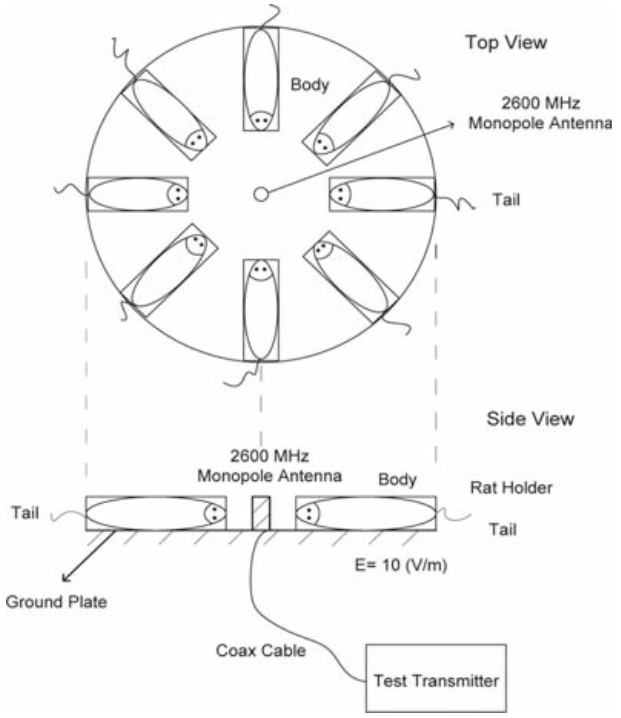


Fig. 1. The experimental setup.

Fig. 2. EMF group during the experiment.

SAR values induced in the tissue using the Time-Lapse Differential (FDTD) method (Tab. 1).

It is accepted that the electric field that mobile phones and wireless communication devices create at the external medium with their antennas is about 10 V/m. For this reason, the device is set to 10 V/m. SAR (Specific Absorption Rate) value on the liver is calculated according to parameters accepted as thicknesses of the skin=1 mm, muscle=10 mm, liver=10 mm and reference values determined according to IFAC (Tab. 2).

The rats were placed in plastic tubes with a diameter of 5.5 cm and a length of 12 cm. The system was arranged so that the rats placed in the tube would be equidistant from the monopole

antenna. In Figure 1. exposure apparatus of rats to the electromagnetic field is provided (Fig. 2).

Preparation of tissue samples

Twenty-four hours after completion of the 30-day experiment, liver tissues were removed by dissection under ketamine-xylazine (Xylazine HCl 10 mg/kg+Ketamine HCl 90 mg/kg) anesthesia in all rats and livers are left in 10 % formaldehyde for 48 hours. Liver samples were then taken to washing step under running tap water overnight. Following routine histological follow-up procedures, tissues are buried in paraffin. Sections with a thickness of 5 µm were cut from the liver tissue using a microtome (Leica RM2125) and the samples were placed in lysine slides.

Histopathological examinations

The liver tissues obtained from all groups were fixed in formalin and paraffin blocks were prepared following routine histologic follow-up procedures. Hematoxylin Eosin staining was then performed for 5 µm thick sections and samples are examined on a Leica DM 500 light microscope (16).

Immunohistochemical examinations

In liver tissue; necrosed cells and apoptotic cells determined by Streptavidin Peroxidase Immunohistochemical Method, using TNF-α for necrosed cells and using caspase-3 and antibodies for apoptotic cells. Liver tissues from each group were fixed in 10 % formaldehyde solution. After routine follow-up, it is embedded in paraffin. Sections taken from paraffin blocks with a thickness of 4–6 µm were taken into polylysine slides. First, deparaffinized tissues were washed with PBS (Phosphate Buffered Saline) and then treated with hydrogen peroxide solution for 5 minutes to prevent endogenous peroxidase activity (17).

Sections were treated with 1 M sodium citrate solution (pH 6.0) for 12 minutes in a microwave to expose antigenic receptors from the cells washed 3 x 5 minutes with PBS. Ultra V Block solution is applied to tissue sections washed several times with PBS to prevent non-specific antibody binding for 5 minutes after that these sections were incubated with primer antibodies TNF-α and caspase-3 for 60 min in a humid environment at room temperature. The tissues were washed with PBS for 3x5 minutes after the application of primary antibodies and incubated with secondary antibody (biotinylated Goat Anti-Polialent) for 30 min. in a humid environment. The tissues were washed with PBS for 3x5 minutes after the application of the secondary antibody and incubated with Streptavidin Peroxidase for 30 minutes in the humidified room at room temperature and then taken into PBS. DAB Substrate Kit solution was instilled into the tissues and the image signal was taken on a light microscope and then washed with PBS simultaneously. Mayer's hematoxylin counterstained tissues were passed through PBS and distilled water and covered with appropriate closure solution. The prepared preparations were examined, evaluated and photographed on a Leica DM 500 microscope. The evaluation of immunohistochemical staining was based on the intensity and extent of staining. The intensity and prevalence of cytoplasmic immunostaining were scored semi-quantitatively (0: no, +1: less, +2: moderate, +3: severe) with a score between 0 to +3.

Malondialdehyde (MDA)

Draper and Hadley's double heating method was used for MDA measurement from lipid peroxidation products. Trichloroacetic acid (TCA) was first added to the samples, vortexed, centrifuged for 10 min after 15 min of boiling. These prepared samples were then boiled again with adding thiobarbituric acid (TBA). Samples were measured for absorbance at 532 nm on a spectrophotometer (Shimadzu UV-1601, Germany) and the results were reported as nmol/gr.Hb and calculated from standard absorbance values obtained from the same samples (18).

Protein determination in tissue

The supernatants of the homogenized samples were measured by manual spectrophotometry using the Bradford method. The optical density-concentration plot generated by the absorbance values of the standards and all samples were evaluated according to this standard graph. The results were divided into microprotein levels and expressed as enzyme activity in the tissue (19).

Superoxide dismutase (SOD)

The principle of this method is the formation of uric acid and O_2-radical from xanthine through a reaction catalyzed by xanthine oxidase and the subsequent formation of red colored formazan through the reaction of INT (2-(4-iodophenol)-5 phenyl tetrazolium chloride and O_2-radical. SOD activity is measured by the degree of inhibition of this reaction. The results obtained were expressed as U/mg protein (20).

Catalase (CAT)

CAT activity was studied according to the Aebi method. The method is based on the principle that the spectrophotometric measurement of absorbance of H_2O_2 during its transformation to water and molecular oxygen in the presence of CAT at 240 nm. Results were expressed as U/mg protein (21).

Tab. 3. Histopathological evaluation of the groups (0: None, +1: Less, +2: Moderate, +3: Severe).

	EMA	EMA+ Quercetin	Sham	Control
Hydropic vacuolar degeneration	+	+	+	±
Dilatation in sinusoids	++	–	+	±
Mononuclear cell infiltration	+	+	+	–
Dilatation in veins	+	+	+	+
Necrotized areas	+	±	+	±

Tab. 4. Caspase 3 and TNF-α immunopositive cell density (0: No +1: Less, + 2: Moderate, + 3: Severe).

	EMA	EMA+ Quercetin	Sham	Control
Caspase-3	+++	++	++	+
TNF-α	+++	++	++	+

Results

Histopathologic and ımmunohistochemical findings

In the light microscope evaluation of the Hematoxylin & Eosin staining, hydropic vacuolar degeneration, sinusoidal dilation, mononuclear cell infiltration, dilatation and necrotized areas were evaluated semi-quantitatively (0: None, +1: less, +2 Moderate, +3 severe). Damage to the liver was observed to be mild in the EMF + quercetin group and the control group and similar scores were obtained.

In the EMF and Sham groups, the damage was found to be higher. In the EMF group, the dilatation of the sinusoids was found to be higher than that of the Sham group. The intensity and prevalence of cytoplasmic immunostaining were scored semi-quantitatively (0: no, +1: less, +2: moderate, +3: severe) with a score between 0 to +3. The concentrations of caspase 3 and TNF-a immunopositive cells were higher in the EMF group (+3) and also in EMF group it observed that immunostaining is strong. In the EMF + Quercetin and Sham groups, moderate levels of caspase 3 and TNF-α immunopositive cells were detected. In the control group, a low level of immune positivity was detected.

In conclusion, it is thought that the stress factors may also be effective due to the damage in the Sham group where EMF effect is minimal and EMF causes moderate level damages in liver tissue. The dose of quercetin antioxidant used in this study is not sufficient (Tabs 3 and 4) (Figs 3–15).

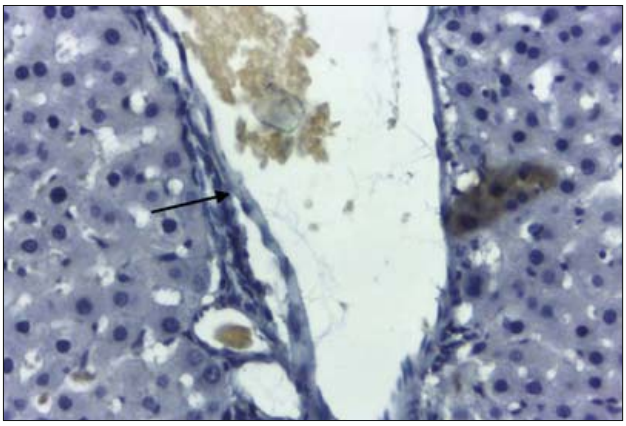


Fig. 5. EMF Group, Dilatation of the veins, H&E, X40.

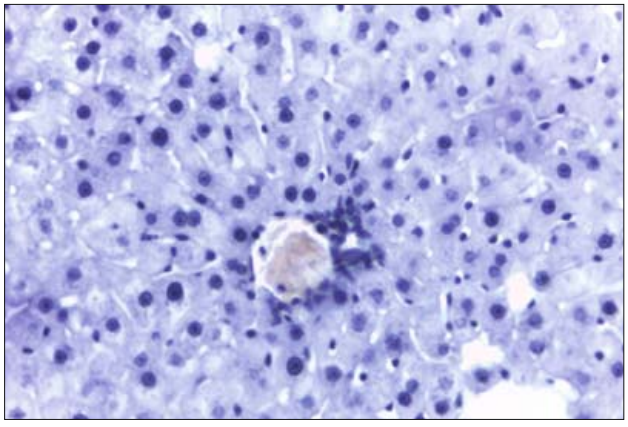


Fig. 3. Control Group, H&E, X40.

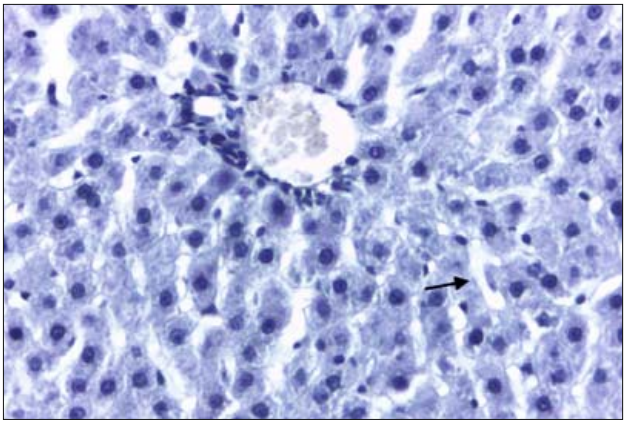


Fig. 6. EMF + Quercetin Group, Dilatation in Sinusoids (–), H&E, X40.

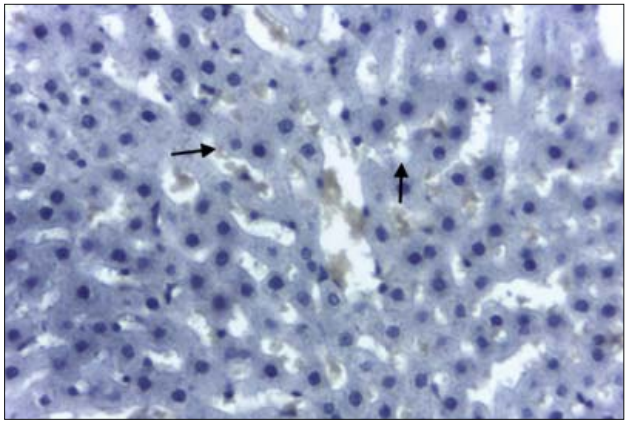


Fig. 4. Sham Group, Mononuclear Cell Infiltration, H&E, X40.

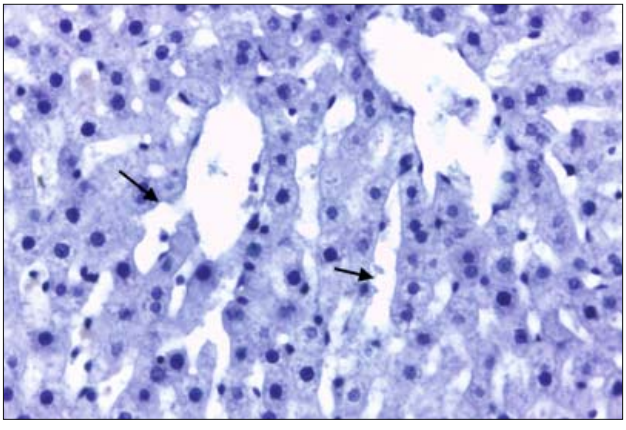


Fig. 7. EMF Group, Dilatation and necrosis areas in sinusoids, H E, X40.

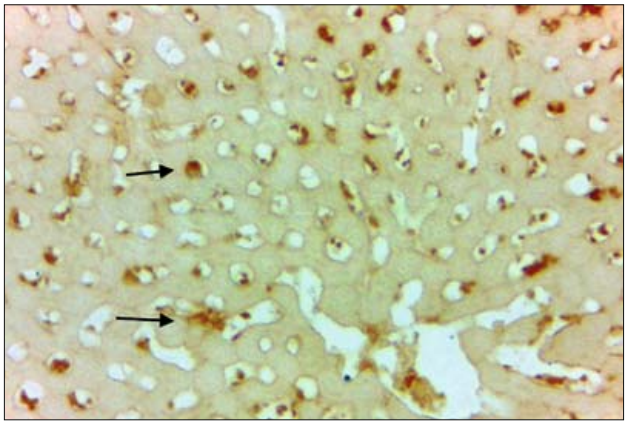


Fig. 8. Control Group, Caspase-3 immunopositive cells (+), H&E, X40.

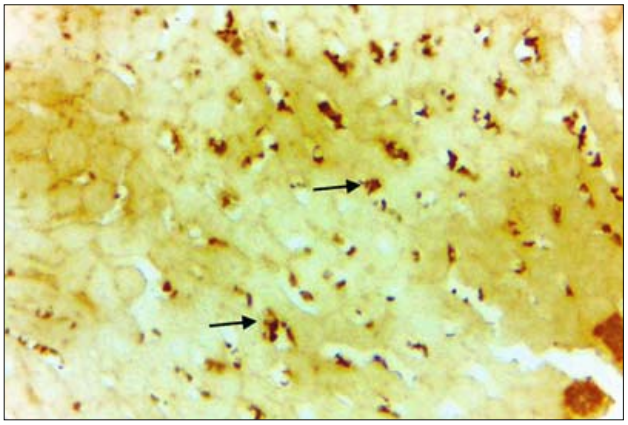


Fig. 11. EMF+Quercetin, Caspase-3 immunopositive cells (++), H&E, X40.

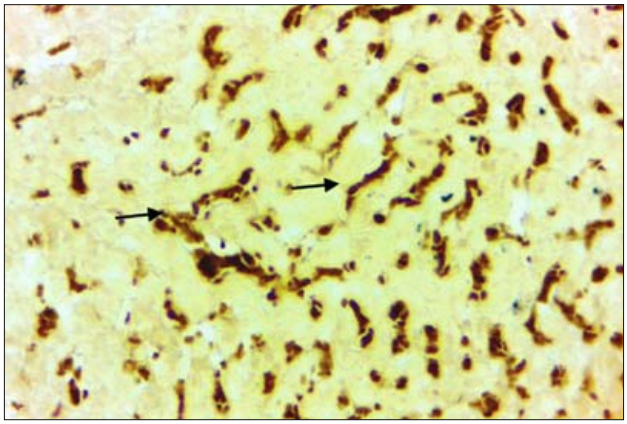


Fig. 9. Sham, Caspase-3 immunopositive cells (++), H&E, X40.

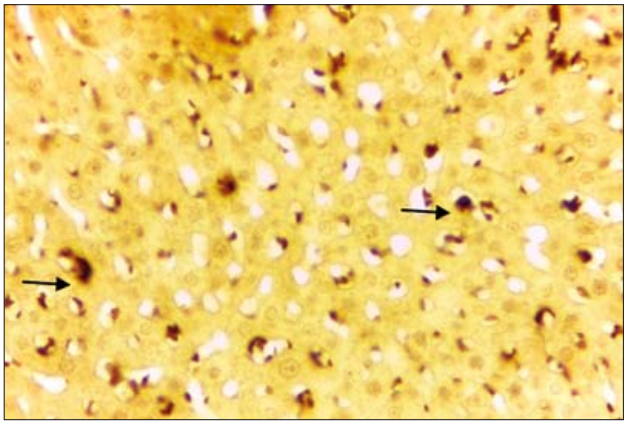


Fig. 12. Control, TNF-α immunopositive cells (+), H&E, X40.

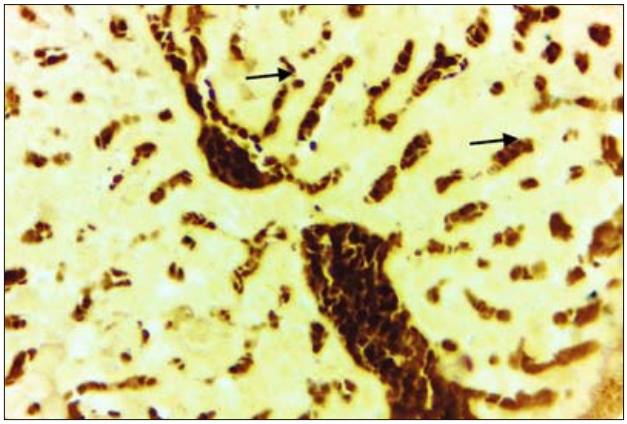


Fig. 10. EMF, Caspase-3 immunopositive cells (+++), H&E, X40.

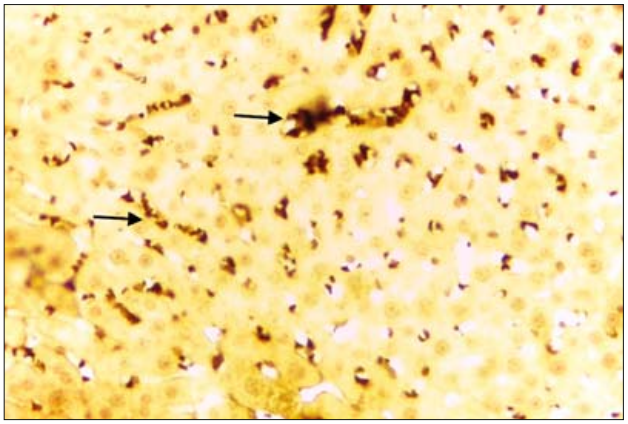


Fig. 13. Sham, TNF-α immunopositive cells (++), H&E, X40.

Oxidant and antioxidant enzyme results

All data were expressed as mean ± ss. Differences between the groups' averages were assessed using the one-way ANOVA post hoc Bonferroni test in the SPSS program. A significance level of $p < 0.05$ was considered.

Kruskal–Wallis test was used for statistical analysis of MDA levels of research groups. The difference between the groups was statistically significant ($p = 0.035 < 0.05$). In terms of MDA values in the comparison between groups; there was a statistically significant increase of MDA in the sham group compared to the

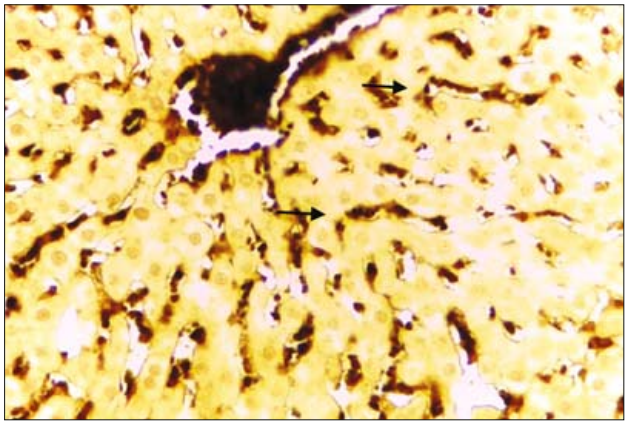


Fig. 14. EMF, TNF-α immunopositive cells (+++), H&E, X40.

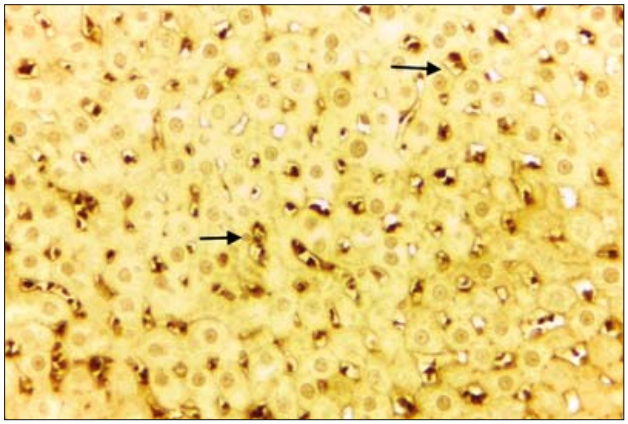


Fig. 15. EMF + Quercetin, TNF-α immunopositive cells (++), H&E, X40.

control group. There was a slight increase of MDA in the EMF group compared to the control group. However, this increase is not statistically significant. In the EMF + Qu group, there was a decrease in MDA levels compared to the EMF group. However, this decrease is not significant (Tab. 5).

One-way ANOVA test was used for statistical analysis of CAT levels of research groups. The difference between the groups was statistically insignificant ($p = 0.526 > 0.05$) in the statistical evaluation applied to the data obtained in terms of CAT values. In comparison, Sham group showed a decrease in CAT levels compared to the control group. However, this decrease was not statistically significant. CAT activity between the control group and the EMF group is not statistically significant. A decrease in the EMF + Qu group compared to the EMF group was observed. However, this difference is not statistically significant (Tab. 5).

Kruskal–Wallis test was used for statistical analysis of SOD levels of research groups. The statistical evaluation of SOD activities of research groups is presented in Table 5. There was no statistically significant difference between the groups on the statistical evaluation applied to the obtained data ($p = 0.332 > 0.05$).

Tab. 5. Oxidant and antioxidant levels of research groups for liver.

	Group I (Control)	Group II (Sham)	Group III (EMA)	Group IV (EMA+Qu)	p
MDA	0.99±0.13	1.43±0.43	1.08±0.11	1.00±0.23	0.035
CAT	1.20±0.86	0.79±0.38	1.08±0.42	1.05±0.84	0.526
SOD	0.68±0.15	0.69±0.02	0.67±0.06	0.73±0.12	0.332

MDA – nmol/gr. Hb, SOD – U/mg, CAT – U/mg
Values bearing different letters on the same line were found to be different from each other

The difference between the control group and the sham group is not significant. The difference between the control group and the EMF group was not statistically significant. There was an increase in SOD activity in the EMF + Qu group compared to the EMF group. However, this increase is not significant.

Discussion

The energy penetration depth decreases with the increase in frequency. For this reason, the majority of the energy from EMFs is absorbed close to the surface. Low frequencies reach far deeper than high frequencies. The results of the studies performed in low-frequency EMFs, such as those performed by Elbaz and Ghonimi are more likely to be due to the fact that the EMFs in the high-frequency range has a less deep effect than EMF's in low frequencies. In our study, the frequency of 2600 MHz is considerably higher than the EMF frequencies used in these studies. For this reason, it is unthinkable that the depth of its effect is higher than the lower frequencies (22).

Elbaz and Ghonimi examined changes in liver tissue structures in mature male albino rats under the effect of 50 Hz, 1 Gaussian EMF applied for 21 days. When the results were examined, severe pathological lesions were seen in the EMF group while a normal histological structure was observed in the liver in the control group. In this study, similar damage to the EMF group was observed, while damage to the liver tissue in the control group was mild (22).

Erpek et al, exposed rats to 6 militesla (mT) (50 Hz, alternating current) EMF for 2 hours a day, 7 days a week for 8 weeks. At the end of the experiment, dilatation of sinusoids, focal inflammatory cell infiltration, decrease of Kupffer cells, fatty degeneration in hepatocytes and decrease of glycogen content were observed in liver sections of some rats exposed to EMF. The sum of histological damage scores of liver and liver of EMF-treated rats was not statistically different from the control group ($p = 0.634$).

In this study, an increase is observed both in histopathological and immunohistochemical findings as well as oxidant enzyme results in the EMF group according to the control group. This is a sign of damage occurred in the EMF group (23).

Devrim et al, examined possible effects of the use of electromagnetic radiation (EMR) in liver tissue on oxidant and antioxidant role in rats and possible protective role of vitamin C in rats. One group was exposed a 900 MHz EMR, another group is exposed to EMR + C vitamin (250 mg/kg/day, 4 weeks). As a result, when compared with the control group, the MDA level in the EMF group increased significantly. In erythrocytes, vitamin

C appears to be partially protective against the oxidative stress. In our study, quercetin was also used as an antioxidant that reduces the oxidative stress caused by EMR. However, the administered dose of quercetin as 100 mg/kg/day was considered inadequate. More meaningful results can be obtained by increasing the amount of quercetin in the next study (24).

Holovska et al, investigated the effect of the 2.45 GHz electromagnetic field on the liver in rats. The study demonstrated the presence of moderate hyperemia, dilatation of the liver sinusoids and small inflammatory foci at the center of the liver lobules. The structure of the hepatocytes has not changed. When examined electron microscopically, necrotizing hepatocytes were also observed. They reach the conclusion that EMF damages the rat liver. Similar to the results of this study in that study, negative results were observed in the EMF group compared to the control group (25).

Koyu et al, conducted a study on the oxidative stress formation on the rat liver tissue as a result of exposure to 900 MHz electromagnetic fields and whether CAPE (caffeic acid phenethyl ester), an active component of the antioxidant propolis extract, reduces the effect of this oxidative stress. As a result; Compared with the EMF group and the control group, an increase in CAT activity and a decrease in SOD activity were observed. They have reached the conclusion that CAPE can prevent oxidative changes in the liver caused by 900 MHz EMF by reducing reactive oxygen species and by increasing antioxidant enzyme activities and strengthening the antioxidant defense system. Since we consider antioxidant use has reduced the harmful effects of EMF, quercetin has also been administered in our study. When compared with the EMF group and the EMF + Qu group, it was observed that 100 mg/kg of quercetin per day caused a decrease in the MDA value, which is an oxidative stress indicator and an increase in antioxidant enzyme activities (CAT value) (26).

Cellat and Kiliçalp exposed guinea pigs to both 900 MHz electromagnetic fields for one month and administered oral green tea extract as a daily dose of 100 mg/kg. There were statistically significant increases ($p \leq 0.001$) in MDA, nitrate and nitrite levels compared to the control group and significant decreases ($p \leq 0.001$) in GSH, SOD and GSH-Px activities in the group receiving electromagnetic radiation. In this study, quercetin administered at 100 mg/kg/day as an antioxidant has not been a damage preventive effect (27).

Conclusion

As a result, according to the results of histopathological and immunohistochemical findings in liver tissue exposed to 2600 MHz electromagnetic field, it has been observed that 2600 MHz EMF cause moderate damage to the liver tissue. Since these damages are also present in the sham group it has been concluded that this damage may occur due to stress factors and do not occur only because of EMF alone. It was also found that the 2600 MHz EMF caused an increase in the oxidative stress indicator MDA in the liver and the difference in SOD and CAT values was not significant in that situation. It is thought that increasing the exposure period may increase the damage. At the same time, it was determined that the dose of quercetin antioxidant used in this study was not sufficient.

References

1. AEid F, MEl-Gendy A, AZahkouk S, AEl-Tahway N, A.El-Shamy S. Ameliorative Effect Of Two Antioxidants On The Liver Of Male Albino Rats Exposed To Electromagnetic Field. Egypt J Hosp Med 2015; 58: 74–93.

2. Sobel E, Davanipour Z, Sulkava R, Erkinjuntti T, Wikstrom J, Henderson VW, Buckwalter G, Bowman JD, Lee PJ. Occupations With Exposure To Electromagnetic Fields: A Possible Risk Factor For Alzheimer's Disease. Am J Epidemiol 1995; 142: 515–524.

3. Coskun O, Comlekci S. The Influence of Pulsed Electric Field on Hematological Parameters in Rat. Toxicol Industr Health 2013; 29 (9): 861–865.

4. Bracken TD, Kheifets LI, Sussman SS. Exposure Assessment For Power Frequency Electric And Magnetic Fields (EMF) And Its Application To Epidemiologic Studies. J Expos Anal Environ Epidemiol 1993; 3: 1–22.

5. Coskun O, Naziroğlu M, Comlekci S, Ozkorucuklu S, Elmas O. Effects of 50 Hertz-1 mT Magnetic Field on Action Potential in Isolated Rat Sciatic Nerve. Toxicol Industr Health, 2011; 27 (2): 127–132.

6. Susman S. Exposure Assessment At Extremely Low Frequencies: Issues, Instrumentation, Modeling And Data. Radio Science 1995; 30: 151–159.

7. Coskun O, Comlekci S. Effect of ELF Electric Field on Some of Biochemistry Characters Serum in Rat. Toxicol Industr Health 2011; 27 (4): 329–333.

8. Khitrov IuA, Kakushkina ML. The Action Of Microwave Radiation On Potassium Ion Transport And Oxygen Consumption In The Perfused Rat Liver. Radiobiologiia 1990; 30 (2): 247–251.

9. D'Andrea JA, Gandhi OP, Lords JL, Durney CH, Johnson CC, Astle L. Physiological And Behavioral Effects Of Chronic Exposure To 2450 MHz Microwaves. J Microwave Power Electromagn Energy 1979; 14 (4): 351–362.

10. Cinar K, Comlekci S, Senol N. Effects of A Specially Pulsed Electric Field on an Animal Model of Wound Healing. Lasers Med Sci 2009; 24: 735–740.

11. Zakaria MMH, Hajipour B, Estakhri R, Saleh BM. Anti–oxidative effect of resveratrol on aluminum induced toxicity in rat cerebral tissue. Bratisl Med J 2017; 118 (5): 269–272.

12. Yurekli AI. Biological Effects of Electromagnetic Fields At Mobile Telecommunication Frequencies. Boğaziçi University, Institute of Biomedical Engineering, Doctor of Philosophy Thesis, 98 page, İstanbul, 2006.

13. Kuzay D, Ozer C, Sirav B, Canseven AG, Seyhan N. Oxidative effects of extremely low frequency magnetic field and radio frequency radiation on testes tissues of diabetic and healthy rats. Bratisl Med J 2017; 118 (5): 278–282.

14. Baghel SS, Shrivastava N, Baghel RS, Agrawal P, Rajput S. A Review Of Quercetin: Antioxidant And Anticancer Properties. World J Pharm Pharmac Sci 2012; 1(1): 146–160.

15. Rahvar M, Owji AA, Mashayekhi FJ. Effect of quercetin on the brain-derived neurotrophic factor gene expression in the rat brain. Bratisl Med J 2018; 119 (1): 28–31.

16. Wang C, Pan Y, Zhang QY, Wang FM, Kong LD. Quercetin and Allopurinol Ameliorate Kidney Injury in STZ-Treated Rats with Regula-

tion of Renal NLRP3 Inflammasome Activation and Lipid Accumulation. Plos One 2012; 7 (6): e38285.

17. Bancroft JD, Stevens A, Turner DR. Theory and Practice of Histological Techniques. pp. 129, London: Churchill Livingstone.

18. Drapper HH, Hadley M. Malondialdehyde Determination As Index Of Lipid Peroxidation. Methods Enzymology 1990; 186: 421–431.

19. Bradford MM, A Rapid And Sensitive Method For The Quantitation Of Microgram Quantities Of Protein Utilising The Principle Of Protein Dye Binding. Analyt Biochem 1976; 72: 248–254.

20. Woolliams JA, Wiener G, Anderson PH, McMurray CH. Variation ın the activities of glutathione peroxidase and superoxide dismutase and ın the concentration of copper ın the blood various breed crosses of sheep. Res Veterin Sci 1983; 34: 69–77.

21. Aebi H. Catalase In Vitro. Methods Enzymol 1984; 105: 121–126.

22. Elbaz A, Ghonimi WA. Exposure Effects of 50 Hz, 1 Gauss Magnetic field on the histoarchitecture changes of liver, testis and kidney of mature male albino rats. J Cytol Histol 2015; 6 (4).

23. Erpek S, Bilgin MD, Doger FK. Effects of electromagnetic field (50Hz, 6mt) on rat liver and kidney. Univ Adnan Menderes Med Fac J 2007; 8 (1): 5 – 11.

24. Devrim E, Erguder IB, Kilicoglu, B, Yaykasli, E, Cetin, R, Durak I. 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; 18: 679–683.

25. Holovska K, Almasiov V, Cigankova V, Benova K, Racekova E, Martoncíkova M. Structural and ultrastructural study of rat liver ınfluenced by electromagnetic radiation. J Toxicol Environ Health 2015; 78: 353–356.

26. Koyu A, Ozguner F, Yilmaz H, Uz, E, Cesur G, Ozcelik N. The protective effect of caffeic acid phenethyl ester (CAPE) on oxidative stress ın rat liver exposed to the 900 MHz electromagnetic field. Toxicol Ind Health 2009; 25 (6): 429–434. Doi: 10.1177/0748233709106821.

27. Cellat M, Kilinç Kiliçalp D. Effect of Green Tea on Prooxidant-Antioxidant Levels in the Kidney of Guinea Pigs Exposed to Electromagnetic Radiation. Univ Firat Veterin J Health Sci 2010; 24 (1): 17–21.

Received March 15, 2018.
Accepted April 13, 2018.