

The impact of electromagnetic radiation (2.45 GHz, Wi-Fi) on the female reproductive system: The role of vitamin C

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Abstract

The present study investigated the effects of applied continuous 2.45 GHz electromagnetic radiation (EMR), which might cause physiopathological or morphological changes in the ovarian, fallopian tubal, and uterine tissues of rats. We proposed that the addition of vitamin C (Vit C) may reduce these severe effects. Eighteen female Sprague Dawley rats were randomly divided into three groups with six animals in each: Sham, EMR (EMR, 1 h/day for 30 days), and EMR + Vit C (EMR, 1 h/day for 30 days 250 mg/kg/daily). Total oxidant status (TOS) and oxidative stress index (OSI) levels increased ($p = 0.011$ and $p = 0.002$, respectively) in the EMR-only group in ovarian tissues. In all tissues, TOS and OSI levels significantly decreased in the Vit C-treated group in ovarian, fallopian tubal, and uterine tissues ($p < 0.05$). Anti-müllerian hormone levels significantly increased in the EMR group ($p < 0.05$) and decreased in the Vit C-treated groups. Estrogen (E2) levels were unchanged in the EMR group, as the differences were not statistically significant. Immunohistochemical examination of the ovaries revealed significant increases in Caspase-3 expressions in the epithelial cells of the EMR group ($p < 0.05$). In the EMR group, hyperemia was observed in uterine tissues. Also, Caspase-3 and Caspase-8 were significantly increased in the EMR group ($p < 0.001$). Caspase-3 was significantly diminished with Vit C application in the ovarian and uterine tissues ($p < 0.05$). Caspase-8 was significantly diminished only in uterine tissues ($p < 0.05$). These results indicate that prolonged EMR exposure induced physiopathological changes in the ovarian, fallopian tubal, and uterine tissues due to oxidative damage. Under the conditions of this study, Vit C may have protective effects on female reproductive system against oxidative damage.

Keywords

Electromagnetic radiation, ovarian, fallopian tubes, uterine, vitamin C

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Introduction

The increasing usage of electrical and electronic equipment in recent years has facilitated our modern life. But, as a result of this, the level of electromagnetic radiation (EMR) in our environment has increased. EMR emitted from these modern devices may lead to the emergence of a variety of biological effects in the human body (Shahin et al., 2013).

Prolonged exposure to 2.45 GHz microwave radiation may lead to chronic kidney damage (Kuybulu et al., 2016), DNA breaks in brain tissue, neurological

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problems (Shahin et al., 2013), changes in cognitive function (Hossmann and Hermann, 2003), brain tumors, neurodegenerative diseases, childhood leukemia, congenital malformation (Khaki et al., 2016), sleep problems, weakness (Bakacak et al., 2015), a decrease in sperm parameters, and increased apoptosis-positive cells in rats (Shokri et al., 2015). Also, the exposure to EMR may cause headaches, fatigue, dizziness, anxiety, sleeplessness, depression, and lack of concentration (Asghari et al., 2016).

During the last decade, studies have shown that long-term exposure to EMR may have adverse effects on the female reproduction system (Gül et al., 2009). Ovaries may be affected more by environmental stressors such as radiation, which may result in germ cell apoptosis. EMR exposure caused infertility, separation of the thecal layer of primary follicles, irregular thickness in the zona layer, and decreased number of ovarian follicles in rats (Bakacak et al., 2015; Khaki et al., 2016).

Oxidative stress plays an important role in the pathophysiology of many diseases leading to membrane damage, apoptotic and necrotic cell death. Environmental factors such as EMR can cause oxidative stress and disrupt antioxidant defense systems (Saygin et al., 2011). Antioxidant defense systems are very important because they provide natural protection for all body tissues against the harmful effects of free radicals (Alchalabi et al., 2015).

Vitamin C (Vit C) is found in many fruits and vegetables. Vit C is also required in many essential metabolic processes in organism and in the physiological protection of the organism. Vit C is known as an important antioxidant that plays a role as a cofactor in many enzymatic reactions and in infection and inflammation, and it protects cells against various oxidative damages. Vitamin C may play an important role in reducing risk of diseases such as cancer, Alzheimer's disease, Parkinson's disease, and other degenerative diseases (Covarrubias-Pinto et al., 2015; Du et al., 2012).

The purpose of this study was to research long-term effects of 2.45 GHz EMR, including on anti-müllerian hormone (AMH) and estrogen levels oxidant/antioxidant status (Total anti oxidant (TAS), total oxidant status (TOS), and the oxidative stress index (OSI)) of ovary, fallopian, and uterine tissues. Also, histopathological and immunohistochemical changes in ovary, fallopian, and uterine tissues were examined in the present study.

Materials and methods

Animal model

All experiments were performed in accordance with the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research of our university (Approval date 3 December 2015). Animals were used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, prepared by our university (Saygin et al., 2011).

There was no significant difference among the average weight of 18 female Sprague-Dawley rats ($n = 18$), weighing 250–300 g, once divided into groups. Rats were housed individually in stainless steel cages in pathogen-free conditions in our laboratory at a temperature of $+24 \pm 3^\circ\text{C}$, with light between 08:00 a.m. and 8:00 p.m., and free access to water and food. They received a commercial chow diet. Environmental average light intensity of 4000 lux was applied and humidity ($40 \pm 10\%$) was maintained.

Study groups

After 1 week adaptation period, all the animals were randomly divided into three equal groups, each consisting of six rats:

Sham ($n = 6$): Sham group

EMR ($n = 6$): 2.45 GHz EMR exposed rats

EMR + Vit C ($n = 6$): 2.45 GHz EMR exposed rats + Vit C group (250 mg/kg/daily orally) (Devrim et al., 2008).

2.45 GHz EMR was applied on EMR and EMR + Vit C groups for 30 days (1 h/daily, 30 days, 9:00–12:00 a.m.).

The 1-h exposure to irradiation in EMR and EMR + Vit C groups was carried out between 9:00 a.m. and 12:00 a.m. every day. The welfare of rats was found normal during the exposure time. The first dose of Vit C administration was given 24 h prior to the experiment. The rats in sham group received isotonic saline solution of an equal volume to that used in EMR + Vit C by oral gavage. The rats were not anesthetized during the exposure. The sham group was kept in their cages under the same environmental condition but without exposure to EMR (Figure 1; Saygin et al., 2009, 2015).

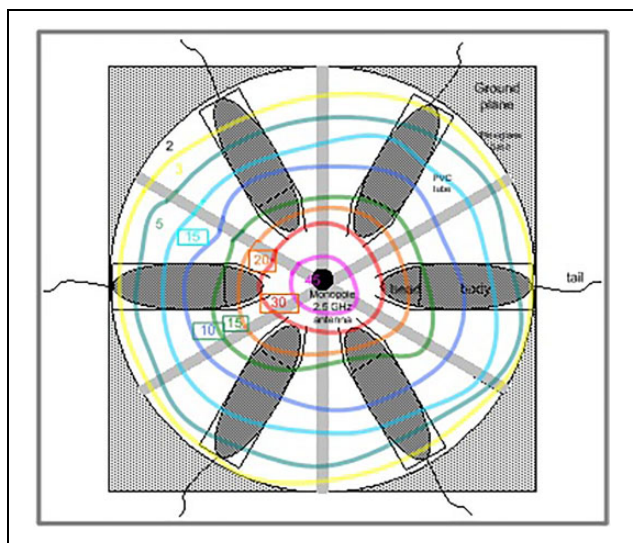


Figure 1. Experimental setup for EMR exposure on the rats. EMR: electromagnetic radiation.

Chemicals

All reagents except the phosphate buffers were prepared on the day of experiment and used fresh. The reagents were equilibrated at room temperature for half an hour before the start, and containers were refilled with reagents. Chemicals for the immunohistochemically and immunopathological analyses were obtained from Sigma-Aldrich Chemical Inc. (St Louis, Missouri, USA), and all organic solvents were from Merck Chemical Inc. (Germany). Vit C, which was used for the treatment group (III), was obtained from Redoxan Tb (Bayer, Germany).

Exposure system and design

Exposure system resource was obtained from SET ELECO (Set Electronic Co./Istanbul, Turkey). Exposure resource was a radio frequency (RF) test generator emitting 2.45 GHz RF, pulsed with 217 Hz by its monopole antenna system (Saygin et al., 2011). This device produces an electric field of 0.1 V/m to 45.5 V/m strength.

All the exposure systems were located in a screened room that had a shielding effectiveness of more than 80 db in the frequency range of 2.0–2.5 GHz. The whole system performance of the exposure device was tested and checked in the laboratory of the Department of Electronics and Communication Engineering. The exposure design and methodology were adapted from Faraone et al. (2006) and Saygin et al. (2011).

The exposure (or Ferris wheel) setup accommodated six rats at the same time. All rats were kept in cylindrical plastic restrainers, as shown in Figure 1.

Each restrainer provided the appropriate and equal exposure conditions for the physical size of one rat (length: 15 cm, diameter: 5 cm). The noses of the rats were positioned in a close contact to the monopole antenna, and the restrained animals were ventilated from head to tail to decrease the stress of the rats while they were in the tube. The health statuses of rats at the time of exposure were found normal for EMR only and EMR + Vit C groups.

All the reflection and exposure measurements were carried out utilizing the Portable RF Survey System (HOLADAY, HI-4417, Eden Prairie, Minnesota, USA) with its standard probe.

Repetition time, frequency, and amplitude of spectrum of RF energy were observed and verified by the satellite level meter (PROMAX, MC-877C, Barcelona/Spain). Specific absorption rate (SAR) value was calculated by an electromagnetic dosimeter (EXTECH Instruments Corporation, USA) using measured electric field intensity (V/m) and digital anatomical models based on the Finite-Difference Time-Domain (FDTD) numerical code. Consequently, SAR value was predicted for the same condition, orientation, and antenna power, using this method as 2.27 mW/kg for uterine and 0.8 mW/kg for ovarian tissue by using the simulation program. Also, the exposure of each group did not affect the other groups.

Anesthesia and tissue sampling

All rats were killed with a mixture of ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg), which were injected intraperitoneally before sacrificing. Then, the ovarian, fallopian tubal, and uterine tissues were removed and placed into a 10% formaldehyde solution (Beltrame et al., 2012) for the immunohistochemical evaluation.

Biochemical analyses

Tissue samples from the fallopian tubes, ovaries, and uterus were collected and homogenized in ice-cold phosphate buffer (pH 7.4) to produce a 10% homogenate (the tissue weight was prepared by adding 9 times phosphate buffer). Tissues were homogenized in a motor-driven tissue homogenizer (IKA Ultra-Turrax T25 Basic; Labortechnik, Staufen, Germany) and sonicator (UW-2070 Bandelin Electronic, Germany) with phosphate buffer (pH 7.4). Unbroken cells, nuclei, and cell debris were centrifuged at $10,000 \times g$ for 10 min at $+4^{\circ}\text{C}$.

TAS and TOS levels were quantified using a commercially available automated colorimetric kit (Rel

Assay[®], Diagnostics kits, Mega Tip, Gaziantep, Turkey) according to the manufacturer's instructions developed by Erel (2004), (2005), which was used for the tissue homogenate. The change of absorbance at 660 nm is related to the total antioxidant level of the sample, which can be measured spectrophotometrically. The results were expressed as mmol Trolox Eq/mg protein. The results are expressed in terms of mM hydrogen peroxide equivalent per g liter (mmol H₂O₂ Equiv/L, mmol H₂O₂ Equiv/mg protein). TAS and TOS were measured spectrophotometrically with the automated chemistry analyzer Beckman Coulter AU5800 (Japan). Estrogen levels were measured by ECLIA analyzer Beckman Coulter Unicel DxI 800 (Fullerton, CA). Estrogen assay results were expressed as ng/dL.

Serum AMH levels were quantified using a commercially available ELISA kit (Elabscience Biotechnology, WuHan, China; catalogue no. E-EL-R0640) according to the manufacturer's instructions. The sensitivity of the assay was 0.1 ng/L with inter- and intra-assay coefficients of variation of <10%. Assay range of AMH was 0.16–10 ng/mL. All samples were analyzed in duplicate. Assay results were expressed as ng/L.

Histopathological analyses

Ovary, fallopian tubes and uterus samples were collected during the necropsy and fixed in 10% buffered formalin. Using standard methods, tissues were embedded in paraffin and cut into 5- μ m thick sections. Tissue sections were stained with hematoxylin-eosin, and examined microscopically. Histopathological changes were graded in a blinded manner.

Microscopic analyses of ovary, fallopian tubes, and uterus were based on the qualitative and quantitative changes under a 40 $\times$ objective of an Olympus CX41 light microscope (Olympus Corporation, Tokyo, Japan). Morphometric evaluation was made using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

Immunohistochemical examination

Sections of 4- to 5- μ m thickness were placed onto polylysine-coated microscope slides. Streptavidin-biotin immunoenzymatic antigen detection system (EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436), (Abcam, Cambridge, UK)) was used as a second antibody.

Selected tissue sections were stained immunohistochemically in order to demonstrate Caspase-3 and Caspase-8 reactions. Commercial kits (Abcam, Cambridge, UK) were used for the immunohistochemical examinations. Genital system tissue samples were immunostained with Caspase-3 (Anti-Caspase-3 antibody (ab4051), 1/100 dilution); and Caspase-8 (Anti-Caspase-8 (ab25901), 1/100 dilution), according to the manufacturer's instructions. Ultimately, sections were counterstained with Harris hematoxylin, mounted in the mounting medium, and examined under the light microscope. All the slides were analyzed for immunopositivity, and a semiquantitative analysis was carried out. Samples were analyzed by examining five different sections in each sample, which were then scored from 0 to 3, according to the intensity of staining (0 = *absence of staining*; 1 = *slight*, 2 = *medium*, and 3 = *marked staining*) (Abdel-Wahhab et al., 1999). Immunohistochemical results were evaluated by one of the pathologists from another center. The animals included in this study were evaluated randomly in a blinded fashion by the researcher without knowing which rat was included into which group.

Statistical analyses

Variables were presented as frequencies, percentages, mean $\pm$ standard deviations (SD), median, or min-max. The Kolmogorov-Smirnov and Shapiro-Wilk tests were used to test for a normal distribution of continuous variables, and the Levene test was used for homogeneity of variance. Data characterized by a normal distribution were expressed as mean $\pm$ SD. Parameters without such distributions were expressed as a median with range. The groups were compared using the non-parametric Kruskal-Wallis test and Mann-Whitney *U* tests. Biochemical parameters were shown to fit a normal distribution, and ANOVA and post hoc LSD tests were used to compare the groups. Calculations were made using the SPSS 15.0 program pack (SPSS Inc., Chicago, Illinois, USA). *p* < 0.05 was set as the value for significance.

Results

Biochemical findings in serum

In serum, a statistically significant increase in AMH levels was found in the EMR group compared with sham group (*p* = 0.017). While a statistically significant

Table 1. AMH and estrogen levels of serum.^a

Groups	AMH (ng/L)		Estrogen (ng/dL)	
	Mean $\pm$ SD	p Value	Mean $\pm$ SD	p Value
Sham	0.71 $\pm$ 0.37	NS	29.80 $\pm$ 14.61	NS
EMR	3.38 $\pm$ 2.44 ^b	b: 0.017	41.00 $\pm$ 14.51	NS
EMR + Vit C	0.49 $\pm$ 0.22 ^c	c: 0.016	23.00 $\pm$ 14.79	NS

EMR: electromagnetic radiation; Vit C: vitamin C; AMH: anti-müllerian hormone; NS: not significant; ANOVA: analysis of variance.

^aValues are presented as means $\pm$ SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. Serum AMH levels were increased statistically significant in EMR group and Vit C treatment decreased the values.

^b $p < 0.05$ versus sham group.

^c $p < 0.05$ versus EMR group.

decrease of AMH levels was observed in EMR + Vit C group compared with EMR group ($p = 0.016$).

Estrogen levels significantly decreased in EMR + Vit C group compared with EMR group ($p = 0.021$; Table 1).

Findings in ovarian tissue

There were significant changes in the ovarian tissue among the groups in terms of TOS and OSI levels ($p < 0.05$) but not TAS levels ($p > 0.05$). In ovarian tissues, a statistically significant increase of TOS levels ($p = 0.011$) and OSI levels ($p = 0.002$) was observed in the EMR group compared with the control group. In contrast, a significant decrease in TOS levels ($p = 0.012$) and OSI levels ($p = 0.001$) was observed in Vit C-treated group compared to the EMR group (Table 2).

The histopathological and immunohistochemical examination of the ovaries revealed no pathological findings in any group. In the sections, hyperemia, inflammatory reactions in parenchymal tissue, degeneration, and necrosis of the cells were assessed. Five different randomly selected areas of the ovaries were examined. In all the groups, the ovaries had normal histological tissue structures (Figure 2). Immunohistochemical examination of the ovaries revealed statistically significant increase in Caspase-3 expressions in EMR group compared to control group in epithelial cells, but there was no difference in Caspase-8 expression among the groups (Table 5).

Findings in fallopian tubes

There were significant changes in fallopian tubes among the groups in terms of TOS and OSI levels

($p < 0.05$) but not TAS levels ($p > 0.05$). A significant decrease was observed in TOS and OSI levels of fallopian tube tissues ($p = 0.027$, $p = 0.008$, respectively) in EMR + Vit C group compared with the EMR group (Table 3).

The histopathological examination of the fallopian tubes revealed normal appearing superficial epithelium and cilia in all the groups. Hyperemia, degeneration or inflammatory reactions were not detected in any group at microscopical examination. There were no significant differences at immunohistochemical examination of the Caspase-3 and Caspase-8 reactions in fallopian tubes among the groups ($p > 0.05$; Table 5).

Findings in the Uterus

There were significant changes in the uterine tissue among the groups in terms of TAS, TOS, and OSI levels ($p < 0.05$). In uterine tissues, a statistically significant decrease of TOS levels ($p = 0.005$) and OSI levels ($p = 0.009$) was observed in the EMR + Vit C group compared with EMR group. Conversely, a significant increase of TAS levels ($p = 0.047$) was observed in Vit C-treated groups compared to the EMR group (Table 4).

Normal endometrial epithelium and gland architecture were observed in all groups' uterine tissues. Only marked hyperemia was observed in the EMR group (Figure 3) at the microscopic examination. Immunohistochemically, while negative immunoreaction was observed in the uterine cells of the control and EMR + Vit C-treated groups, marked immunopositive reaction was observed in endometrial, uterine gland, myometrial, and connective tissue cells in the EMR group (Figures 4 to 5). Results were statistically significant between EMR and control or EMR + Vit C groups (Table 5).

Discussion

The present study showed that the exposure to 2.45 GHz EMR caused oxidative stress in ovarian, fallopian tubes, and uterine tissues. EMR increased the level of oxidative stress markers in ovarian tissues. In addition, the Vit-C treated group showed a decreased oxidative damage in ovarian, fallopian tubes, and uterus tissues.

Because of the short estrous cycle in the rats, the stage of the cycle could not easily be determined from the appearance of the ovarian follicles (Yuan and

Table 2. TAS-TOS-OSI levels of ovarium tissues.^a

Groups	TAS (mmol Trolox equivalents/L)		TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv./L)		OSI	
	Mean $\pm$ SD	p Value	Mean $\pm$ SD	p Value	Mean $\pm$ SD	p Value
Sham	0.75 $\pm$ 0.27	NS	14.99 $\pm$ 8.52	NS	1.67 $\pm$ 0.29	NS
EMR	0.60 $\pm$ 0.23	NS	21.18 $\pm$ 7.76 ^b	b: 0.011	3.02 $\pm$ 0.62 ^b	b: 0.002
EMR + Vit C	0.82 $\pm$ 0.27	NS	7.60 $\pm$ 1.46 ^c	c: 0.012	1.22 $\pm$ 0.28 ^c	c: <0.001

EMR: electromagnetic radiation; Vit C: vitamin C; TAS: total antioxidant levels; TOS: total oxidant levels; OSI: oxidative stress index; NS: not significant.

^aValues are presented as means $\pm$ SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. While TAS levels were not affected by EMR exposure, TOS and OSI statistically increased. Vit C treatment ameliorated the levels in ovaries.

^b $p < 0.05$ versus sham group.

^c $p < 0.05$ versus EMR group.

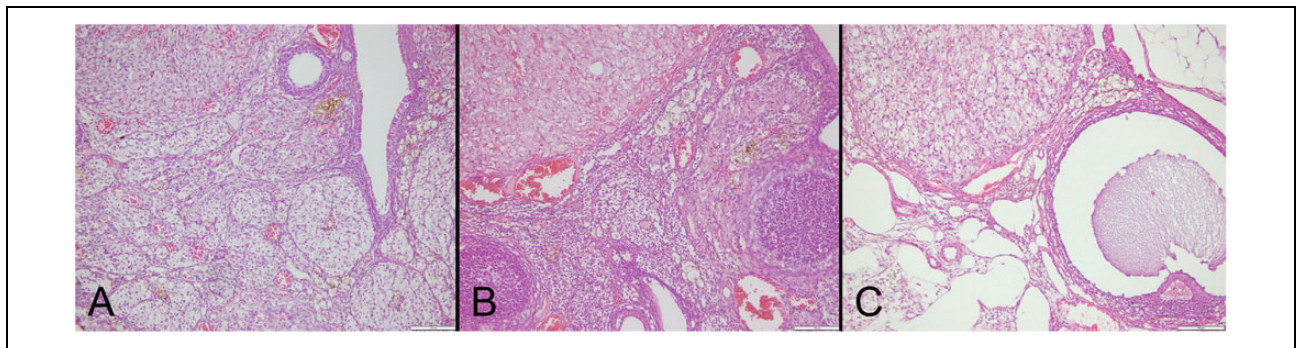


Figure 2. Normal histological architecture of the ovarium tissue in EMR + Vit C (a), EMR (b), and control (c) groups, HE, bar = 100 μm . Ovaries were the most resistant tissue of female genital system. EMR: electromagnetic radiation; Vit C: vitamin C; HE: hematoxylin-eosin.

Table 3. Oxidative stress markers of fallopian tubes.^a

Groups	TAS (mmol Trolox equivalents/L)		TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv./L)		OSI	
	Mean $\pm$ SD	p Value	Mean $\pm$ SD	p Value	Mean $\pm$ SD	p Value
Sham	0.60 $\pm$ 0.28	NS	4.90 $\pm$ 1.07	NS	0.63 $\pm$ 0.14	NS
EMR	0.57 $\pm$ 0.18	NS	5.49 $\pm$ 2.20	NS	0.80 $\pm$ 0.20	NS
EMR + Vit C	0.78 $\pm$ 0.14	NS	3.28 $\pm$ 0.35 ^b	b: 0.027	0.50 $\pm$ 0.15 ^b	b: 0.008

EMR: electromagnetic radiation; Vit C: vitamin C; TAS: total antioxidant levels; TOS: total oxidant levels; OSI: oxidative stress index; NS: Not significant; ANOVA: analysis of variance.

Values are presented as means $\pm$ SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. Although EMR insignificantly increased the TOS and OSI, Vit C significantly decreased the levels in fallopian tubes.

^a $p < 0.05$ versus EMR group.

^b $p < 0.05$ versus sham group.

Foley 2002). The duration of the estrous cycle (typically 4–5 days) of rats is a primary rationale for using these species in toxicology studies. Even within one strain of rat, recording of the stage of the cycle for routine screening toxicity studies is not necessary (Dixon et al., 2014). The main reason to choose rats for this study was its short estrous cycle. To minimize

any effect of the cycle, the rats were randomly divided into groups.

Previous studies have investigated different effects of EMR on ovarian and uterine tissues, but they did not investigate the effects on the fallopian tubes. Alchalabi et al. (2016), investigated the effect of 1800 MHz electromagnetic fields (EMF) on oxidative

Table 4. Oxidative stress markers of uterus.^a

Groups	TAS (mmol Trolox equivalents/L)		TOS ($\mu\text{mol H}_2\text{O}_2$ Equiv./L)		OSI	
	Mean $\pm$ SD	p value	Mean $\pm$ SD	p value	Mean $\pm$ SD	p value
Sham	0.45 $\pm$ 0.11	NS	5.70 $\pm$ 3.04	NS	1.45 $\pm$ 0.65	NS
EMR	0.38 $\pm$ 0.12	NS	7.57 $\pm$ 0.67	NS	2.26 $\pm$ 1.08	NS
EMR + Vit C	0.63 $\pm$ 0.23 ^b	b = 0.047	3.29 $\pm$ 1.35 ^b	b: 0.005	0.64 $\pm$ 0.14 ^b	b: 0.009

EMR: electromagnetic radiation; Vit C: vitamin C; TAS: total antioxidant levels; TOS: total oxidant levels; OSI: oxidative stress index; NS: not significant; ANOVA: analysis of variance.

Values are presented as means $\pm$ SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. Statistically significant amelioration was observed in oxidative stress markers in uterus by Vit C.

^ap < 0.05 versus sham.

^bp < 0.05 versus EMR group.

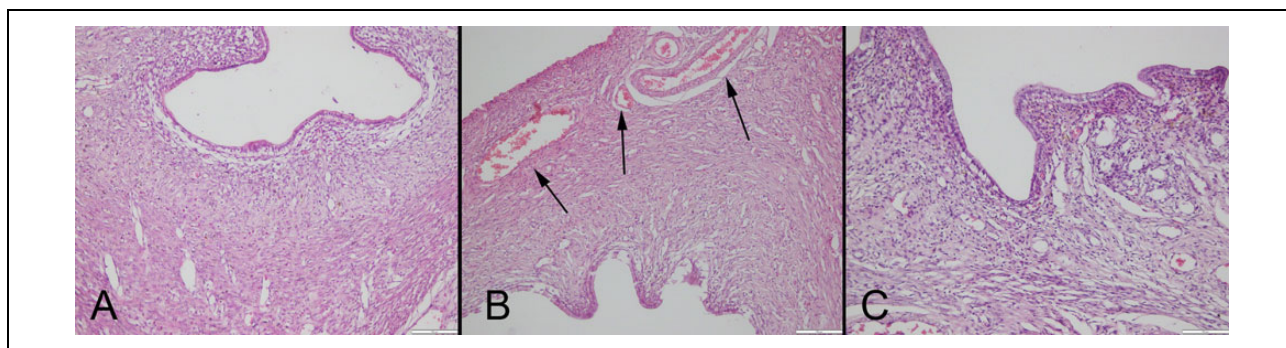


Figure 3. Histopathological appearance of the uterus. (a) Normal histology in EMR + Vit C group, (b) marked hyperemia in uterine vessels (arrows) in EMR group, (c) normal microscopical appearance of the uterus in control group, HE, bar = 100 μm . EMR caused slight inflammatory reaction in uterus. EMR: electromagnetic radiation; Vit C: vitamin C; HE: hematoxylin-eosin.

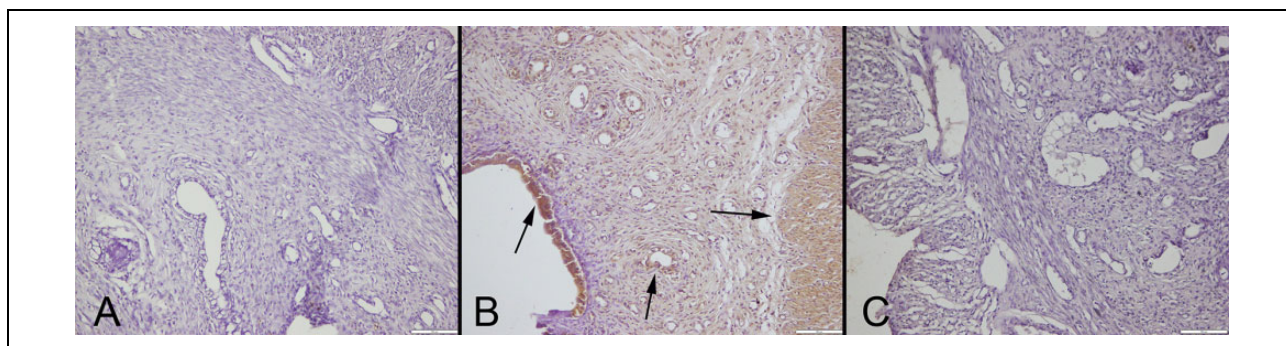


Figure 4. Caspase-3 immunoreaction between the groups. (a) Negative immunoreaction in EMR + Vit C group; (b) marked expression in endometrial, uterine gland, and myometrial cells (arrows) in EMR group; (c) negative reaction in control group, Streptavidin Biotin Peroxidase method, bar = 100 μm . While EMR caused apoptotic reaction in endometrium, Vit C has ameliorative effects. EMR: electromagnetic radiation; Vit C: vitamin C.

stress and histopathological markers in ovarian and uterine tissues and observed increased MDA levels and decreased GSH-Px activity. In another study, Sangun et al. (2015) performed 2.45 GHz EMR exposure for 1 h/day (whole body SAR value of 0.1 W/kg) during pregnancy and growth in Wistar rats. In this

study, TAS, TOS, OSI levels, and increased TOS and OSI were evaluated in ovarian tissues. Oral et al. (2006), investigated the effect of 900 MHz EMF on endometrial apoptosis in female Wistar albino rats, and they found an increase in MDA levels. We observed a significant increase in TOS levels and OSI

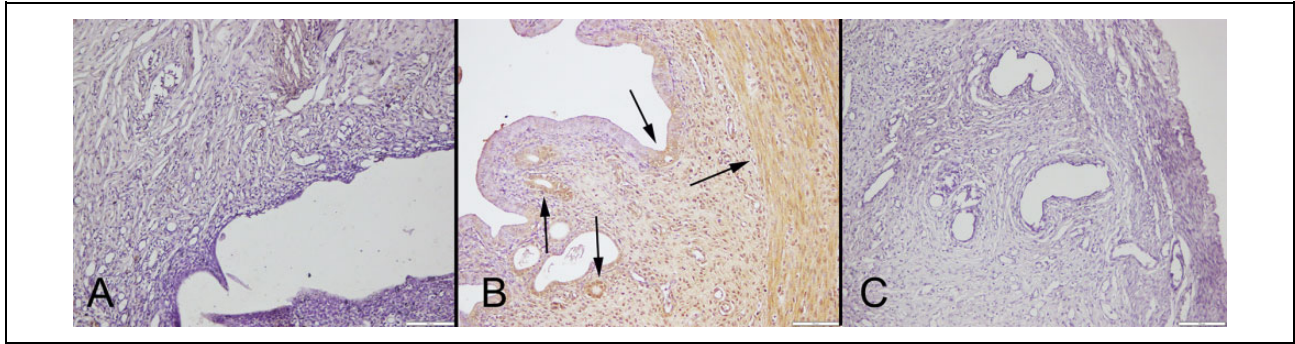


Figure 5. Caspase-8 immunoexpression between the groups. (a) Negative immunoreaction in EMR + Vit C group; (b) marked expression in endometrial, uterine gland, and myometrial cells (arrows) in EMR group; (c) negative reaction in control group, Streptavidin Biotin Peroxidase method, bar = 100 μ m. While EMR caused apoptotic reaction in myometrium and uterine glands, Vit C has ameliorative effects. EMR: electromagnetic radiation; Vit C: vitamin C.

Table 5. Statistical analysis of the immunohistochemical scores.^a

	Caspase-3			Caspase-8		
	Ovary	Fallopian tube	Uterus	Ovary	Fallopian tube	Uterus
Sham	0.20 $\pm$ 0.13	0.10 $\pm$ 0.01	0.20 $\pm$ 0.13	0.20 $\pm$ 0.13	0.20 $\pm$ 0.13	0.30 $\pm$ 0.15
EMR	2.10 $\pm$ 0.23 ^b	0.10 $\pm$ 0.01	2.10 $\pm$ 0.34 ^b	0.20 $\pm$ 0.13	0.20 $\pm$ 0.13	2.10 $\pm$ 0.27 ^b
EMR + Vit C	0.12 $\pm$ 0.12 ^c	0.00 $\pm$ 0.00	0.25 $\pm$ 0.16 ^c	0.25 $\pm$ 0.16	0.12 $\pm$ 0.12	0.25 $\pm$ 0.16 ^c

EMR: electromagnetic radiation; Vit C: vitamin C; NS: not significant.

^aValues are presented as means $\pm$ SD. The relationships between groups and results of immunohistochemistry markers are assessed by one-way ANOVA. Immunohistochemical scores showed that EMR caused apoptotic activity in ovaries and uterus and Vit C has ameliorative effects.

^b $p < 0.05$ versus sham.

^c $p < 0.05$ versus EMR group.

index in ovarian, fallopian, and uterine tissues in adult female rats caused by the exposure to 2.45 GHz. However, in the uterus tissues, there were no changes in TOS levels and OSI index compared with the control group. Thus, the damage of ovarian, fallopian, and uterus tissues following exposure to 2.45 GHz EMF was demonstrated in other researches. The development of primordial follicles is an important step in the development of the oocyte. These follicles are responsible for the formation of the ovarian reservoir (Gaytan et al., 2014). Roushangar et al. (2014), applied 3 mT EMF 4 h/day during the pregnancy period in mice and showed that EMF affected oocyte differentiation, folliculogenesis, and decreased the ovarian reservoir. Ahmadi et al. (2016) showed that the exposure to a magnetic field (50 Hz) caused atresia in ovarian follicles. In another study, EMR (900 Hz), which was applied for 1 h/day during pregnancy, increased atretic follicle numbers and apoptotic index levels. Histopathological examination detected severe follicle degeneration and vasocongestion (Türedi et al.,

2016). Bakacak et al. (2015) detected a significant decrease in the number of ovarian follicles in rats after exposure to EMF. Another study suggested that the exposure to EMF affected reproductive potential and decreased follicular capacity (Cecconi et al., 2000).

Serum AMH level is a marker used to determine the status of the primordial follicle reservoir. AMH level is moderately increased in polycystic ovarian syndrome and excessive response to gonadotropins and ovarian hyperstimulation syndrome (Salmassi et al., 2015). Thus, AMH is an important marker for granulosa cell activity. This marker may be used for the diagnosis of ovarian granulosa cells tumors. Two to 5% of all ovarian tumors are granulosa cell tumors. Granulosa cell tumors increase estrogen levels, in addition to abnormal uterus bleeding and endometrial hyperplasia in women (Hill et al., 2013). In a study on women, the increase in AMH levels showed usefulness in the early detection of human ovarian tumors (Salmassi et al., 2015).

In this study, we researched AMH and E2 levels for providing histopathological and immunohistochemical

observations after the exposure to EMR. AMH and E2 levels increased significantly after the exposure to 2.45 GHz EMF. Our study findings are similar to other studies in the literature (Hill et al., 2013). Long exposure to 2.45 GHz EMF causes hyperstimulation of granulosa cells.

Recent studies reported that immunohistochemical and histopathological changes in the female reproductive system may affect fertility (Roushangar et al., 2014; Türedi et al., 2016). In the study, rats were exposed to 1800 MHz during 2 h/day (30 and 60 day, whole body SAR of 0.974 W/kg). Investigating the effect of 1800 MHz on immunohistochemical and histopathological changes in ovarian and uterine tissue, Alchalabi et al. (2016) observed vacuolation in interstitial, granulosa, and luteal cells and ooplasm of ovarian tissues. Also, there was disorientation of the corona radiata and disruption, and thinning of the zona pellucida. This study demonstrated apoptosis in the luminal epithelial cells and changes in glandular epithelium ranging from desquamation of epithelial cells to degenerative processes in uterine tissue. Roushangar et al. (2014) investigated the effect of low-frequency EMR on oocyte differentiation and follicular development in neonatal and adulthood ovaries. The study reported decreased follicular formation (primary, secondary, and antral follicles) and increased atretic follicles. Also, it showed rupturing and thinning in the zona pellucida and separation of the granulosa layer, vacuolization, and decrease nuclei of oocytes and irregular shapes. However, Sanguin et al. (2015) reported no adverse effect of 2.45 GHz EMR (1 h/day, whole body SAR value of 0.1 W/kg) on histologic parameters and E2 values. Histopathological findings of our present study did not show hyperemia, inflammatory reaction in parenchymal tissue, degeneration, and necrosis of the cells in ovarian tissues. Only immunohistochemical examination of the ovaries revealed statistically significant increases in Caspase-3 expressions in epithelial cells. Histopathological examination of the fallopian tubes revealed normal appearing superficial epithelium and cilia in all groups. Hyperemia, degeneration or inflammatory reactions were not detected in any group upon microscopic examination. There were no significant differences at immunohistochemical examination of the Caspase-3 and Caspase-8 reactions in fallopian tubes among the groups. In addition, the exposed groups were showing hyperemia and increased number of apoptotic cells in uterus.

Caspase-3, a pivotal effector caspase, is an essential protease of the apoptotic pathway. It proteolytically cleaves a number of death substrates and activates endonucleases, leading to internucleosomal deoxyribonucleic acid fragmentation, a hallmark of apoptosis (Nicholson, 1999). Caspase-8 is an apical initiator caspase, which has been linked to receptor-induced apoptosis (Siegel, 2006). The activation of Caspase-8 suggests that EMR may induce apoptosis via the receptor-mediated extrinsic pathway. Caspase-3 is an effector molecule that is stimulated by active Caspase-8 during the extrinsic process. To evaluate the effect of EMR on the female reproductive system, these caspases were selected and used for this study.

Exposure to 2.45 GHz EMR might cause oxidative stress (Shahin et al., 2013). Oxidative stress may affect the maturation of primordial follicles. Strengthening of the antioxidant system in treatment can reduce the infertility that is caused by imbalance (Abbasi et al., 2016). Vit C is known as the most important antioxidant. It protects body tissues against oxidative damage. Nasr et al. (2014) showed the ameliorating effect of vit C after ischemia-reperfusion in adult rat ovary. Abbasi et al. (2016) investigated the effect of vit C on monosodium glutamate inducing changes in the rats ovary. Decreased weight of the ovary, granulosa cell degeneration, vascular congestion, and increased number of primary follicles were detected in the exposed group. Ameliorating effects of vit C on ovarian histomorphology were shown in the study. Another study, Oral et al. (2006) investigated the effects of vit C and vit E on endometrial apoptosis induced by a 900 MHz mobile phone. In the treated groups, MDA levels and Caspase-3 and Caspase-4 activities were decreased compared to the exposed groups. In our study, the administration of vit C showed the decreased TOS levels and OSI index in ovarian, fallopian tubes, and uterus tissues. TAS levels were significantly increased in uterus, but the difference was not significant in the ovarian and fallopian tissues. Histopathologic analyses showed normal anatomical configuration in ovarian, fallopian tubes, and uterus. In our immunohistochemical analyses, Caspase-3 was decreased significantly in ovaries and uterus. Caspase-8 was significantly reduced in uterus in the Vit C-treated group compared to the group exposed to EMR. Our study findings are similar to those of Oral et al. (2006). In addition, this study observed the reduction in serum AMH and E2 levels by the co-administration of vit C compared to the exposed groups. Our findings demonstrated that prior

administration of vit C prevented ovarian damage induced of by 2.45 GHz EMR.

Conclusions

According to our findings, within the safety limits of this study, we concluded that 2.45 GHz EMR may cause oxidative degeneration of the ovarian, fallopian tubes, and uterus tissues. Oxidative damage may be a reason for infertility. Also, AMH was increased in the group exposed to EMR. Vit C may protect the ovarian, fallopian tubes, and uterus tissues against the exposure to 2.45 GHz EMR.

Further investigation is needed to explain the biological effects of EMR exposure on rat ovaries, fallopian tubes, and uterus tissues. An ameliorative or therapeutic role for vit C should also be investigated in the future researches, and the details of the relationship between 2.45 GHz EMR exposure, AMH, and E2 should be examined.

Study limitations

The exposure to EMR, in particular, causes oxidative damage and apoptosis. This chronic condition causes much tissue damage. It is recognized as a close model for daily used devices related to infertility.

Despite these potential limitations, we feel that this approach is useful in assessing the pharmacological roles of Vit C, with the aim of determining the protective effects of Vit C regulating antioxidant (TAS) and antiapoptotic (Caspase-3 and -8) on the female reproductive system.

Our protocol was specifically designed to define and characterize the effects of EMR on the female reproductive system, rather than to address pathophysiological mechanisms. Once the responses of TAS, TOS, Caspase-3, and -8 following EMR exposure are clearly demonstrated, hypotheses may be generated to inform the additional research needed to clarify the mechanism involved.

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