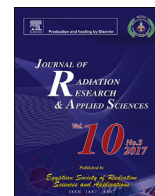


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The ameliorative effect of gallic acid on pancreas lesions induced by 2.45 GHz electromagnetic radiation (Wi-Fi) in young rats



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ABSTRACT

The aim of this study was to investigate the effects of electromagnetic radiation (EMR) on the pancreas tissue of young rats and the ameliorative effect of Gallic acid (GA). Six-week-old, 48 male rats were equally divided into four groups: Sham group, EMR group (2.45 GHz), EMR (2.45 GHz)+GA group (30 mg/kg/daily) orally and GA group (30 mg/kg/daily). After 30 days, serum and pancreatic tissue samples were harvested for biochemical, histopathological and immunohistochemical analysis. Serum amylase, lipase, glucose, and tissue malondialdehyde, total oxidant status and oxidative stress index were increased, whereas total antioxidant status decreased in the EMR group. The histopathological examination of the pancreases indicated slight degenerative changes in some pancreatic endocrine and exocrine cells and slight inflammatory cell infiltrations in the EMR group. At the immunohistochemical examination, marked increase was observed in calcitonin gene related protein and Prostaglandin E2 expressions in pancreatic cells in this group. There were no changes in interleukin-6 expressions. GA ameliorated biochemical and pathological findings in the EMR+GA group. These findings clearly demonstrate that EMR can cause degenerative changes in both endocrine and exocrine pancreas cells in rats during the developmental period and GA has an ameliorative effect.

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1. Introduction

Especially in developed countries, the use of Wi-Fi and radiation-emitting wireless devices has increased dramatically in the recent century (Kumar, Behari, & Sisodia, 2013; Naziroglu, Yuksel, Ozkaya & Kose, 2013). Reports of harmful effects of EMR from Wi-Fi, mobile phones and computers are increasing rapidly in the last few decades. Living organisms are continually exposed to radiation during their life. The deterministic effects of electromagnetic radiation (EMR) are related to the exposure time and dose. The level of damage on the cell structure might vary among cells and be based on their metabolic activity. EMR can cause detrimental effects on cell function and chromosomal aberrations

in exposed tissues (Al-Damegh, 2012; Lai & Singh, 1997; Moussa, 2005; Ozorak et al., 2013; Tsurita, Uno, Tsuno, Nagawa, & Muto, 1999; Valberg, Kavet, & Rafferty, 1997).

Because of the large amount of mobile phone and computer users, investigating, understanding, and monitoring any possible potential public health impacts are important (Naziroglu, Tokat, & Demirci, 2012; Turker et al., 2011). Another problem that causes EMR exposure is the decrease in the ozone layer, and this issue leads to an increase in the amount of dangerous ultra violet (UV) radiation that reaches the earth's surface (Thiele, Dreher, Maibach, & Packer, 2003). Due to EMR that originates from reduced ozone layer thickness and technological devices, it is estimated that numerous diseases will increase in the near future because of UV radiation and EMR. (Al-Damegh, 2012; Mayer, 1992; Moussa, 2005; Ozorak et al., 2013; Verma et al., 2011). Electromagnetic fields can alter the energy levels and spin orientation of electrons and increase the production of reactive oxygen species (ROS). Accordingly, exposure to EMR is associated with enhanced ROS production in cells and tissues (Naziroglu et al., 2012; Turker et al., 2011).

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Although the knowledge of EMR damage in the pancreas is newly reported, studies on this subject have increased rapidly. Sakurai et al. reported that glucose-stimulated insulin secretion was decreased by EMR exposure (Sakurai, Koyama, Komatsubara, Jin, & Miyakoshi, 2005). Turker (2015) showed that EMR can cause ultrastructural changes in endocrine cells in the pancreas. EMR has also been shown to increase the diabetogenic effect of alloxan in mice (Garyae, Kokaya, Mukhina, Leonova-Garyaeva, & Kokaya, 2007). There is no knowledge on the effects of EMR on exocrine cells and the curability of the harmful effects of radiation. The purpose of this study was to investigate the effects of 2.45 GHz EMR on both endocrine and exocrine pancreas cells in young rats and the potential ameliorative effect of Gallic acid (GA) on lesions using biochemical, histopathological and immunohistochemical methods.

2. Materials and methods

We examined the oxidative stress status, histopathological effects and immunohistochemical findings of direct exposure to EMR in rats during the developmental period. The experiments were performed in accordance with the guidelines for animal research of the National Institutes of Health and were approved by the Committee on Animal Research at Suleyman Demirel University, Isparta. Animals were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by Suleyman Demirel University (18/02/2016-19).

2.1. Rats

There was no significant difference in the average weight of the groups of forty-eight 6-week-old male Sprague Dawley rats, which weighed 150–170 g. The number of rats in each group was twelve because this experimental setup is suitable for homogeneous irradiation occurring at the same time (Fig. 1). A long term study might affect rat welfare, and the rats may die during the long experiment period. Rats were housed individually in stainless-steel cages in pathogen-free conditions in our laboratory at 24 ± 3 °C with light from 08:00 to 20:00 and free access to water and food. They received a commercial chow diet (Korkuteli Yem, Korkuteli-Turkey). The environmental average light intensity was 4000 lux, and the humidity was $40 \pm 10\%$.

2.2. Experimental design

The rats were randomly allocated into 4 groups of 12 animals each. The sham group was the control, and no treatment was given to this group. A total of 2.45 GHz EMR was exposed to rats in the EMR group. The EMR+GA group was exposed to 2.45 GHz EMR and treated with GA 30 mg/kg/daily given orally (Mansouri et al., 2013). Only GA was given 30 mg/kg/daily orally in the GA group. EMR was applied to the groups of EMR and EMR+GA for 3 h/daily during the 30 days.

The source of EMR exposure was a radio frequency (RF) test generator SET ELECO (Set Electronic Co., Istanbul, Turkey), which provided 2.45 GHz RF emission, pulsed with 217 Hz and its monopole antenna system (Saygin et al., 2011). This device has 0.1 V/m to 45.5 V/m electric field strength. All exposure systems were located in a screened room, which has a shielding effectiveness better than 80 db in the frequency range of 2.0–2.5 GHz. The system performance of the exposure device was tested and checked in the Laboratory of the Department of Electronics and Communication Engineering (Suleyman Demirel University, Isparta, Turkey). The exposure design and methodology were adapted from previous studies (Saygin et al., 2011, 2015).

A carousel (or Ferris Wheel) setup contained twelve rats at the same time. All rats were kept in cylindrical, plastic restrainers, as seen in Fig. 1. Each restrainer provided 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 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 the rats at the time of exposure were normal in all groups.

All of the reflection and exposure measurements were conducted utilizing a Portable RF Survey System (HOLADAY, HI-4417, Minnesota, USA) with its standard probe. Repetition time, frequency, and amplitude of the spectrum of RF energy was observed and verified by a satellite level meter (PROMAX, MC-877C, Barcelona/Spain). The electromagnetic dosimetry was calculated using measured electric field intensity (V/m) and digital anatomical models based on the FDTD numerical code. Using this method, the SAR value was predicted for the same condition, orientation, and antenna power as that of the whole body, which is 50 mW/kg. Additionally, the exposure of each group did not affect the other groups. This study focused on the developmental process and EMR exposure time and duration, and the SAR value simulated of daily exposure in humans (Saygin et al., 2011, 2015). Young people are used to Wi-Fi connections, and we simulated the same conditions in this study. This SAR value was the threshold for pathophysiological changes of pancreatic tissue.

On day 30, blood samples were collected from the tail vein. Then rats were anesthetized with a cocktail of ketamine hydrochloride (HCl) (90 mg/kg) and xylazine HCl (10 mg/kg), which were administered intraperitoneally before sacrificing. After an abdominal incision was made, pancreatic tissues were quickly removed, and divided equally into two longitudinal sections. Then, half of the pancreas tissues were fixed in a 10% neutral formalin solution for histopathological and immunohistochemical examinations. The other half of the pancreas tissues were kept at -80 °C for biochemical and oxidant-antioxidant enzyme analyses. Blood samples were extracted to determine the serum levels of glucose, amylase, and lipase.

2.3. Biochemical methods

The pancreatic tissue was homogenized in ice-cold phosphate buffer (pH 7.4) to produce 10% homogenate. 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 sedimented by centrifugation at 10,000g for 10 min at $+4$ °C. The levels of protein, malondialdehyde (MDA), total oxidant status (TOS), total antioxidant status (TAS) and oxidative stress index (OSI) were determined in the supernatants.

Pancreas tissues were processed according to the method of Bradford (1976) The MDA levels in the tissues were determined from the homogenate by following the double heating method of Drapper and Hadley (1990).

A Rel Assay, a novel automated colorimetric kit which was developed by Erel (2005), was used to determine the TOS of the tissue samples. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules shown in the sample. The results are expressed in terms of mM hydrogen peroxide equivalent per g liter (mmol H₂O₂ Equiv/L, mmol H₂O₂ Equiv/mg protein).

A Rel Assay, a novel automated colorimetric kit developed by Erel (2004), was used to determine the total antioxidant status of the tissue samples. The change in absorbance at 660 nm is related

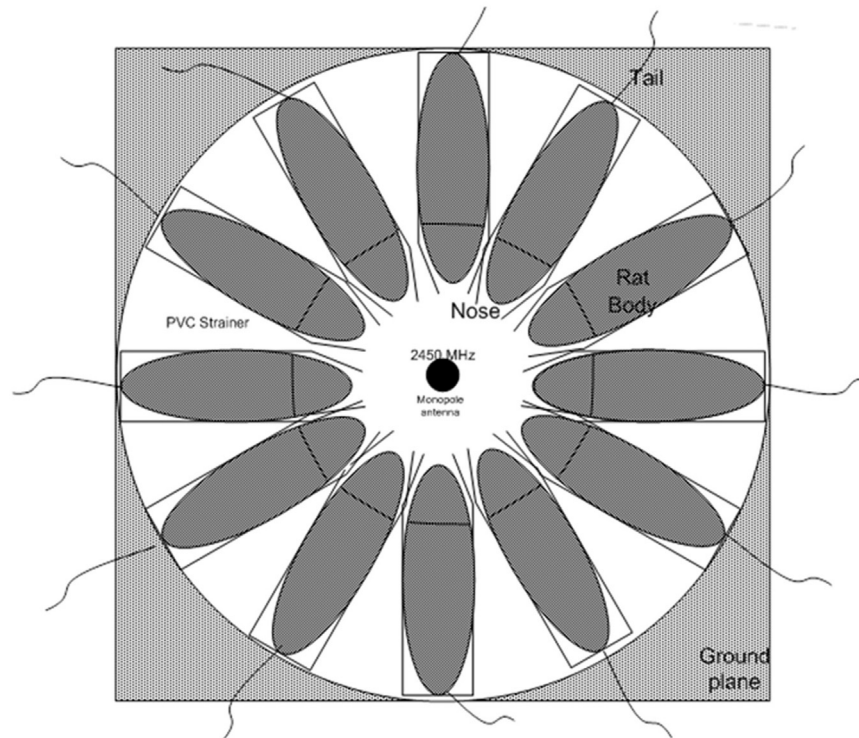


Fig. 1. Experimental setup for irradiation of the rats.

to the total antioxidant level of the sample, which can be measured spectrophotometrically. The levels were expressed as mmol Trolox Eq/mg protein. OSI is an indicator of oxidative stress, and the ratio of TOS to TAS was accepted as the OSI (Demirbag et al., 2007). OSI was calculated using the following equation:

$$\text{OSI (arbitrary unit)} = \left[\frac{(\text{TOS}, \mu\text{mol/L})}{(\text{TAS}, \mu\text{mol Trolox equivalent/L})} \times 100 \right]$$

TAS and TOS were measured with an automated chemistry analyzer (Beckman Coulter AU5800, Japan). An autoanalyser (Beckman Coulter AU 680, California, USA) was used to determine the glucose, amylase and lipase levels spectrophotometrically.

2.4. Histopathological method

After routine processing of the formalin fixed samples, tissues were embedded in paraffin, 5- μm sectioned by a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany), stained with hematoxylin-eosin (HE) and examined microscopically. Histopathological changes were graded in a blinded manner. Microscopic lesions were scored to evaluate the pathological findings. Scores were made according to the number of degenerative cells. To evaluate the percentage of degenerated cells, 10 different areas were examined in both the endocrine and exocrine sections of the pancreas in each rat under the 40X objective of an Olympus CX41 light microscope. Morphometric evaluation was performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

2.5. Immunohistochemical method

Pancreas samples were then immunostained with primary antibodies. All antibodies were purchased from Abcam, Cambridge,

UK. Selected tissue sections were immunostained using CGRP [Anti CGRP antibody [4901] (ab81887)]; Prostaglandin E₂ [Anti-Prostaglandin E₂ antibody (ab2318)] and IL-6 [Anti-IL-6 antibody (ab6672)] antibodies according to the manufacturer's instructions. All of the slides were analyzed for immunopositivity, and a semi-quantitative analysis was conducted. 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). For negative control primary antibody was omitted.

2.6. Statistical analysis

One-way analysis of variance test was used to determine significant differences between the groups. The groups were compared using the non-parametric Kruskal-Wallis test and Dunnett test. Biochemical parameters that fit to the normal distribution were shown, and ANOVA and post hoc LSD, Bonferroni, and Tukey tests were used to compare groups. To determine differences between groups in the histopathological and immunohistochemical analyses, the Bonferroni-Dunn multiple comparison method was used. Calculations were made using the SPSS 15.0 program. $p < 0.05$ was set as the level of significance.

3. Results

Pancreases from all groups were macroscopically normal in appearance. No hemorrhages, inflammation or necrosis were observed in any groups. The histopathological examination of the pancreases of the EMR group indicated slight degenerative changes in some pancreatic endocrine and exocrine cells and slight inflammatory cell infiltrations. General hydropic degeneration and vacuolar degeneration in some cells were observed in both endocrine and exocrine cells. In the affected cells, vacuolation of the

cytoplasm, decreased nuclei, and chromatin condensation were noticed. A small amount of scattered cells showed necrotic changes such as karyopyknosis and scant karyolysis. The pancreata in the EMR+GA group showed normal histology (Fig. 2). The statistical analysis results showed significant differences between the EMR and other groups according to number of degenerative cells. Generally, the most affected cells were those in the endocrine pancreas (Table 1).

PGE₂, IL-6 and CGRP expressions were detected using immunohistochemical methods. Immunohistochemistry of the pancreatic tissues of the EMR group revealed a marked increase in PGE₂ and CGRP expression in the Langerhans islet and in some exocrine cells, whereas no differences in IL-6 expression were found between the groups. An ameliorative effect of GA was observed in the pancreases of the EMR+GA group (Figs. 3–4). Immunopositivity was generally localized in the endocrine cells of the Langerhans islet. Immunohistochemical scores are shown in Table 1.

In the EMR group, a significant increase was observed in tissue MDA, TOS and OSI levels, whereas TAS levels decreased. Gallic acid treatment ameliorated these levels in the EMR+GA group, and the results are shown in Table 2.

Serum glucose, lipase and amylase levels were higher in the EMR group, and the results were significant, indicating both endocrine and exocrine cell damage. Gallic acid treatment (EMR+GA group) decreased these levels, and they were higher in the sham and GA groups (Table 3).

4. Discussion

Numerous electrical devices that utilize Wi-Fi are used in daily life. The most common communication devices are generally spreading EMF in the 2.45 GHz frequency. Currently, the use of wireless networks has increased very quickly across the world. Wireless local area network (WLAN) systems constitute an

important part of our daily lives, even though there is some evidence of its possible harmful effects on health (Saygin et al., 2011). The average age of those using electronic devices has decreased in the last few decades, and children are exposed to EMR at a very early ages. The possible harmful effects of Wi-Fi on cells may be more severe during developmental stages. For that reasons, to better evaluate the effect of Wi-Fi on young rat pancreatic cells, we selected 2.45 GHz dose in this study. Our results showed that EMR might be causing pancreatic lesions in both endocrine and exocrine cells and that GA may have an ameliorative effect on EMR-caused pancreatic injury in young rats. The main mechanism of the injury was related the oxidative damage and inflammatory process produced by EMR, in addition, PGE₂ and CGRP had an important role in EMR-related pancreatic damage during the developmental period.

The pancreas is a multifunctional organ. The exocrine pancreas is a part of the gastrointestinal system and makes and secretes digestive enzymes into the intestine. The endocrine part of the organ makes and secretes hormones into the blood to control energy metabolism and storage throughout the body. Because of the complex and susceptible structure of the organ, numerous factors can cause lesions and damage in the pancreas (Ozmen, Sahinduran, & Mor, 2010; Yilmaz et al., 2009). Langerhans islets play a fundamental role in regulating the blood glucose levels of the body through the secretion of hormones such as insulin, glucagon, somatostatin, and pancreatic polypeptide. The release of insufficient amounts of these hormones is the basis of various forms of diabetes that have become worldwide epidemics in the last few decades. Therefore, it is important to assess the effects of EMR exposure, which is common in the daily life of humans, on pancreatic islet function (Sakurai, Satake, Sumi, Inoue, & Miyakoshi, 2004, 2008). Although there are a few reports of the effect of EMR on endocrine pancreatic cells (Sakurai et al., 2004, 2005, 2008), there is no knowledge about the effect of Wi-Fi during the developmental

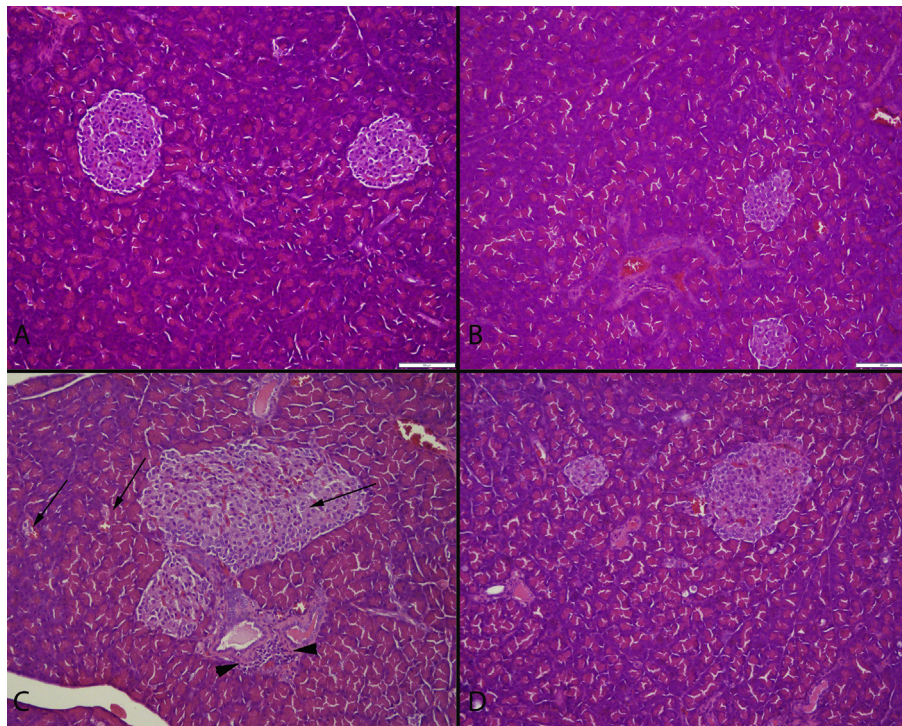


Fig. 2. Histopathological appearance of the pancreas, no marked pathological findings in the (A) sham group; (B) GA group; (D) EMR+GA group, (C) Inflammatory cell infiltrations (arrow heads), degeneration in cells (arrows) and marked hyperemia in Langerhans islet in EMR group; HE, Bar = 100 μ m.

Table 1Statistical analysis results of histopathological and immunohistochemical scoring results of the pancreas.^a

	SHAM	GA	EMR	EMR+GA	P Value
Degenerated endocrine cell (%)	2.11 ± 0.92	1.88 ± 1.05	4.50 ± 1.64	2.87 ± 1.24	EMR-SHAM (<0.001) EMR-EMR+GA (<0.001) SHAM-GA (NS) ^b
Degenerated exocrine cell (%)	2.55 ± 0.72	2.00 ± 1.32	4.50 ± 2.27	2.50 ± 0.92	SHAM-EMR+GA (NS) EMR-SHAM (<0.001) EMR-EMR+GA (<0.001) SHAM-GA (NS)
CGRP	0.55 ± 0.52	0.55 ± 0.52	1.90 ± 0.56	0.50 ± 0.53	SHAM-EMR+GA (NS) EMR-SHAM (<0.001) EMR-EMR+GA (<0.001) SHAM-GA (NS)
PGE2	0.33 ± 0.50	0.33 ± 0.50	2.60 ± 0.51	1.37 ± 0.51	SHAM-EMR+GA (NS) EMR-SHAM (<0.001) EMR-EMR+GA (NS) SHAM-GA (NS)
IL-6	0.33 ± 0.50	0.22 ± 0.44	0.60 ± 0.69	0.50 ± 0.75	SHAM-EMR+GA (<0.001) EMR-SHAM (NS) EMR-EMR+GA (NS) SHAM-GA (NS) SHAM-EMR+GA (NS)

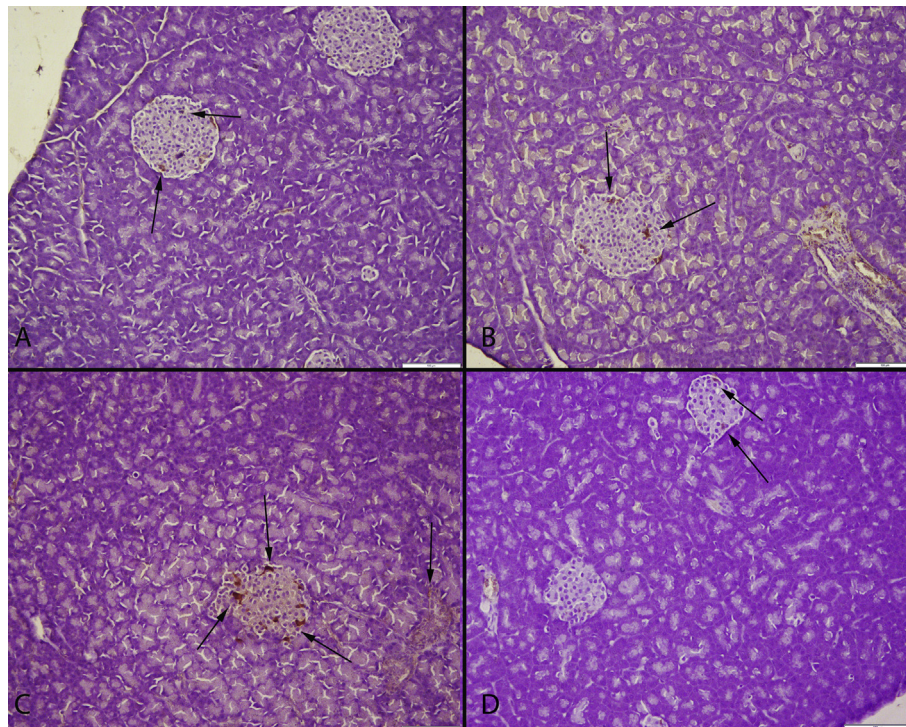
^a Values represent mean ± standard deviation.^b NS: Non significant.

Fig. 3. CGRP immunoreaction in the pancreas of the rats. (A) Slight CGRP immunopositive cells in the Langerhans islet (arrows) and no immunopositivity in exocrine part in sham group; (B) CGRP expression in Langerhans islet and no reaction in exocrine pancreas in GA group; (C) Increase in CGRP positive cells in Langerhans islets and exocrine pancreas (arrows) in EMR group; (D) Normal CGRP expression in cells in Langerhans islet and no expression in exocrine part in EMR-GA group, Streptavidin biotin peroxidase method, Bar = 100 μ m.

period on pancreatic cells. This study supported the idea of harmful effects of EMR on the endocrine pancreas, but our findings showed that the harmful effect of EMR on the exocrine pancreas may be more severe in the developmental stage.

Diabetes is increasing at an alarming rate worldwide, which can mainly be attributed to life style and environmental effects on the pancreas (Biddinger & Kahn, 2006). Diabetes mellitus is a metabolic life-threatening disease characterized by complete or partial deficiency of insulin, resulting in chronic hyperglycemia (Abou-Seif &

Youssef, 2004; Hasselbank, Glatz, & Luiken, 2003). Hyperglycemia is a principal cause of elevated free radical levels, which when followed by production of ROS can lead to increased lipid peroxidation and altered antioxidant defenses and thus further impair glucose metabolism in biological systems (Balasubashini, Rukkumani, Viswanathan, & Menon, 2004; Kuyvenhoven & Meinders, 1999; West, 2000). Increased oxidative stress increases the rate of development and the progression of diabetes and its complications (Balasubashini et al., 2004). The connection between

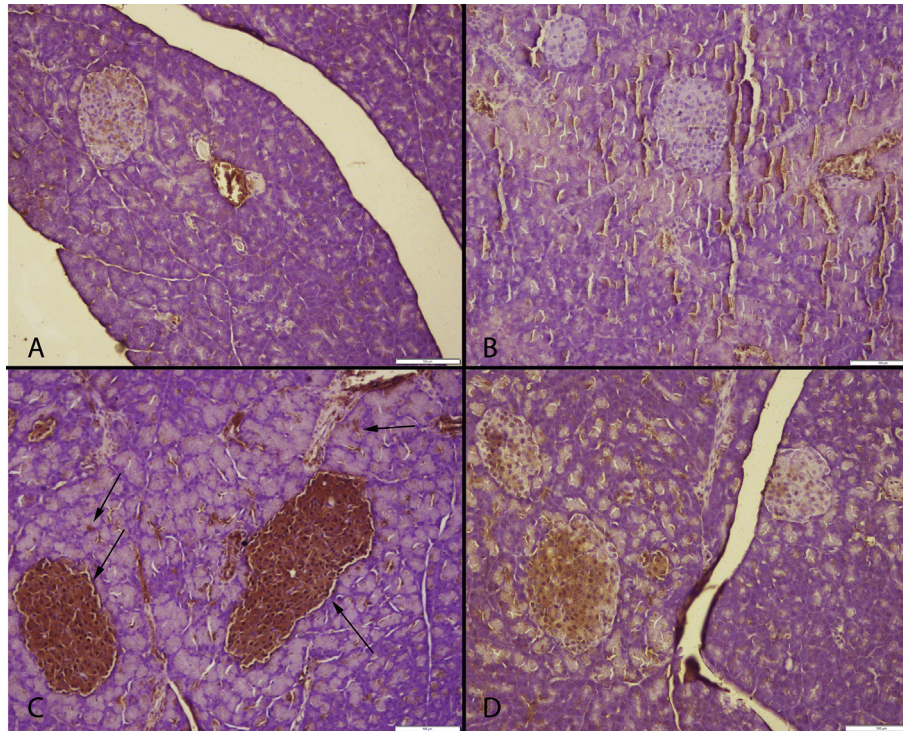


Fig. 4. PGE₂ expression of the pancreas, (A) Slight PGE₂ expression of pancreatic cells in sham group and (B) no expression in GA group; (C) marked increase of PGE₂ immunoreaction of Langerhans islet and exocrine cells in EMR group; (D) Moderate immunoreaction in Langerhans islet in EMR-GA group, Streptavidin biotin peroxidase method, Bar = 100 μ m.

Table 2
Statistical analysis results of MDA, TAS, TOS and OSI levels in the pancreas.^a

	SHAM	GA	EMR	EMR+GA	P Value
MDA (μ mol/mg protein)	0.05 \pm 0.02	0.03 \pm 0.01	0.08 \pm 0.03	0.03 \pm 0.01	EMR-SHAM (<0.001) EMR-EMR+GA (<0.001) SHAM-GA (NS) ^b SHAM-EMR+GA (NS)
TAS (mmol Trolox E/L)	1.73 \pm 0.36	1.82 \pm 0.42	0.45 \pm 0.11	1.58 \pm 0.42	EMR-SHAM (<0.01) EMR-EMR+GA (<0.01) SHAM-GA (NS) SHAM-EMR+GA (NS)
TOS (μ mol H ₂ O ₂ E/L)	18.52 \pm 8.58	18.89 \pm 10.04	31.82 \pm 8.55	21.05 \pm 8.17	EMR-SHAM (<0.01) EMR-EMR+GA (<0.01) SHAM-GA (NS) SHAM-EMR+GA (NS)
OSI	1.63 \pm 0.37	1.87 \pm 0.71	2.25 \pm 0.77	1.36 \pm 0.52	EMR-SHAM (<0.05) EMR-EMR+GA (<0.05) SHAM-GA (NS) SHAM-EMR+GA (NS)

^a Values represent mean \pm standard deviation.

^b NS: Not significant.

exposure to artificial EMR and the changing health of diabetic sufferers is an established fact that has been demonstrated beyond doubt in numerous scientific studies (Sakurai et al., 2004, 2005, 2008). Previously, endocrine pancreatic cell damage from EMR has been described using cell culture or histological levels (Sakurai et al., 2005, 2008, 2004; Farahna et al., 2014), but there is no study related pathogenesis of the damage. Accordingly, our study was the first to study the pathological effects of EMR on both endocrine and exocrine pancreatic cells during the developmental period at the histopathological, immunohistochemical and biochemical levels. This study indicated that EMR causes harmful effects on Langerhans islets and can cause diabetes or increased diabetes predisposition at a young age. We detected high glucose levels,

degenerated exocrine cells and marked expiration of PGE₂ and CGRP in addition to increased oxidative stress markers in the EMR group.

This study results also revealed that EMR not only causes endocrine but also exocrine damage in the pancreas at young age. In addition to high degenerated exocrine cell numbers, inflammatory reactions in the pancreas and increases in serum amylase and lipase levels (which are characteristic of exocrine pancreas damage) were observed in this study. The biochemical and pathological findings of the present study showed that exposure to EMR was able to induce pancreatic tissue damage in the developmental period. However, GA decreased the damage caused by EMR exposure.

Table 3
Statistical analysis results of serum glucose, lipase and amylase, levels.^a

	SHAM	GA	EMR	EMR-GA	P Value
Glucose (mg/dl)	120.69 ± 51.67	133.84 ± 55.14	259.06 ± 66.59	182.81 ± 36.01	EMR-SHAM (<0.001) EMR-EMR+GA (<0.01) SHAM-GA (NS) ^b SHAM-EMR+GA (<0.01) EMR-SHAM (<0.05) EMR-EMR+GA (<0.001) SHAM-GA (NS) SHAM-EMR+GA (NS)
Lipase (U/L)	6.31 ± 1.90	6.48 ± 2.68	9.68 ± 2.42	6.88 ± 3.40	EMR-SHAM (<0.05) EMR-EMR+GA (<0.001) SHAM-GA (NS) SHAM-EMR+GA (NS)
Amylase (U/L)	242.92 ± 10.29	253.00 ± 62.27	858.68 ± 50.98	490.83 ± 64.48	EMR-SHAM (<0.001) EMR-EMR+GA (<0.001) SHAM-GA (NS) SHAM-EMR+GA (NS)

^a Values represent mean ± standard deviation.

^b NS: Non significant.

Cellular damage due to EMR might result from an increase in lipid peroxidation and a decrease in the activity of antioxidant enzymes of the body with the consequent damage of cellular membranes (Mansouri et al., 2013; Saada & Azab, 2001). Degenerative changes in cells due to radiation resulting in significant dysfunction might decrease the cell's processes and distort its structure. Our results were supported and in agreement with previous studies, but this study indicated that the harmful effect of EMR is more severe during the developmental period. Increased MDA, TOS and OSI levels indicated that oxidative stress is important in the occurrence of pathological lesions in the pancreas exposed to EMR. Garyae et al. (2007) reported that EMR enhanced the degenerative effect of alloxan in pancreatic beta cells. In the present study, we found both endocrine and exocrine pancreas damage caused by EMR. Serum amylase, lipase and glucose levels supported the histopathological and immunohistochemical findings.

The results of this study indicated that a possible interaction may occur in diabetes mellitus and exocrine pancreas disease caused by EMR in the developmental period. Diabetes and exocrine pancreas diseases have been increasing in the last few decades, parallel to the use of technological devices which produce EMR, and the diagnosis of pancreatic diseases has been rapidly increasing in young people. These study results indicate that endocrine cells of the pancreas are more susceptible to EMR than exocrine cells. The rats that we used this study were young, and the marked increase in lesions may be related to this factor. Further studies are necessary to evaluate the effects of EMR on the pancreas at various ages. Specifically, EMR may be more harmful in young animals. The powerful antioxidant GA has a protective effect of EMR-induced pancreatic lesions. This study suggests a possible relationship between EMR exposure at a young age and pancreatic diseases. This study results thought that any possible relation with EMR exposure and pancreas related disease such as diabetes in children.

References

- Abou-Seif, M. A., & Youssef, A. A. (2004). Evaluation of some biochemical changes in diabetic patients. *Clinica Chimica Acta*, 346, 161–170.
- Al-Damegh, M. A. (2012). Rat testicular impairment induced by electromagnetic radiation from a conventional cellular telephone and the protective effects of the antioxidants vitamins C and E. *Clinics*, 67, 785–792.
- Balashubhini, M. S., Rukkumani, R., Viswanathan, P., & Menon, V. P. (2004). Ferulic acid alleviates lipid peroxidation in diabetic rats. *Phytotherapy Research*, 18, 310–314.
- Biddinger, S. B., & Kahn, C. R. (2006). From mice to men: Insights into the insulin resistance syndromes. *Annual Review of Physiology*, 68, 123–158.
- Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.
- Demirbag, R., Gur, M., Yilmaz, R., Kunt, A. S., Erel, O., & Andac, M. H. (2007). Influence of oxidative stress on the development of collateral circulation in total coronary occlusions. *International Journal of Cardiology*, 116, 14–19.
- Drapper, H. H., & Hadley, M. (1990). Malondialdehyde determination as index of lipid peroxidation. *Methods in Enzymology*, 186, 421–431.
- Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, 37, 277–285.
- Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, 38, 1103–1111.
- Farahna, M., Omer, M. A. A., Garalnabi, M. E. F., Al-Ganim, A. A., Abdelkareem, S., & Busharaa, Y. M. (2014). The effects of static magnetic field on rats brain, lungs, liver, pancreas and blood electrolytes. *NeuroQuantology*, 21, 230–236.
- Garyae, P. P., Kokaya, A. A., Mukhina, I. V., Leonova-Garyaeva, E. A., & Kokaya, N. G. (2007). Effect of electromagnetic radiation modulated by biostructures on the course of Alloxan-induced Diabetes Mellitus in rats. *Bulletin of Experimental Biology and Medicine*, 143, 197–199.
- Hasselbaink, D. M., Glatz, J. F. C., & Luiken, J. J. F. P. (2003). Ketone bodies disturb fatty acid handling in isolated cardiomyocytes derived from control and diabetic rats. *Biochemical Journal*, 371, 753–760.
- Kumar, S., Behari, J., & Sisodia, R. (2013). Influence of electromagnetic fields on reproductive system of male rats. *International Journal of Radiation Biology*, 89, 147–154.
- Kuyvenhoven, J. P., & Meinders, A. E. (1999). Oxidative stress and diabetes mellitus. Pathogenesis of long term complications. *European Journal of Internal Medicine*, 10, 9–19.
- Lai, H., & Singh, N. P. (1997). Acute exposure to a 60 Hz magnetic field increase DNA strand breaks in rat brain cells. *Bioelectromagnetics*, 18, 156–165.
- Mansouri, M. T., Farbood, Y., Sameri, M. J., Sarkaki, A., Naghizadeh, B., & Rafeirad, M. (2013). Neuroprotective effects of oral gallic acid against oxidative stress induced by 6-hydroxydopamine in rats. *Food Chemistry*, 138, 1028–1033.
- Mayer, S. J. (1992). Stratospheric ozone depletion and animal health. *Veterinary Record*, 131, 120–122.
- Moussa, E. A. (2005). Effect of electromagnetic field on liver and kidney tissues of Swiss albino mice. *Journal of the Egyptian-German Society of Zoology*, 48, 29–53.
- Naziroglu, M., Tokat, S., & Demirci, S. (2012). Role of melatonin on electromagnetic radiation-induced oxidative stress and Ca²⁺ signaling molecular pathways in breast cancer. *Journal of Receptors and Signal Transduction*, 32, 290–297.
- Naziroglu, M., Yuksel, M., Ozkaya, M. O., & Kose, S. A. (2013). Recent reports of Wi-Fi and mobile phone-induced radiation on oxidative stress and reproductive signaling pathways in females and males. *The Journal of Membrane Biology*, 246, 869–875.
- Ozmen, O., Sahinduran, S., & Mor, F. (2010). Pathological and immunohistochemical examination of the pancreas in subacute endosulfan toxicity in rabbits. *Pancreas*, 39, 367–370.
- Ozorak, A., Naziroglu, M., Celik, O., Yuksel, M., Ozcelik, D., Ozkaya, M. O., et al. (2013). Wi-Fi (2.45 GHz)- and mobile phone (900 and 1800 MHz)-induced risks on oxidative stress and elements in kidney and testis of rats during pregnancy and the development of offspring. *Biological Trace Element Research*, 156, 221–229.
- Saada, H. N., & Azab, K. S. (2001). Role of lycopene in recovery of radiation induced injury to mammalian cellular organelles. *Europe PMC (Die Pharmazie)*, 56, 239–341.
- Sakurai, T., Koyama, S., Komatsubara, Y., Jin, W., & Miyakoshi, J. (2005). Decrease in glucose-stimulated insulin secretion following exposure to magnetic fields. *Biochemical and Biophysical Research Communications*, 332, 28–32.
- Sakurai, T., Satake, A., Sumi, S., Inoue, K., & Miyakoshi, J. (2004). An extremely low frequency magnetic field attenuates insulin secretion from the insulinoma cell line, RIN-m. *Bioelectromagnetics*, 25, 160–166.
- Sakurai, T., Yoshimoto, M., Koyama, S., & Miyakoshi, J. (2008). Exposure to extremely low frequency magnetic fields affects insulin-secreting cells. *Bioelectromagnetics*, 29, 118–124.
- Saygin, M., Asci, H., Ozmen, O., Cankara, F. N., Dincoglu, D., & Ilhan, I. (2015). Impact of 2.45 GHz microwave radiation on the testicular inflammatory pathway

- biomarkers in young rats: The role of gallic acid. *Environmental Toxicology*, 31, 1771–1784.
- Saygin, M., Caliskan, S., Karahan, N., Koyu, A., Gumral, N., & Uguz, A. (2011). Testicular apoptosis and histopathological changes induced by a 2.45 GHz electromagnetic field. *Toxicology and Industrial Health*, 27, 455–464.
- Thiele, J. J., Dreher, F., Maibach, H. I., & Packer, L. (2003). Impact of ultraviolet radiation and ozone on the transepidermal water loss as a function of skin temperature in hairless mice. *Skin Pharmacology and Applied Skin Physiology*, 16, 283–290.
- Tsurita, G., Uno, S., Tsuno, N. H., Nagawa, H., & Muto, T. (1999). Effects of exposure to repetitive pulsed magnetic stimulation on cell proliferation and expression of heat shock protein 70 in normal and malignant cells. *Biochemical and Biophysical Research Communications*, 26, 689–694.
- Turker, H. (2015). The effect of ultraviolet radiation of pancreatic exocrine cells in mole rats: An ultrastructural study. *Journal of Radiation Research and Applied Sciences*, 8, 49–54.
- Turker, Y., Naziroglu, M., Gumral, N., Celik, O., Saygin, M., Comlekci, S., et al. (2011). Selenium and L Carnitine reduce oxidative stress in the heart of rat induced by 2.45- GHz radiation from wireless devices. *Biological Trace Element Research*, 143, 1640–1650.
- Valberg, P. A., Kavet, R., & Rafferty, C. N. (1997). Can low level 50/60 HZ electric and magnetic field cause biological effects. *Radiation Research*, 148, 2–12.
- Verma, P., Sharma, P., Parmar, J., Sharma, P., Agrawal, A., & Goyal, P. K. (2011). Amelioration of radiation-induced hematological and biochemical alterations in Swiss albino mice by Panax ginseng extract. *Integrative Cancer Therapies*, 10, 77–84.
- West, I. C. (2000). Radicals and oxidative stress in diabetes. *Diabetic Medicine*, 17, 171–180.
- Yilmaz, M., Topsakal, S., Herek, O., Ozmen, O., Sahinduran, S., Buyukoglu, T., et al. (2009). Effects of Etanercept on sodium taurocholate–induced acute pancreatitis in rats. *Translational Research the Journal of Laboratory and Clinical Medicine*, 154, 241–249.