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ORIGINAL ARTICLE



Radiofrequency radiation emitted from Wi-Fi (2.4 GHz) causes impaired insulin secretion and increased oxidative stress in rat pancreatic islets

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ABSTRACT

Purpose: There is a great concern regarding the possible adverse effects of electromagnetic radiation (EMR). This study investigated the effects of EMR induced by Wi-Fi (2.45 GHz) on insulin secretion and antioxidant redox systems in the rat pancreas.

Materials and methods: Adult male Sprague–Dawley rats in the weight range of 230–260 g were divided into control, sham, Wi-Fi exposed groups. After long-term exposure (4 h/day for 45 days) to Wi-Fi EMR, plasma levels of glucose and insulin during intraperitoneal glucose tolerance test were measured. Islet insulin secretion and content, lipid peroxidation, and antioxidant status in pancreas of rats were determined.

Results: Our data showed that the weight gain in the Wi-Fi exposed group was significantly lower than the control group ($p < .05$). Wi-Fi (2.45 GHz)-exposed group showed hyperglycemia. Plasma insulin level and glucose-stimulated insulin secretion from pancreatic islet were significantly reduced in the Wi-Fi-exposed group. EMR emitted from Wi-Fi caused a significant increase in lipid peroxidation and a significant decrease in GSH level, SOD, and GPx activities of the pancreas.

Conclusions: These data showed that EMR of Wi-Fi leads to hyperglycemia, increased oxidative stress, and impaired insulin secretion in the rat pancreatic islets.

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KEYWORDS

Insulin secretion; oxidative stress; electromagnetic radiation; Wi-Fi

Introduction

Several epidemiological and experimental studies have described the relationship between the effects of exposure to electromagnetic fields (EMFs) and biological health (Sage and Carpenter 2009; Sivani and Sudarsanam 2012; Li et al. 2017). Wireless local area network (WLAN) system is one of fast-growing technologies in the world. Currently, the access to the internet and the use of Wireless Fidelity or Wi-Fi (is a type of WLAN) are inevitable. It has widespread usage in working and living areas and the environment is now loaded with radiofrequency electromagnetic radiation (RF-EMR) from this device. Exposure to radiofrequency signal of Wi-Fi may lead to the leakage of radiation from it and absorption of some of the transmitted energy by living cells of the body. Several studies have reported that acute and chronic exposure of EMR can produce morphological alterations in biological cells and tissues (Misa Agustino et al. 2012; Shokri et al. 2015; Çelik et al. 2016). Pancreas is an important secretory organ in the human body and endocrine parts of this organ, islets of Langerhans, secrete insulin that plays an essential role in metabolism and glucose homeostasis. Pancreatic β -cell dysfunction causes impaired glucose homeostasis and has an important role in the pathogenesis of

diabetes. There are a few studies that suggest that EMR affect the morphology and insulin release of the pancreatic islets. Khaki and Ali-Hemmati (2015) have shown that in the EMF-exposed group (with a frequency of 50 Hz and intensity of 3 mT, 4 h a day for 6 weeks), the plasma insulin level was significantly decreased. It has been shown that exposure to extremely low-frequency EMFs reduces insulin secretion from the insulinoma cell line (Sakurai et al. 2004). Mortazavi et al. (2016) have shown that radiofrequency radiations emitted from the mobile phone could not alter the plasma level of insulin, but it causes a mild damage in the islet cells of the pancreas. The studies about the possible effects of exposure of EMF emitted from Wi-Fi on the secretory function of the pancreas are scarce, and it is not clear that this radiofrequency radiation directly influences the pancreatic islet insulin release and content. Therefore, one of aims of this study was to investigate the insulin secretion of the pancreatic isolated islet in the animals that exposed to RF-EMR emitted from Wi-Fi.

Many physiological functions such as mitochondrial functions can produce reactive oxygen substances (ROS). In animal body, the ROS are controlled and scavenged by enzymatic and non-enzymatic antioxidants. Imbalance in oxidative redox system results in an increase in ROS production

that can cause oxidative injuries in the cellular biomolecules such as nucleic acids, lipids, and proteins (Devasagayam et al. 2004; Yin et al. 2011). A number of recent reports have suggested that EMR induces excessive amounts of ROS eliciting oxidative stress damage that results in abnormal function in the biological system and disease (Consales et al. 2012; Çiğ and Nazıroğlu 2015; Yakymenko et al. 2016). Because the pancreas has a weak antioxidant defense system and it is sensitive to oxidative stress, it was of interest to investigate whether Wi-Fi radiation induces oxidative stress in the pancreas. Therefore, the present study has been designed to evaluate the effects of Wi-Fi radiation on the isolated islet insulin secretion and content, antioxidant enzymes activities, and lipid peroxidation in pancreas.

Materials and methods

Chemicals

Collagenase P was obtained from Roche Diagnostic (Mannheim, Germany); all other reagent-grade chemicals were purchased from Sigma (StLouis, MO, USA) or Merck (Darmstadt, Germany). The serum levels of insulin were determined using rat insulin kit (Mercodia, Uppsala, Sweden). The serum levels of glucose were measured using a commercial kit available from Pars Azmoon Co, Iran.

Animals and study design

Sprague–Dawley male, age-matched rats (12 weeks old), in the weight range of 230–260 g and belonging to a local stock bred in the animal facility of the Research Institute of Shiraz University of Medical Sciences (Shiraz, Iran) were used. Animals were kept in controlled conditions of light (12 h–12 h light–dark cycles), temperature ($22 \pm 3^\circ\text{C}$), and relative humidity ($24 \pm 6\%$) with free access to food and water. Rats were randomized into three groups (details as follows), eight rats in each group.

1. Wi-Fi_{2.4 GHz} group: Animals were placed in Plexiglas restrainer without free moving and exposed to 2.4 GHz EMR from Wi-Fi.
2. Sham group: Animals were placed in Plexiglas restrainer without free moving and treated exactly as exposed group except that the Wi-Fi turned off during the exposure.
3. Control group: The control animals were kept in standard cages in the animal room, without any treatment or restraint of any kind.

It should be noticed that the sham and Wi-Fi_{2.4 GHz} groups were transferred to an exposure room and placed in Plexiglas restrainer but the control group was kept in the animal room, without being transferred to the exposure room.

This study was performed and approved according to ethical guidelines of the Animal Experimentation Ethics Committee of Shiraz University of Medical Sciences.

The exposure system

In this study, the exposure system for RF-EMR from Wi-Fi was a D-Link Wi-Fi router (D-Link DIR-600L, D-Link Corporation, Taiwan) that has been used in previous study (Mahmoudi et al. 2015). The RF-EMR group was exposed to 2.4 GHz EMF irradiation for 4 h per day (4 pm to 8 pm) for 45 days. In present study, the duration of exposure and distance from Wi-Fi router is also chosen based on the study of Mahmoudi et al (2015). During the RF-EMR exposure, the animals were placed in Plexiglas cage that restrain the movement of the rats. The Wi-Fi router operated on power level of 0.1 W, and the Plexiglas cage was placed 30 cm away from this EMF exposure source. During the exposure time, the data were exchanged between modem and a laptop computer that was located 5 meter away from the Wi-Fi router.

Body and pancreatic weight

During the experiment, the body weights of all animals were measured every week. At the end of the experimental period, after deep anesthesia induced by intraperitoneal (IP) injection of pentobarbital sodium, 100 mg/kg, the pancreas was removed, cleaned from fat tissue, and weighed. The difference between initial and final body weight was calculated as the weight gain of the animals.

Intraperitoneal glucose tolerance test

For intraperitoneal glucose tolerance test (IPGTT), after overnight (10–12 h) fasting, the first blood samples were collected by cutting the tail tip at time zero under general anesthesia. After that, IP injection of glucose at a dose of 2 g/kg body weight was done, and blood samples were taken from the tail vein at 15, 30, 60, 90, and 120 min after glucose injection. Plasma levels of glucose were assayed by the glucose oxidase method (Pars Azmoon Co., Tehran, Iran), and plasma insulin concentrations were measured by the ultrasensitive rat insulin enzyme-linked immunosorbent assay method (ELISA, Mercodia, Uppsala, Sweden). The following formula (Farrokhi et al. 2014) was used for calculation of homeostasis model assessment of insulin resistance (HOMA-IR):

$$\text{HOMA IR} = \text{Glucose (mmol/l)} \times \text{Insulin (U/ml)} / 22.5.$$

Islet isolation

Islet isolation was performed based on the method described in a previous study (Safayee et al. 2016). Briefly, after anesthesia (60 mg/kg pentobarbital I.P.), 10 ml ice-cold HBSS containing 0.5 mg/ml of collagenase P was injected through the bile duct. The inflated pancreas was removed and digested for 17 min at 37°C . After four washes with cold HBSS under a stereomicroscope, the islets were hand-picked.

Insulin secretion and content

In vitro study was performed for evaluation of insulin secretion; batches of five islets were cultured in 1 ml RPMI-1640 media and incubated with different glucose concentrations (2.8, 16.7 mmol/l) for 60 min in a 37°C CO₂ incubator. The incubation medium was removed and kept at -20°C until the insulin measurement. The insulin content was determined with acid-ethanol extraction, as described with detail in a previous study (Karbalaei et al. 2016).

Measurement of oxidative stress markers in the pancreas

On day 45, the animals were killed under anesthesia and the pancreases were removed, weighted, washed with cold saline, and immediately frozen at -80°C. Pancreases were sliced and homogenized in cold sodium phosphate buffer (pH 7.4) containing 1 mM EDTA. The homogenates were then centrifuged at 4000 rpm for 15 min at 4°C. The supernatants of the pancreas tissue homogenates were separated and used to assay enzyme activities and protein determination. Marklund and Marklund (1974) and Hafeman et al.'s (1974) methods were used to assay superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities, respectively. Catalase (CAT) activity was measured spectrophotometrically using the method described by Aebi (1983). This method is based on the content of H₂O₂ which was degraded. Lipid peroxidation (TBARS) according to the method of Hagar et al. (2006) was determined by measuring thiobarbituric acid reactive substances based on spectrophotometric measurement of the color produced during the reaction to thiobarbituric acid (TBA) with MDA. The Bradford (1976) method was used for assessment of protein content of the supernatant using bovine serum albumin (BSA) as standard.

Statistical analysis

Statistical analysis of all data was performed using GraphPad Prism software, version 6.0 (Graphpad Software, La Jolla, CA, USA) and expressed as means ± S.E.M. For multiple comparisons, one-way ANOVA (post hoc Tukey) was done. Repeated

measures two-way ANOVA followed by the Bonferroni post hoc test was used to analyze the plasma glucose and insulin before and after 15, 30, 45, 60, 90, and 120 min glucose injection in several groups during IPGTT. A value of $p < .05$ was considered statistically significant.

Results

Effect of Wi-Fi radiation on body and pancreatic weights

As shown in Table 1, initial body weight did not differ among the groups. At the end of the study, Wi-Fi_{2.4GHz} group had lower body weight and weight gain as compared with the control and sham groups ($p < .05$). There were no statistically significant differences in the pancreas weight of the rats among the three experimental groups.

Effect of Wi-Fi radiation on plasma glucose and insulin concentrations, HOMA-IR, and islet insulin content

As shown in Table 2, there were no significant differences between all experimental groups in the fasting plasma glucose and insulin levels, HOMA-IR, and islet insulin content.

Effect of Wi-Fi radiation on plasma glucose concentrations during intraperitoneal glucose tolerance test

Glucose tolerance test results for all groups are shown in Figure 1. Our results showed that the mean plasma glucose levels during IPGTT in the Wi-Fi_{2.4GHz} group at 15, 45, 60, 90, and 120 min after injection of glucose (2 g/kg body weight) were higher than those of the control and sham groups (Figure 1(A)). As shown in Figure 1(B), the area under the curves (AUC) for plasma glucose concentration during IPGTT in Wi-Fi_{2.4GHz} group was also significantly higher as compared with the control and sham groups. Before and after glucose injection, there were no significant differences in the plasma glucose concentrations and plasma glucose AUC between the control and sham groups.

Table 1. Effects of Wi-Fi radiations on body and pancreas weights.

Group	Initial body weight (g)	Final body weight (g)	Body weight gain (%)	Pancreatic weight (g)
Control	246.8 ± 2.8	311.8 ± 6.7	26 ± 2.3	1.08 ± 0.05
Sham	245.4 ± 3.2	308.5 ± 6.3	25 ± 1.1	1.12 ± 0.03
Wi-Fi _{2.4 GHz}	246.3 ± 2.3	292.0 ± 3.1 ^{a,b}	18 ± 1.2 ^{a,b}	1.13 ± 0.06

Values are expressed as means ± S.E.M. ($n = 8$).

^a $p < .05$, statistically significant differences compared to control.

^b $p < .05$ statistically significant differences compared to sham group.

Table 2. Effects of Wi-Fi radiations on fasting plasma glucose and insulin, HOMA-IR, and islet insulin content.

Group	Fasting glucose (mmol/l)	Fasting insulin (U/ml)	HOMA-IR	Islet insulin content (pmol/mg protein)
Control	5.20 ± 0.2	7.76 ± 1.30	1.81 ± 0.36	1423.1 ± 147.2
Sham	5.05 ± 0.28	8.59 ± 0.45	1.92 ± 0.14	1305.7 ± 137.5
Wi-Fi _{2.4 GHz}	4.80 ± 0.25	8.45 ± 1.77	1.89 ± 0.44	1658.06 ± 165.4

Values are expressed as means ± S.E.M. ($n = 8$).

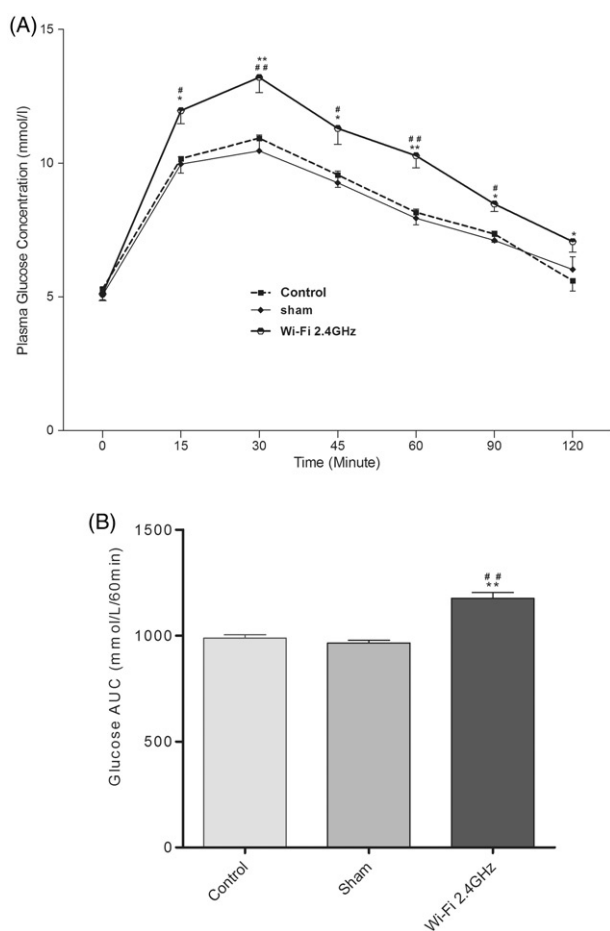


Figure 1. Mean plasma glucose (A) and the total area under the respective glucose curve (B) during intraperitoneal glucose tolerance test in the control, sham, and Wi-Fi-exposed groups. Data are expressed as means $\pm$ S.E.M., $n = 8$. Significant differences were assessed by two-way ANOVA and Bonferroni post hoc test. $**p < .01$, $*p < .05$, statistically significant differences compared with control. $###p < .01$, $##p < .05$, statistically significant differences compared with sham group.

Effect of Wi-Fi radiation on plasma insulin levels during intraperitoneal glucose tolerance test

The results of insulin levels before and during the IPGTT for all groups are demonstrated in Figure 2. As shown in Figure 2(A), after injection of intraperitoneal glucose, the insulin levels were significantly reduced at all time periods except 90 and 120 min in the Wi-Fi_{2.4GHz} group as compared with the control group. The plasma insulin AUC in the Wi-Fi radiation-exposed group was also significantly lower than the control and sham groups (Figure 2(B)). There were no significant differences in the plasma insulin concentrations and area under the curves for insulin between the control and sham groups.

Effect of Wi-Fi radiation on glucose-stimulated insulin secretion of pancreatic islet

In this study, pancreatic islet insulin release in response to two different glucose concentrations (2.8 and 16.7 mmol/l) was measured. As shown in Figure 3(A), in low glucose concentrations of 2.8 mmol/l, insulin release by the pancreatic islets in Wi-Fi_{2.4GHz} group was not altered compared to the control and sham groups. In response to glucose

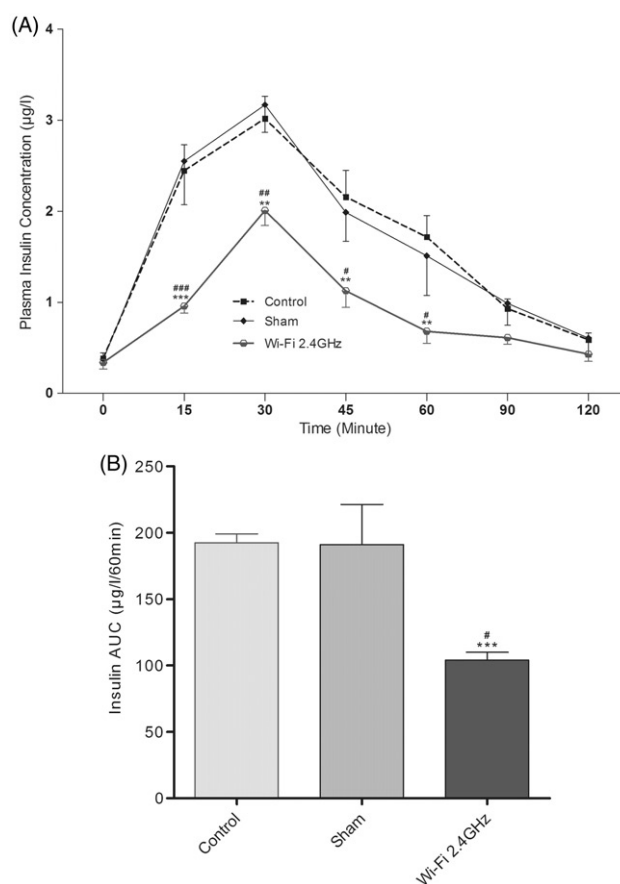


Figure 2. Mean plasma insulin (A) and the total area under the curve for plasma insulin (B) during intraperitoneal glucose tolerance test in the control, sham, and Wi-Fi-exposed groups. Data are expressed as means $\pm$ S.E.M., $n = 8$. Significant differences were assessed by two-way ANOVA and Bonferroni post hoc test. $***p < .001$, $**p < .01$, statistically significant differences compared with control. $###p < .001$, $##p < .01$, $#p < .05$, statistically significant differences compared with sham group.

concentration of 16.7 mmol, insulin secretion by the islets of pancreas in Wi-Fi exposed rats was significantly reduced (Figure 3(B)). In comparison with the controls, pancreatic islet insulin secretion in the sham group was not changed.

Effect of Wi-Fi radiation on pancreatic oxidative stress markers

Pancreatic tissue MDA, GSH, SOD, GPx, and CAT levels are presented in Figure 4. The exposure to radiofrequency radiation emitted from Wi-Fi had a significant adverse effect on the pancreas redox status. A significant elevation in MDA concentration as a marker of lipid peroxidation was observed in the pancreatic tissues of the Wi-Fi-exposed rats when compared with the sham and control rats (Figure 4(A)). In comparison with the controls, a significant reduction in GSH level (Figure 4(B)) and antioxidant enzyme activities of SOD (Figure 4(C)) and GPx (Figure 4(D)) was indicated in the Wi-Fi_{2.4GHz} group. We observed no significant differences in CAT activity among all the three groups (Figure 4(E)). On the other hand, there were no significant differences between the control and sham groups in lipid peroxidation and antioxidants parameters.

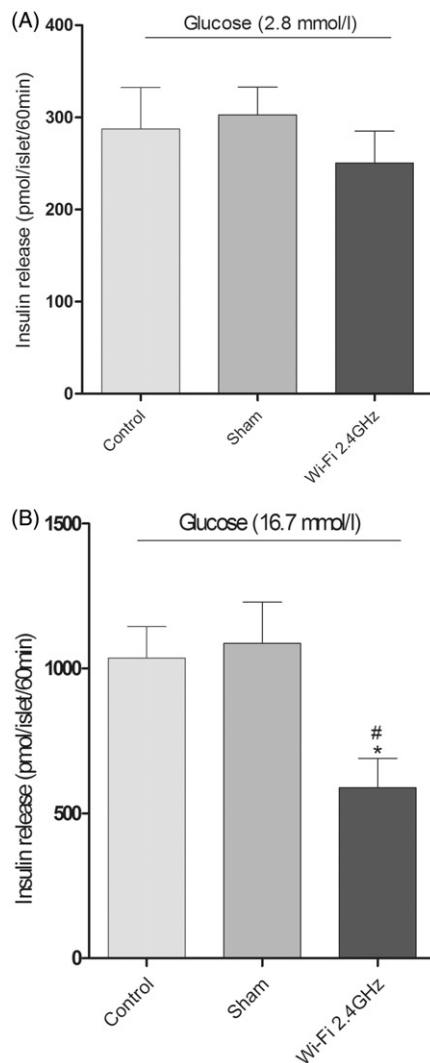


Figure 3. Insulin secretion by islets in glucose concentrations of 2.8 mmol/l (A) and 16.7 mmol/l (B) in the control, sham, and Wi-Fi-exposed groups. Each bar presents the mean $\pm$ SEM from 12 batches of five islets from 5 rats, incubated for 60 min in 1 ml of medium containing different glucose concentrations. Differences were analyzed by one-way ANOVA. * $p < .05$, statistically significant differences compared with control. # $p < .05$, statistically significant differences compared with sham group.

Discussion

WLANs have widespread use in public areas, schools, workplaces, and homes. Therefore, our environment is loaded with electromagnetic pollution and the study of the health effect of EMF radiations on living cells is vital. Some studies have reported that EMF radiations at different frequencies and durations can cause serious biological effects in some tissue of experimental animals (Fahmy et al. 2015; Gokcek-Sarac et al. 2017; Türedi et al. 2017). Uncontrolled usage of wireless technologies such as Wi-Fi can have potential adverse effects on the biological system. The aim of present study was to evaluate the effect short distance and long-term exposure from Wi-Fi EMF radiation on insulin secretion and oxidative stress in pancreas of rats. This study indicated that radiofrequency radiation emitted from Wi-Fi (2.45 GHz) induced impaired glucose tolerance and reduced pancreatic insulin secretion in rats. Our data also showed that 2.45 GHz RF-EMR emitted by this device lead to changes in the

antioxidative activities and increase the ROS production and lipid peroxidation in the pancreatic islet cells.

Our data showed that exposure to RF-EMR from Wi-Fi decreased the body weight gain. The effects of radiofrequency exposure on body weight in different studies are inconsistent. In line with the present results, Salah et al. (2013) indicated that exposure to Wi-Fi radiofrequency radiation for 1 h/day/21 days reduced the body weight. In contrast to our data, Fahmy et al. (2015) showed that exposure to Wi-Fi radiation for 24 h/40 days increased the body weight gain in rats. These controversial results can be attributed to the intensity, frequency of use, duration of the exposure, and distance from exposure source which vary from individual to individual (Çiğ and Nazıroğlu 2015). It is suggested that EMR can affect the food intake and change body metabolism that influences the body weight gain (Tsuji et al. 1996).

Our data showed radiation exposed animals had impaired glucose tolerance test so that during IPGTT the mean plasma levels of glucose were significantly higher in Wi-Fi-exposed group compared with the control and sham groups. The result of this study also showed that Wi-Fi radiation can cause a significant decrease in the mean plasma levels of insulin during IPGTT. For the high level plasma glucose, there are two possibilities: first, decreased insulin secretion and second, reduced sensitivity of the peripheral tissues to insulin called insulin resistance. In further explorations, HOMA-IR index (in fasting state) and the islet insulin secretion were measured. Although there was no difference in HOMA-IR index between Wi-Fi radiation-exposed group and control, but islet insulin secretion in response to high concentration of glucose was significantly decreased in Wi-Fi radiation-exposed animals. As known, the insulin secretion from the pancreas is critical for the glucose homeostasis. Therefore, elevation of the blood glucose induced by Wi-Fi radiofrequency radiation can be related to reduced insulin secretion from pancreatic islet. Some possible mechanisms such as conformational changes in insulin, insulin receptors, and glucose transport proteins were proposed for hyperglycemia induced by EMF-RF radiations (Kleiber 2017).

The mechanism of glucose stimulated insulin secretion (GSIS) from pancreatic β -cells is approximately clear. By the glycolytic pathway and oxidative phosphorylation, glucose is metabolized to produce ATP. Increase of the cytoplasmic ATP/ADP ratio leads to closure of ATP-sensitive potassium (KATP) channels, depolarization of the plasma membrane, and the opening of voltage-sensitive Ca^{+2} channels. Increased Ca^{+2} influxes result in insulin release from β -cells (Wiederkehr and Wollheim 2006; Jensen et al. 2008). It has been suggested that there is a link between GSIS and ROS production in pancreatic β -cells and the same pathways involved in the activation of GSIS can enhance the ROS production (Fridlyand and Philipson 2004). Although low concentrations of ROS are necessary for biochemical process and the maintenance of physiological functions of the cells and tissues, free radicals have a role in cellular signaling (Forman et al. 2014). However, excessive production of ROS can be highly cytotoxic and can cause oxidative damage of cellular macromolecules such as proteins, lipids, and nucleotides that

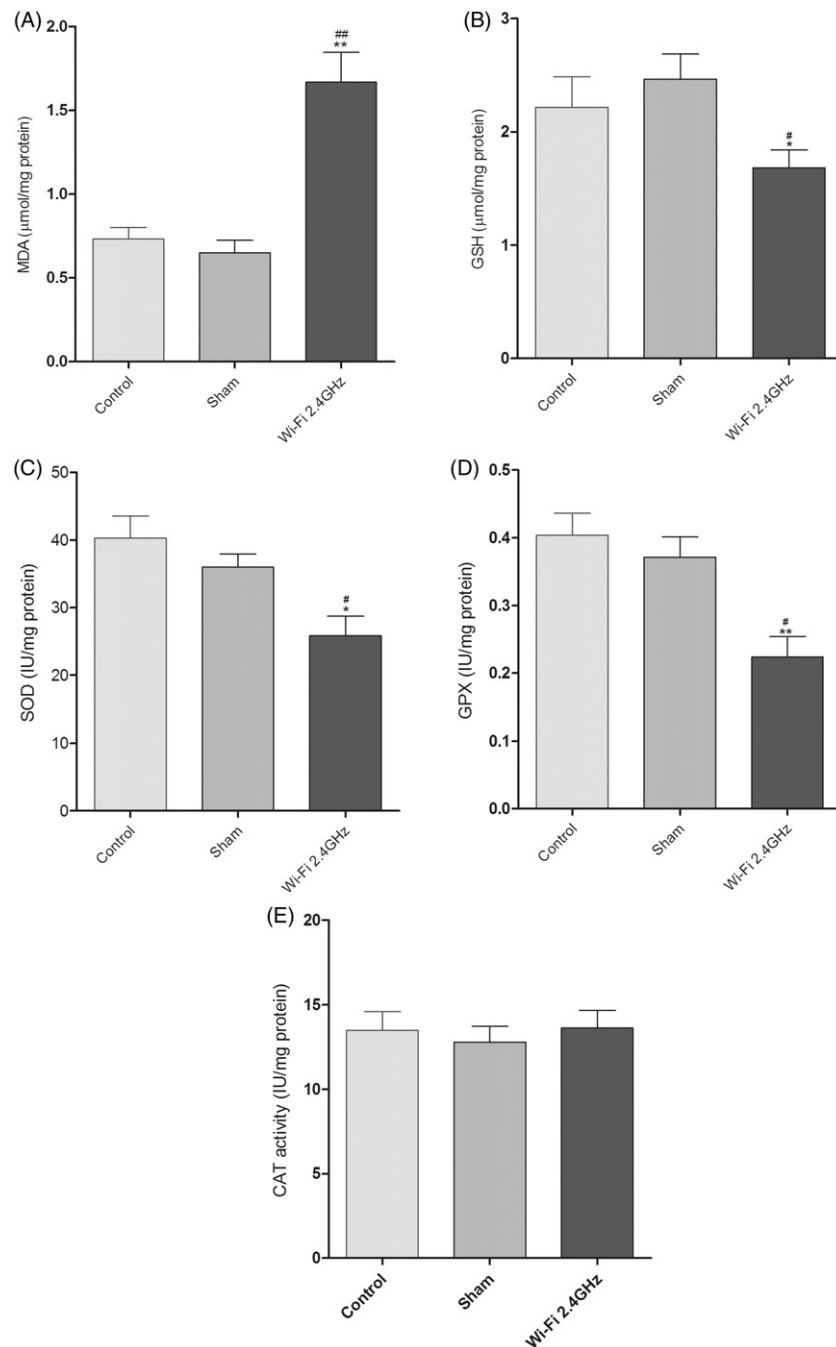


Figure 4. MDA level (A), GSH content (B), enzymatic activities of SOD (C), GPx (D), and CAT (E) in pancreas of control, sham, and Wi-Fi-exposed rats. Each bar presents the mean $\pm$ SEM from 8 rats. Differences were analyzed by one-way ANOVA. ** $p < .01$, * $p < .05$, statistically significant differences compared with control. ## $p < .01$, # $p < .05$, statistically significant differences compared with sham group.

leads to cell and tissue damage. The pancreatic islets have low levels of antioxidant enzyme concentrations and activities and increased ROS generation or oxidative stress is one of the most important factors in progression of pancreatic beta-cell dysfunction, impaired insulin secretion, and diabetes (Kajimoto and Kaneto 2004; Robertson and Harmon 2007). In the present study, we investigated biomarkers of oxidative stress and antioxidant in the pancreas of rats after exposure to Wi-Fi (2.45 GHz) for 4 h/day during 45 consecutive days. Compared with the control and sham groups, our result showed that Wi-Fi radiation group increased lipid peroxidation (LPO)/MDA. The level of GSH and activities of antioxidant enzymes such as SOD and GPx were significantly

reduced in the Wi-Fi group. There are contradictory reports about the effects of RF-EMR exposure on free radical generation in living cells; this controversy is attributed to different intensities, frequency and duration of radiation exposure, and also the kind of experimental animals (Yakymenko et al. 2016). In accordance with our results, several studies have shown that radiofrequency radiation has significant and positive effects on ROS production (Deshmukh et al. 2013; Tök et al. 2014; Ragy 2015; Yakymenko et al. 2016), so that pretreatment of animals with scavengers of free radical before exposure has reduced EMF-RF-induced DNA damage (Ozguner et al. 2006; Guney et al. 2007; Aynali et al. 2013). In contrast, a few studies reported that EMF-RF exposure has no significant effect

(Dasdag et al. 2003; Demirel et al. 2012). It is proposed that radiofrequency radiation induced ROS production in the living cells by Fenton reaction (an iron-mediated process), cellular membrane NADPH oxidase activation, and mitochondrial ROS overproduction (Friedman et al. 2007; Desai et al. 2009; Burlaka et al. 2013; Yakymenko et al. 2016).

In conclusion, our data showed that the EMF–RF emitted from Wi-Fi caused a high level of plasma glucose and low-level plasma insulin. Pancreatic islet insulin secretion was decreased in the Wi-Fi_{2.4GHz}-exposed groups. Excess production of ROS or reduced antioxidant defense system may be possible reasons for reduced insulin secretion in the Wi-Fi_{2.4GHz} group.

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Disclosure statement

The authors report no conflicts of interest.

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