

Ameliorative effects of vitamin C against hepatic pathology related to Wi-Fi (2.45 GHz electromagnetic radiation) in rats

O. Ozmen^{1*} and O. Kavrik²

¹Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology, Burdur, Turkey

²Suleyman Demirel University, Faculty of Medicine, Department of Physiology, Isparta, Turkey

ABSTRACT

► Original article

*Corresponding authors:

Ozlem Ozmen, Ph.D.,

E-mail:

ozlemozmen@mehmetakif.edu.tr

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Background: The aim of this study was to investigate hepatic lesions induced by the 2.45 GHz electromagnetic radiation (EMR) of Wi-Fi and the protective effects of Vitamin C (Vit C) in rats. **Materials and Methods:** Eighteen Sprague-Dawley female rats were randomly distributed into three groups, each containing six rats. The groups were Control, EMR (EMR, 1 h/day for 30 days) and EMR + Vit C (EMR, 1 h/day for 30 days and Vit C, 250 mg/kg/daily). At the end of the study, blood and liver tissue samples were collected for laboratory examinations. **Results:** Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were increased in the EMR group compared with the control group. Liver histology of rats in the EMR group revealed severe hyperemia, slight degeneration, and lipidosis. Caspase-3, hypoxia-inducible factor-1 (HIF-1), and HIF-2 immunoexpression was increased, and Sirtuin-1 (SIRT-1) expression was markedly decreased in hepatocytes in the EMR group. Vit C treatment ameliorated both biochemical and pathological findings. **Conclusion:** The results of this study showed that 2.45 GHz (Wi-Fi) EMR can cause liver damage in rats, but Vit C has an ameliorative effect.

Keywords: Wi-Fi, vitamin C, liver, pathology, caspase-3, HIF-1a, HIF-2a, SIRT-1, immunohistochemistry.

INTRODUCTION

Recently, humans have become increasingly affected by electromagnetic waves, and this situation is escalating rapidly. Numerous technical and electrical devices are sources of electromagnetic waves ^(1, 2). Most communication devices that use Wireless Fidelity (Wi-Fi) emit 2.45 GHz electromagnetic radiation (EMR) ⁽³⁾. Such an increase in EMR in the environment has raised scientific interest in the biological effects and possible health outcomes of EMR exposure ⁽⁴⁾. Recent studies showed that EMR may lead to pathological effects in different organs and systems in the human and animal bodies ^(5, 6).

EMR may affect biological systems by increasing the release of free radicals and by

changing the antioxidant defense systems of tissues, thus leading to oxidative stress ⁽⁷⁾. So, antioxidant treatments in animals and human could be beneficial in preventing or reducing some complications of EMR ⁽⁸⁾.

Apoptosis is a selective, controlled, and programmed cell death process that occurs as a result of normal cellular differentiation and development. Caspases belong to a family of highly conserved cysteine-dependent aspartate-specific acid proteases that use a cysteine residue as a catalytic nucleophile and share a stringent specificity for cleaving their substrates after aspartic acid residues in target proteins in the process of apoptosis ⁽⁹⁾. Caspases are categorized as proinflammatory or proapoptotic, depending upon their participation in these cellular responses.

Caspase 3, the prototype of a proapoptotic caspase and a pivotal effector caspase, plays a central role in the apoptotic process ⁽¹⁰⁾. It proteolytically cleaves a number of death substrates and activates endonucleases, leading to internucleosomal deoxyribonucleic acid (DNA) fragmentation, a hallmark of apoptosis ⁽¹¹⁾.

Hypoxia-inducible factor 1 (HIF-1) is a key transcription factor for adaptive responses to hypoxic conditions and regulates the expression of various genes involved in oxygen delivery, cellular growth, and redox homeostasis in many tissues and organs ⁽¹²⁾. HIF-1 is a heterodimeric factor consisting of an oxygen-sensitive α -subunit and a constitutively expressed β -subunit. The expression of HIF-1 α is upregulated under hypoxic conditions ⁽¹³⁾. HIF-1 α expression can represent an acute response to low pO₂, whereas HIF-2 α levels may increase over time in hypoxia and play a role during chronic hypoxia ⁽¹⁴⁾.

Sirtuins are a family of seven nicotinamide adenine dinucleotide-dependent class III histone deacetylases that are present in nearly all subcellular compartments ⁽¹⁵⁾. SIRT-1 is generally regarded as an important regulator that protects cells against stress and apoptosis. By regulating various targets, SIRT-1 has roles in protein and cellular homeostasis during stress, thereby protecting cells from oxidative and genotoxic damage ⁽¹⁶⁾.

Nowadays, both humans and animals are commonly affected by EMR, and harmful effects of EMR on different tissue and organs are increasingly reported. The aim of this study was to examine the effect of 2.45 GHz Wi-Fi EMR on the liver; the pathogenetic roles of caspase-3, HIF-1 α , HIF-2 α , and SIRT-1 in this damage; and the possible protective role of Vit C in rats.

MATERIALS AND METHODS

This study was performed in 18 female Sprague-Dawley rats divided into three groups. Rats were 8 weeks of age with a body weight range of 250-300 g, and there was no significant difference in body weight among the groups.

Rats were housed individually in stainless-steel cages under pathogen-free conditions in the laboratory at a temperature of $+24 \pm 3$ °C with light between 08:00 a.m. and 20:00 p.m. and free access to water and food. They received a commercial chow diet. An environmental average light intensity of 4000 lux was applied, and a humidity level of $40 \pm 10\%$ was maintained. The 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 Suleyman Demirel University. Animals were used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory Animals, prepared by this university.

After a 1-week adaptation period, all animals were randomly divided into three equal groups. The control group (CON) (n = 6) was not exposed to EMR. The EMR (n = 6) group was exposed to 2.45 GHz EMR. The EMR + Vit C group was treated orally with 250 mg/kg/daily Vit C in conjunction with EMR exposure for 30 days ⁽¹⁷⁾.

The 1-hour exposure to irradiation in the EMR and EMR+Vit C groups was carried out between 9:00 a.m. and 12:00 a.m. every day. The welfare of the rats was found to be normal during the exposure period. The first dose of Vit C administration was given 24 h prior to the experiment. Rats in the CON group received isotonic saline solution of an equal volume to the Vit C used in EMR+Vit C group by oral gavage. The rats were not anesthetized during the exposure. The CON group was kept in their cages under the same environmental conditions without stress and EMR exposure ^(18,19).

The exposure system was obtained from SET ELECO (Set Electronic Co./Istanbul, Turkey). The EMR source was a radio frequency (RF) test generator emitting 2.45 GHz RF, pulsed at 217 Hz by its monopole antenna system ⁽¹⁹⁾. This device produces an electric field with a strength of 0.1 V/m to 45.5 V/m.

All the exposure systems were located in a screened-in room which 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 Saygin *et al.* ⁽¹⁹⁾ and Faraone *et al.* ⁽²⁰⁾.

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

The repetition time, frequency, and amplitude of the spectrum of RF energy were observed and verified by using a satellite level meter (PROMAX, MC-877C, Barcelona/Spain). The specific absorption rate (SAR) value was calculated by the electromagnetic dosimeter using a measured electric field intensity (V/m) and digital anatomical models based on the FDTD numerical code. Consequently, the SAR value was predicted for the same condition, orientation, and antenna power, using this method as 4.88 mW/kg for liver tissue by using the simulation program.

At the end of the study, rats were anesthetized with a mixture of ketamine hydrochloride (HCl) (90 mg/kg) and xylazine (10 mg/kg), which were injected intraperitoneally before sacrifice, and then a necropsy was performed. Blood samples were collected for biochemical analyses. Liver tissues were removed and fixed in 10% formaldehyde solution for histopathological and immunohistochemical examinations.

An autoanalyzer (Beckman Coulter AU 680, California, and USA) was used to determine aspartate aminotransferase (AST) and alanine aminotransaminase (ALT) levels.

Formalin-fixed liver samples were then routinely processed by using automated tissue-processing equipment (Leica ASP300S, Wetzlar, Germany). Then, the tissues were embedded in paraffin and sectioned to a 5- μ m thickness by using a Leica RM2155 rotary microtome (Wetzlar, Germany). Tissue sections were stained with hematoxylin-eosin (HE) and examined microscopically. All sections were examined similarly for all samples. Histopathological changes were graded in a blinded manner.

Then, 5- μ m thick liver sections were mounted onto polylysine microscope slides. A streptavidin-biotin immunoenzymatic antigen detection system [EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436), (Abcam, Cambridge, UK)] was used as a secondary antibody. Liver sections were stained immunohistochemically in order to demonstrate caspase-3, HIF-1 α , HIF-2 α , and SIRT-1 expression. Commercial kits (Abcam, Cambridge, UK) were used for immunohistochemical examination. Liver samples were immunostained with caspase-3 [Anti-caspase-3 antibody (ab4051), 1/100 dilution]; HIF-1 α [Anti-HIF-1-alpha (H1alpha67) Grade ab1 antibody - ChIP, 1/100 dilution], HIF-2 α [Anti-HIF2 alpha antibody (ep190b; ab8365)], and Sirtuin [Anti-SIRT1 antibody (E104; ab32441), 1/100 dilution], according the manufacturer's instructions. Finally, sections were counterstained with Harris hematoxylin, mounted in mounting medium, and examined under a 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). Immunohistochemical results were evaluated by one of the pathologists from another center. The rats included in this study were evaluated randomly by a researcher who was blinded to group allocation. For morphometric examination of the liver, an Olympus CX41 light microscope was used. Morphometric evaluation was carried out by using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

Statistical analyses

One-way analysis of variance was used to detect significant differences between groups. SPSS 15.0 software for Windows (SPSS Inc, Chicago, IL, USA) was used for data analysis. Variables were presented as frequencies, percentages, and means $\pm$ standard deviations. The Bonferroni test was used for the

analysis of biochemical parameters and immunohistochemical scores. The overall number of positive cells or the intensity of staining were noted and compared between groups. One-way analysis of variance was used to detect significant differences between the groups. The threshold for significance was set as $p<0.05$.

RESULTS

No rats died during the study, and no marked necropsy findings were observed in any group. Serum AST and ALT levels were increased by EMR exposure, but Vit C ameliorated these levels. Biochemical analysis result of serum samples for AST and ALT are given in table 1.

Liver histology of rats in the EMR group

showed marked hyperemia, slight degeneration, and lipidosis (figure 1). Lipid droplets in hepatocytes was characterized as empty spaces varying in diameter. Neither necrosis nor infiltration were diagnosed in the livers of any groups. Swollen hepatocytes were irregular in shape and arranged in anastomosing cords around the central veins.

Immunohistochemical examination revealed that while slight or no immunoreaction was observed in the Vit C and Control groups, marked increases in all markers except SIRT-1 were seen in hepatocytes in the EMR group. SIRT-1 expression was decreased by EMR expression. Vit C had an ameliorative effect on EMR-induced immunohistochemical expression (figures. 2-5). The results of statistical analysis on liver immunohistochemistry scores are shown in table 2.

Table 1. Results of statistical analysis on liver enzyme biochemistry.

	AST (U/L)	ALT (U/L)
CON	96.16±43.76	84.00±21.96
EMR	197.57±29.68	136.00±37.55
Vit C	94.60±21.75	78.40±19.56
	CON-EMR<0.001	CON-EMR<0.05
	CON-Vit C (NS)	CON-Vit C (NS)
	EMR-Vit C<0.01	EMR-Vit C<0.01

*Values are presented as means±SD. The relationships between groups and results of biochemical markers are assessed by one-way ANOVA. **NS: Non significant; EMR: Electromagnetic radiation; CON: Control; ALT: Alanine aminotransaminase; AST: Aspartate aminotransferase.

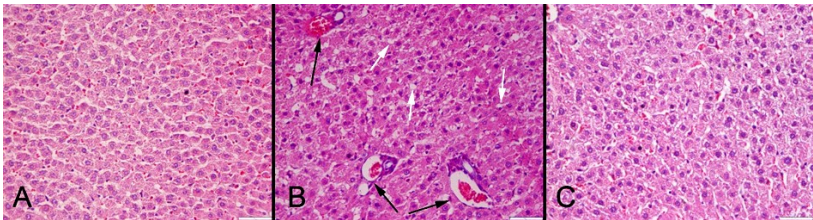


Figure 1. (A) Normal liver histology of the Vit C group; (B) marked hyperemia in the liver (black arrows) and lipidosis in hepatocytes (white arrows) in the EMR group; (C) normal microscopic appearance of the liver in the Vit C group; HE, bars = 50 µm.

Figure 2. Caspase-3 immunoreaction in the livers of rats treated with EMR with or without Vit C. (A) no immunoexpression in the Vit C group; (B) marked immunopositive reaction (arrows) in livers in the EMR group; (C) negative immune reaction in hepatocytes in the Control group, Streptavidin biotin peroxidase method, bars = 50 µm.

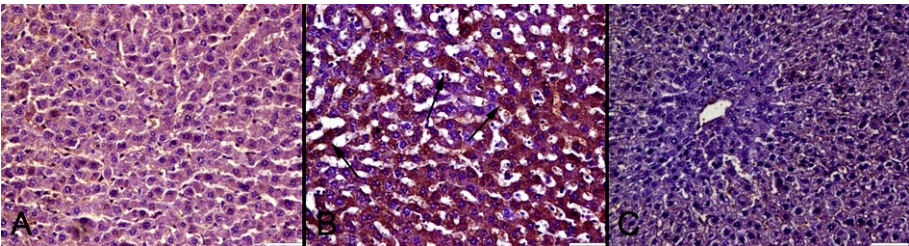


Figure 3. Effect of EMR and Vit C on HIF-1a immunoreaction in the livers of rats. (A) no expression in the Vit C group; (B) marked immunopositive reaction (arrows) in hepatocytes in the EMR group; (C) negative immunoreaction in the Control group; streptavidin biotin peroxidase method, bars = 50 µm.

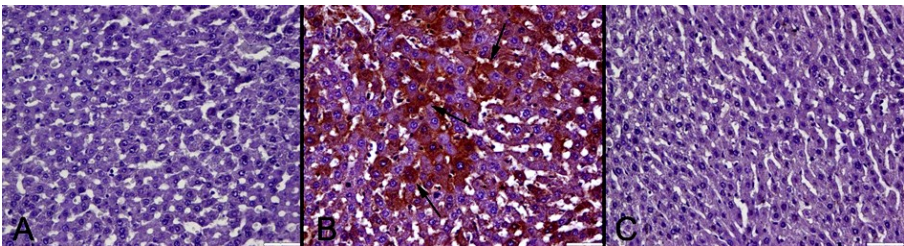


Figure 4. Effect of EMR and Vit C on HIF-2a immunoreaction in the livers of rats. (A) no expression in the Vit C group; (B) marked immunopositive reaction (arrows) in hepatocytes in the EMR group; (C) negative immunoreaction in the Control group; streptavidin biotin peroxidase method, bars = 50 µm.

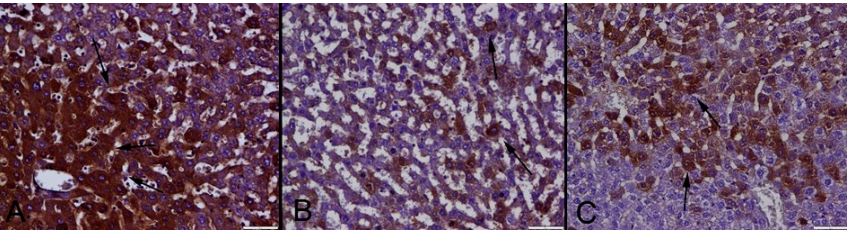
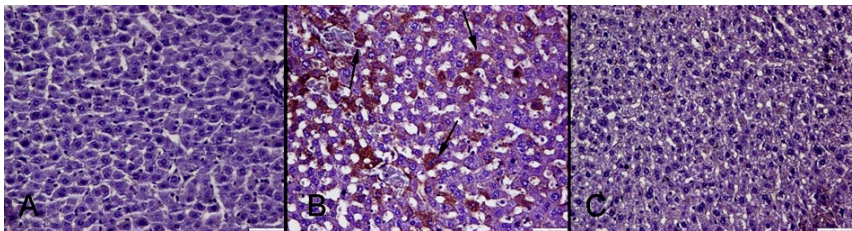


Figure 5. Effect of EMR and Vit C on SIRT-1 immunoreactivity in the livers of rats. (A) marked expression in hepatocytes (arrows) in the Vit C group; (B) decreased immunopositive reaction in hepatocytes (arrows) in the EMR group; (C) increased immunoreaction in hepatocytes (arrows) in the control group compared with the EMR group; streptavidin biotin peroxidase method, bars = 50 µm.

Table 2. Results of statistical analysis on immunohistochemical scores.

	Caspase-3	HIF-1a	HIF-2a	SIRT-1
Vit C	0.25±0.16	0.00±0.00	0.25±0.16	2.25±0.16
EMR	2.30±0.21	2.40±0.26	1.60±0.30	0.80±0.24
Cont	0.20±0.13	0.10±0.01	0.20±0.13	1.20±0.13
P value	Vit C-EMR (<0.001) Vit C-Cont (NS) EMR-Cont (<0.001)	Vit C-EMR (<0.001) Vit C-Cont (NS) EMR-Cont (<0.001)	Vit C-EMR (<0.001) Vit C-Cont (NS) EMR-Cont (<0.001)	Vit C-EMR (<0.001) Vit C-Cont (<0.01) EMR-Cont (<0.001)

• Values presented as mean±SD. • One-way ANOVA Bonnferroni test. • NS: non-significant

DISCUSSION

Studies on the influence of EMR generated by mobile phones or the other commonly used technology products that are rapidly increasing in prevalence in our daily lives have expanded in recent decades. Examination of the effects of EMR in humans solely for experimental reasons would be against medical ethics. For that reason, this study was conducted to examine the effects of EMR on the livers of rats. In accordance with commonly accepted norms aimed at reducing the suffering of laboratory animals, the study was conducted on a small yet representative group of rats (18 animals) divided into three subgroups of 6 rats each.

Mobile phones emit EMR in the microwave frequency range (900–2450MHz), which may pose a danger to human health (21,22). Although studies recently reported on the harmful effects of EMR, the use of technological devices that emit EMR is increasing. This study also showed

the harmful effects of mobile phones emitting 2.45 GHz EMR on rat livers at the cellular level. Due to the deleterious effects of EMR, numerous prophylactic agents have also been examined for their ability to protect cells from the effects of EMR. Because oxidative stress plays an important role in the effects of EMR exposure on organs and tissues (23), the main choice for prophylactic measures are antioxidant agents. This study revealed that Vit C may be an excellent agent for preventing EMR-induced damage to liver cells.

One of the main findings of this study was related to the effects of specific caspase-3 activation during EMR exposure. In this study, active caspase-3 was found to be more abundant in the livers of rats treated with EMR. Vit C treatment caused a decrease in caspase-3 expression. The results of the current study demonstrate that caspase-3 activation in hepatocytes plays a central role in EMR-related hepatic damage.

Recent studies have suggested that the expression levels and deacetylase activity of SIRT-1 are constitutively decreased under pathological conditions and that small noncoding microRNAs play a key role in this abnormal regulation ^(24,25). The results of this study were also in agreement with previous studies. A statistically significant decrease was observed in SIRT-1 expression in the hepatocytes of EMR-exposed rats.

HIFs regulate numerous signaling events by binding to specific DNA sequences, known as hypoxia-responsive elements, in target genes, resulting in their increased or decreased transcription ⁽²⁶⁾. Numerous studies reported that regions of hypoxia develop in the liver after acute liver injury caused by, for example, alcohol, monocrotaline, or acetaminophen treatment ⁽²⁷⁻³⁰⁾. This study showed that exposure to EMR, one of the liver-damaging agents, caused increases in HIF-1a and HIF-2a expression.

The biological effects of exposure to EMR from mobile phones were reported to be variable and to depend on many factors, including the duration of exposure, distance from the various sources, species, and tissues, as well as the conditions of exposure ⁽³¹⁻³⁴⁾. Although this study showed that Wi-Fi caused EMR-related hepatic damage, further studies are needed to evaluate dose- or duration-related changes in the harmful effects of EMR on the liver.

The role of HIFs in EMR has not been fully investigated yet. In this study, we provide evidence for an effect of HIF-1 α and HIF-2a on hepatic lipid accumulation in EMR exposure. These results showed a possible relationship between EMR exposure and hypoxia in the liver and a specific pathophysiological role in EMR-induced liver damage. Hepatic fibrosis is an important problem and has many causes whereby damaging agents produce some extent of hepatocellular injury, which initiates a reparative process. Part of this process involves differentiation of hepatic stellate cells into myofibroblasts, and then portal fibroblasts become activated. In chronic damage, collagen deposition continues, and fibrosis occurs ⁽³⁵⁻³⁷⁾.

HIFs play a critical role in the development of liver fibrosis ⁽³⁸⁾. An important result of this study is that HIF-1a and HIF-2a activation may be related to hepatic fibrosis in chronic EMR exposure.

Recently, EMR-induced damage in different organs or tissues has been frequently reported. However, the mechanisms of this damage have not yet been clarified. The present study data suggest that the development of EMR-related hepatic damage in rats may be associated with the downregulation of SIRT-1 and upregulation of HIF-1a, HIF-2a, and caspase-3. Our study provides direct evidence for the relationship between the development of EMR-induced liver damage and the prevention of this damage with Vit-C treatment.

In conclusion, EMR emitted from mobile phones might produce impairments in some biochemical and pathological changes in liver tissues of rats. Withdrawal from EMR could definitely overcome the deleterious effects of EMR exposure. Further experiments are still necessary with the purpose of explaining the frequency, intensity, exposure time, and other parameters involved in EMR, especially concurrent with environmental pollutants, so that humans may protect ourselves from these harms.

Conflicts of interest: Declared none.

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