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Endurance exercise exerts hepatoprotection against electromagnetic radiation via targeting oxidative stress in male rats

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► ***Original article***

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ABSTRACT

Background: The effects of electromagnetic radiation (EMR) on the body are an emerging field of research. The evidence supports the production of oxidative stress (OS) through EMR and the positive role of exercise in reducing liver damage. The evidence supports the production of OS through EMR and the positive role of exercise in reducing liver damage. ***Materials and Methods:*** 32 Wistar male rats were randomly divided into four groups: control, EMR, endurance training (ET), and EMR+ET. The ET and EMR+ET groups ran on the treadmill with the maximum speed and duration of 30 m/ min and 35 min, respectively (5 sessions/weeks, during four weeks). The ET and EMR+ ET groups were exposed to a 2.45GHZ Wi-Fi router for four weeks (4 h/day, 7days/weeks). Forty-eight hours after the last training session, the blood and liver tissue of the animals were sampled for histological, biochemical evaluations and measurement of oxidative stress markers. ***Results:*** Histopathological findings confirmed the protective role of ET in rats exposed to EMR. The results showed that in the EMR group, the concentration of malondialdehyde (MDA) and the serum levels of liver enzymes (AST, ALT, and ALP) increased significantly, and the activity of antioxidant enzymes decreased significantly ($P<0.05$). In the ET and EMR+ET groups, compared to the EMR group, the concentration of MDA and the serum levels of liver enzymes significantly decreased, and the activity of antioxidant enzymes increased significantly ($P<0.05$). ***Conclusion:*** Our findings show that ET can improve the state of OS and liver damage caused by EMR.

INTRODUCTION

One of the important challenges with modern technology is electromagnetic pollution. Although this technology helps to make life easier, the adverse effects of electromagnetic radiation (EMR) increase the possibility of reducing the quality of life [1]. Wi-Fi is used to connect devices and access the Internet in homes, schools, workplaces, and public transportation [2]. Despite the advantages of using this technology, there are risks involved. Exposure to EMR raises the possibility of tissue damage, which could result in conditions like cancer, neurological disorders, and cardiovascular problems [3, 4]. The liver is one of the organs that studies have shown to be vulnerable to the emission of EMR [5-7]. The liver is essential for sustaining the body's homeostasis during both rest and physical activity, and it is one of the tissues that experiences oxidative stress [8]. Wi-Fi may play a significant role in causing cellular damage through the oxidative stress pathway [9]. Research has shown that electromagnetic fields (EMFs) induce the formation of reactive oxygen species (ROS) in cells. ROS are participated in numerous biological processes in the human body. Nevertheless, they are dangerous for the biological and physiological substances of cells. With an excessive increase in ROS, cell damage occurs, which can lead to a wide range of diseases. Free radicals (FR) react with various biological molecules, including DNA, and lead to changes in their structure [10]. Oxidative stress occurs when there is no balance between the production of FR and the ability of cells to clear them. Oxidative stress, as a harmful process, can destroy cellular structures such as plasma membrane, lipids, proteins, lipoproteins, and DNA [11]. Protective mechanisms, including antioxidants and antioxidative enzymes, regulate the physiological levels ROS within

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cells. Meanwhile, both external and internal factors influence ROS levels by altering the activity of enzymes responsible for producing and degrading ROS [12]. The primary defense against oxidative stress is with antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) [13]. Antioxidant defense mechanisms are weakened by exposure to agents such as EMF that cause excessive ROS production [10]. Weakening the defense mechanism of antioxidant enzymes and producing oxidative stress in the liver tissue can affect the activity of liver enzymes [14]. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are liver enzymes whose serum levels indicate the state of liver cells. In such a way that there is a high level of these enzymes in the liver, and after liver damage, their serum level increases [15]. Based on the findings, experts recommend that people include physical activity and exercise regularly in their lifestyle because it reduces the risk of chronic diseases and mortality. At the same time, it is a means of primary prevention of diseases [16]. Endurance exercise is a strategy to increase exercise capacity and health in clinical and healthy individuals [17]. The positive effect of exercise on preventing injuries caused by exposure to EMR in some organs has been reported [18-21]. It is essential to mention that oxygen consumption increases in high-intensity exercise; therefore, with the increase of FR in this type of activity, there is a possibility of weakening the antioxidant capacity, and as a result, acute and high-intensity exercise increases oxidative stress. While continuous exercise with moderate intensity strengthens antioxidant capacity and ultimately reduces oxidative stress [22, 23]. For example, several studies have demonstrated the beneficial impact of exercise on decreasing oxidative stress [24, 25]. While in another study, an increase in oxidative stress due to exercise has been shown [26]. Although several certain research have examined the impact of exercise on the performance of some tissues exposed to EMR, there remains a paucity of knowledge regarding the effects of endurance exercise on liver damage induced by EMR, and these effects are still unknown. Given the limited understanding of the protective role of endurance training against EMR-induced liver damage, this study uniquely aims to elucidate the histopathological and biochemical effects of ET on oxidative stress in hepatic tissues. We hypothesize that by reducing FR and improving the antioxidant defense mechanism, endurance training (ET) reduces decreases cellular caused by EMR emission and, therefore, can prevent the destruction of liver tissue and help improve its function. Therefore, this research was conducted to investigate the influence of ET on histopathological, histochemical alterations, and liver oxidative stress in male rats exposed to EMR emitted from a Wi-Fi$_{2.45GHZ}$ device.

MATERIAL AND METHODS

Animals

For the research, a total of 32 male Wistar rats, aged 8 weeks and weighing between 180 and 220 grams, were sourced from the Pasteur Institute of Iran and chosen as the study samples. Following weighing, the rats were randomly assigned to four groups: control (C), EMR, ET, and EMR+ET. To acclimate to the laboratory setting, the animals were housed for a period of two weeks. The rats were kept in standard laboratory conditions (temperature 22 ± 2 °C, humidity 50 ± 5 with a 12-h light/dark cycle) and in standard transparent polycarbonate cages (two rats from the same group in each cage) and All of them had free access to laboratory animal food and water. All ethical guidelines for the study were adhered to in accordance with the principles for working with laboratory animals set by Iran University of Medical Sciences (Ethical number: IR.IUMS.AEC. 1401.033). Additionally, the study procedures were conducted following the guidelines established by the National Institutes of Health (NIH).

Endurance training program

To minimize stress and get familiar with the training program, rats in the training groups ran on a treadmill (Tajhiz Gostar Omid Iranian, Iran) for one week before starting the main protocol, participating in five sessions of 5 minutes each at a speed of 10 m/min with a 0% incline [27]. At the beginning and end of each ET session, the rats warm up and cool down for 5-3 min at a speed of 5-7 m/min. Following the familiarization period, ET and EMR+ET groups implemented the ET program for four weeks. During the first week, the rats ran on the treadmill for 25 minutes at a speed of 20 m/min, while in the second week, they increased to 30 minutes at 25 m/min. In the third and fourth weeks, the rats continued with exercise training at the same duration but at a speed of 30 m/min for 35 minutes [28]. In addition, the Wi-Fi router was disabled while performing endurance exercise during the study.

EMR Exposure

In the present research, rats in the EMR and EMR+ET groups were subjected to EMR emitted by a Wi-Fi router operating at 2.45 GHz (D-Link Corporation, DWR-M920, Taiwan) for four hours each day over a duration of four weeks. During the four hours of EMR emission, the rats were housed in their cages, positioned 30 cm away from the Wi-Fi router. Additionally, data exchange occurred between the router and a laptop, involving download activities [29, 30]. The specific absorption rate (SAR) was calculated using the equation $SAR = \frac{\sigma . E^2}{\rho} \ (W/kg)$. here E (V/m) represents the electric field strength, σ (S/m) denotes the electrical conductivity of the tissue, and ρ (Kg/m^3) indicates the mass density of the tissue [31].

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The magnitude of the electric field was measured to be 4.53 V/m using a field meter (Electromagnetic Radiation Tester Model ERT2000, Taiwan). Consequently, the measured SAR value for the whole body was found to be 0.03 W/kg. In addition, to reduce and prevent the effects of EMR, the study was conducted under the same laboratory conditions, first on two groups that were not exposed to EMR. Then the two groups that were exposed to EMR were studied.

Blood and liver tissue sample

Rats were anesthetized with a mixture of ketamine (30-50 mg/kg) and xylazine (3-5 mg/kg) 48 hours following the final ET session. After anesthesia, a blood sample (10 cc) was taken from the heart of each rat using a syringe, and then the liver tissue was carefully separated. Blood samples were centrifuged at 3000 rpm for 10 minutes, and the resulting serum samples were stored at -80°C for the measurement of liver enzymes. Following blood collection, the liver sample was isolated and washed, then immediately frozen in liquid nitrogen. Then, they were stored at -80°C to measure oxidative stress markers. A fragment of liver tissue intended for histopathological evaluation was fixed in a 10% formalin-buffered solution, dehydrated using ethanol, and embedded in paraffin. The samples were then sectioned to a thickness of 5 µm using a microtome and subsequently stained with hematoxylin-eosin (H&E). A light microscope did photography, and histological parameters were examined.

Liver specific enzymes

Serum levels of AST, ALT, and ALP were assessed using commercial kits from Pars Azmun (Pars Azmun, Iran), with evaluations conducted on a BioSystem A25 (BioSystem, Spain), which utilizes the colorimetric assay method. The results were reported in international units per liter (IU/L).

Measurement of oxidative stress

To prepare the sample, 100 mg of tissue was homogenized in 1 ml of phosphate buffer (pH 7.0) using a homogenizer. The resulting homogenate was then centrifuged at 4000 rpm for 20 minutes at 4°C, and the supernatant was collected for further analysis. Malondialdehyde (MDA) is the major product of lipid peroxidation. The MDA concentration measurement method is based on the reaction with thiobarbituric acid and using a special measurement kit (MDA ELISA Kit=ZB 0156-R9648, Germany), and the results were reported as µM. The activities of SOD (SOD ELISA Kit=ZB-0168-R9648, Germany) and CAT (CAT ELISA Kit=ZB-0869-R9648, Germany) enzymes were measured by ELISA kits of ZellBio company, and the results were expressed as IU/mL and nM/min/mL, respectively.

Statistical analysis

The Shapiro-Wilk test and Levene's Test were employed to assess the normality of the data and the homogeneity of variances, respectively. To compare mean variables across different groups, one-way ANOVA was utilized, followed by Tukey's post hoc test for further analysis of group differences. The findings were reported as mean ± standard error of the mean (SEM). Statistical analyses were conducted using SPSS software version 23 (IBM, United States). A significance level of P values <0.05 was considered.

RESULTS

Histopathological considerations

Figure1 shows transverse 6 micrometer sections of hepatic tissue stained with H&E and histopathological changes were evaluated at study groups. According to our finding, apoptotic cells, sinusoid expansion, fat droplets and deposition of collagen fibers (as marker of fibrosis) in EMR group were presented clearly and all of these pathological parameters show a very low presence in the ET group.

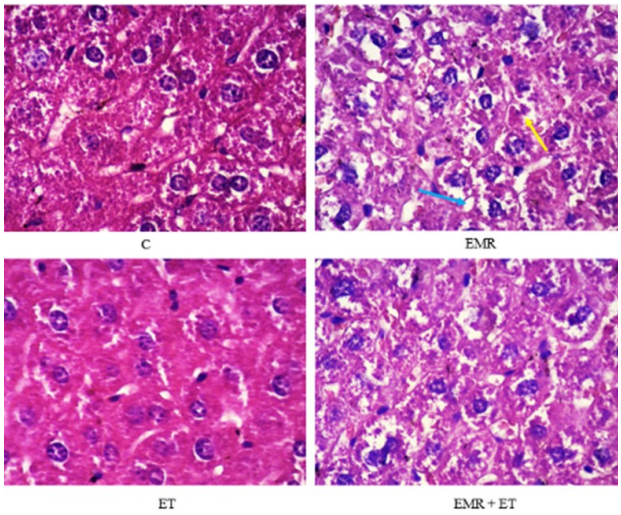


Figure 1. 6 micrometer transvers hepatic sections stained with H&E in different study groups (n= 4). C: control, EMR: electromagnetic radiation, ET: endurance training, EMR + ET: electromagnetic radiation + endurance training. (H&E×40). Yellow arrow: Apoptotic cell, blue arrow: Fat Droplet.

Oxidative stress

The average activity of SOD, CAT, and MDA concentration in liver tissue is presented in Figure 2. There was a significant difference in the mean values of oxidative stress markers between groups (P<0.05). In the EMR group, CAT activity decreased significantly, and MDA concentration increased significantly compared to C and EMR+ ET groups. (P<0.05), while no significant difference was found in SOD activity between these three groups. Endurance exercise in the ET group compared to the C group

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significantly increased CAT activity and significantly decreased MDA concentration (P<0.05). However, the changes in SOD activity were non-significant in comparing the two groups. The activity of SOD, CAT, and MDA concentration in the EMR+ ET group was not statistically significant compared to the C group. In the ET group compared to the EMR group, SOD and CAT activity increased significantly, and MDA concentration decreased significantly (P<0.05). The amount of CAT activity and MDA concentration in the ET group increased and decreased significantly compared to the EMR+ET group, respectively (P<0.05); however, the average SOD changes were not statistically significant when comparing the two groups together.

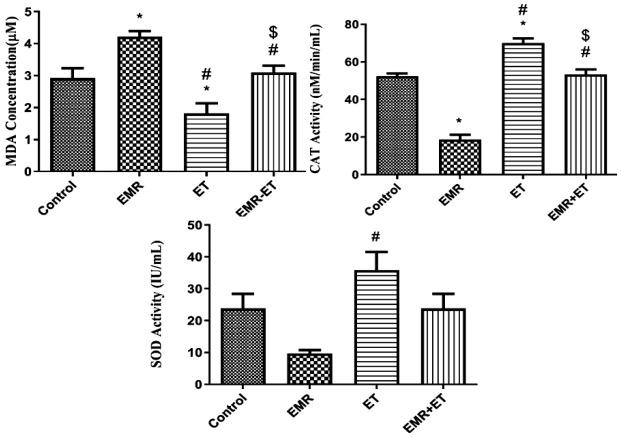


Figure 2. Changes in the activity of antioxidant enzymes and lipid peroxidation in liver tissue in different study groups after four weeks of exposure to electromagnetic radiation and endurance training. C: control, EMR: electromagnetic radiation, ET: endurance training, EMR + ET: electromagnetic radiation + endurance training. Values are presented as mean ± SEM (n=5). * Significant difference compared to C group (P<0.05) # Significant difference compared to EMR group (P<0.05). $ Significant difference compared to the ET group (P<0.05).

Serum liver enzymes levels

The average serum levels of AST, ALT, and ALP of the groups are shown in Figure 3. There was a significant difference in the mean serum levels of AST, ALT, and ALP between the groups. (P<0.05). The serum levels of AST, ALT, and ALP increased significantly in the EMR group compared to the C group (P<0.05). Endurance exercise in ET and EMR+ET groups led to a significant decrease in liver enzymes compared to the EMR group (P<0.05). The serum levels of all three liver enzymes in the ET and EMR+ET groups were non-significant compared to the C group. It should be noted that serum ALT levels in the ET group decreased significantly compared to the EMR+ET group (P<0.05). The difference in mean serum levels of AST and ALP between ET and EMR+ET groups was not statistically significant.

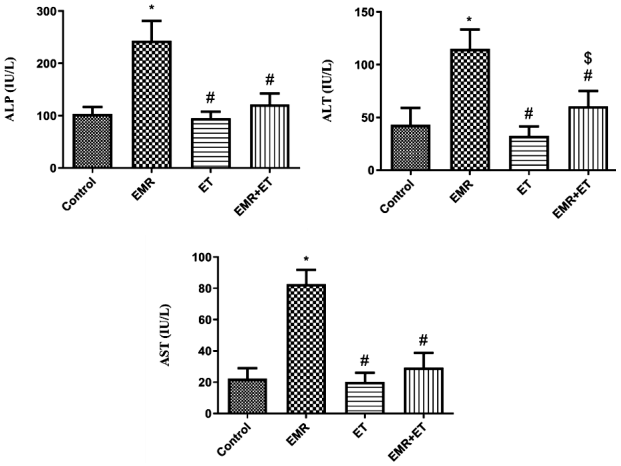


Figure 3. Changes in the serum levels of liver enzymes in different study groups after four weeks of exposure to electromagnetic radiation and endurance training. C: control, EMR: electromagnetic radiation, ET: endurance training, EMR + ET: electromagnetic radiation + endurance training. Values are presented as mean ± SEM (n=5). * Significant difference compared to C group (P<0.05) # Significant difference compared to EMR group (P<0.05). $ Significant difference compared to the ET group (P<0.05).

DISCUSSION

In this research, we examined the impact of ET on oxidative stress, Liver enzyme levels in serum, and histopathology of liver tissue in male rats exposed to Wi-Fi$_{2.45GHZ}$. In the liver tissue of rats that were exposed to EMR emitted from a Wi-Fi$_{2.45GHZ}$ device for 4 weeks, the increase of vacuolated hepatic cells with unclear nucleus and nuclear membrane, apoptotic cells, sinusoids expansion, fat droplets in hepatocytes and deposition of collagen fibers were observed. However, ET improved the histopathological alterations in the liver of rats exposed to EMR. The results of previous studies are consistent with these data [14, 18, 32]. The findings of this research indicated that exposure to EMR induces oxidative stress in the liver by diminishing antioxidant enzyme levels and elevating MDA concentration, while during this period, aerobic exercise was able to improve the oxidative stress caused by this EMR. Antioxidant enzymes such as SOD and CAT are responsible for protecting cells against the increase of superoxide radicals. SOD plays a role in catalyzing the conversion of superoxide to hydrogen peroxide. After that, hydrogen peroxide is cleaned through the activity of enzymes such as CAT [33]. EMFs, which are the cause of excessive production of ROS, impair antioxidant systems, and lead to oxidative stress. Oxidative stress significantly influences DNA damage, general and specific gene expression of apoptosis [10, 34]. As shown in this study, oxidative stress in liver tissue increased due to exposure of rats to EMR, which is agreement

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with previous reports [5, 7, 14, 35]. Fahmy et al. (2020) demonstrated a reduction in antioxidant enzyme activity and an elevation in MDA levels inside the tissue in the group exposed to EMR (24h/day, during 40 days) [5]. The findings in another study showed a decrease in SOD, CAT and GPx activity and an elevation in MDA concentrations in liver tissue owing to exposure to EMR released from mobile phones operating at a frequency of 1800 MHz for 2 hours per day over 8 weeks [7]. Also, the results of this research showed the reduction of oxidative stress on the effect of ET. There is a close relationship between the production of ROS and exercise intensity. Usually, high-intensity aerobic exercise is associated with high amounts of anaerobic metabolism and hypoxia. High-intensity exercise leads to a disparity between free radical generation and the antioxidant defense mechanism, and increases lipid and protein oxidation and induces apoptosis. Whereas moderate intensity aerobic exercise leads to increased antioxidant defense and reduces oxidative stress. Continuous aerobic exercise reduces the level of oxidative damage indicators of lipids and proteins, and this positive effect is probably due to the increase in the activity of antioxidant enzymes, which can increase the body's antioxidant defense capacity [36]. Numerous research has demonstrated the advantageous impact of exercise on oxidative stress markers. [17, 18, 37]. For example, Ahmadian et al. (2018) observed a decrease in hepatic oxidative stress after 3 weeks (5 sessions/weeks) of running rats on a treadmill [17]. Amiri *et al*. (2023) also showed that swimming exercise (5 sessions/weeks, 30 min/day, during 4 weeks) improved oxidative stress caused by EMR release [18]. The present study showed that the serum levels of liver enzymes (AST, ALT, and ALP) changed significantly and an increase in these enzymes was observed in the group exposed to EMR compared to other groups. After reviewing the findings in some studies, it can be said that the production of free radicals increases due to the emission of EMR, so the increase in oxidative stress may result in heightened serum levels of liver enzymes by damaging the hepatocytes and destroying the cell membrane [5, 18, 35]. EMR penetrates the body and affects the tissue and changes the cell membrane potential and dipole ion distribution. It is possible that these changes affect the biochemical processes of the cell [35]. Alterations in liver enzyme levels indicate the damage caused by EMR emitted from the Wi-Fi$_{2.45GHZ}$ device in liver cells. Liver cell damage leads to elevated serum levels of liver enzymes [15]. The findings of this study regarding the rise of liver enzymes following exposure to EMR are consistent with previous findings [32, 38, 39]. Likewise, the results of Adebayo et al. (2019) research showed that liver enzyme levels were markedly elevated in rats exposed to EMF$_S$ for 5 weeks [32]. Also, Poladi *et al*. (2018) reported a significant elevation in the serum levels of liver aminotransferases resulting from the exposure of rats to Wi-Fi$_{2.45GHZ}$ (8h/day, During 1 to 4 weeks) [15]. In their study, the observed increase in the levels of enzymes was time-dependent and the serum levels of liver aminotransferases reached the maximum level based on the time of exposure to Wi-Fi$_{2.45GHZ}$ (from the first to the fourth week). In addition, the findings of the current study indicated a considerable reduction in serum levels of the liver's enzymes (AST, ALT, and ALP) decreased significantly through ET. It is possible that aerobic exercise can lead to the improvement of liver condition through positive effect on metabolic changes. Also, continuous aerobic exercise enhances antioxidant capacity, hence diminishing hepatic cellular damage. [40]. The present investigation demonstrates that the decrease in liver enzymes post-exercise aligns with findings from prior research [24, 25, 41]. For example, Fathi *et al*. (2020) examined the impact of exercise on ethanol-induced liver damage in rats. Their results showed a significant decrease in the liver's enzyme levels in aerobic exercise groups (5 sessions/weeks, during 8 weeks) [25]. Also, in the study of Kohestani *et al*. (2020), a decrease in liver enzymes due to continuous aerobic exercise (5 sessions/weeks, during 8 weeks) was observed in rats with Non-alcoholic fatty liver disease [42]. In interpreting the results of this study, attention to some limitations is essential, which will be referred to below. One major limitation of this research was that it only looked at how exercise and electromagnetic radiation affected liver damage and oxidative stress indicators; other organs were not evaluated. Additionally, the effects were only evaluated over the short period of four weeks, with long-term effects not being considered. Furthermore, only oxidative stress markers were used for analysis, and other potential mechanisms and pathways were not explored. Therefore, there is a need for more comprehensive and broad studies to be conducted that examine additional markers, organs, and mechanisms over longer timeframes.

CONCLUSION

It can be concluded that continuous and regular ET safeguards the liver of rats against EMR-induced damage by enhancing antioxidant enzyme activity and decreasing free radicals. Furthermore, ET seems to improve levels in the serum of liver injury indicators and histological changes by reducing oxidative stress. Therefore, our findings showed that ET is a cost-effective strategy without side effects that can improve liver damage caused by EMR.

Conflict of Interest: The authors declare that there are no conflicts of interest.

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***Authors' Contributions*:** N.A. designed the study; H.A., R. Z. F. performed the experiments, analyzed the data, and drafted the article; K.R. contributed for histological analysis. F.S., K.R. interpreted the data. H.A. wrote the paper. N.A. formulated the idea, revised the work, and guaranteed the accuracy of the data analysis. All authors participated in preparing the final draft of the manuscript, revised the manuscript, and critically assessed the academic contents. All authors have read and approved the manuscript's content and confirmed the accuracy or integrity of any part of the work.

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