

Impact of L-carnitine and Selenium Treatment on Testicular Apoptosis in Rats Exposed to 2.45 GHz Microwave Energy

M Saygin¹, S Caliskan², MF Ozguner¹, N Gumral¹, S Comlekci³, N Karahan⁴

ABSTRACT

Objective: It has been suggested that electromagnetic radiation (EMR) by wireless devices (2.45 GHz) induces testicular apoptosis. We investigated if supplemental selenium (Se) and L-carnitine may reduce this adverse effect.

Material: Twelve-week old male Wistar albino rats were used in this study. Twenty-four rats were equally divided into four groups which were named as: sham group, EMR-only, EMR+L-carnitine (1.5 mg L-carnitine/kg/day) and EMR+Se (1.5 mg Se/kg/every other day).

Results: The level of Bcl-2, Bax, caspase-3 and -8 were compared and a significant difference was found between the sham and EMR-only groups ($p < 0.05$), and Bcl-2, Bax, caspase-3 and -8 expressions increased in the EMR-only group. The level of Bcl-2, Bax, tumour necrosis factor-alpha (TNF- α), caspase-3 and -8 were compared and a significant difference was found between the sham and EMR+L-carnitine groups ($p < 0.05$) and Bcl-2, Bax, TNF- α , caspase-3 and -8 expressions increased in the EMR+L-carnitine group. The level of Bcl-2, Bax, TNF- α , caspase-3 and -8 were compared and a significant difference was found between the sham and EMR+Se groups ($p < 0.05$) and Bcl-2, Bax, TNF- α , caspase-3 and -8 expressions increased in the EMR+Se group. When the expression of caspase-8 was compared, a significant difference was found between the EMR-only and EMR+Se groups ($p < 0.05$). Caspase-8 expression decreased in EMR+Se group compared with EMR-only group.

Conclusion: Electromagnetic radiation exposure resulted in testicular apoptosis in rats, mainly by the intrinsic pathways by down-regulated expression of caspase-8. Reduction in the activation of the intrinsic pathway of apoptosis was found higher with selenium administration compared with L-carnitine administration.

Keywords: Apoptosis, L-carnitine, selenium, spermatogenesis, testes, Wi-Fi

Impacto de la L-carnitina y Tratamiento con Selenio en la Apoptosis de Ratas Expuestas a Energía de Microondas de 2.45 GHz

M Saygin¹, S Caliskan², MF Ozguner¹, N Gumral¹, S Comlekci³, N Karahan⁴

RESUMEN

Objetivo: Se ha sugerido que la radiación electromagnética (EMR) de dispositivos inalámbricos (2.45 GHz) induce apoptosis testicular. Investigamos si el selenio suplementario (Se) y la L-carnitina pueden reducir este efecto adverso.

Material: Ratas albinas Wistar machos de doce semanas de edad fueron utilizadas en este estudio. Veinticuatro ratas fueron divididas igualmente en cuatro grupos nombrados como sigue: grupo de simula-

From: ¹Department of Physiology, Faculty of Medicine, Suleyman Demirel University, Isparta, Turkey, ²Department of Physiology, Faculty of Medicine, Pamukkale University, Denizli, Turkey, ³Department of Electronics and Communication, Faculty of Engineering, Suleyman Demirel University and ⁴Department of Pathology, Faculty of Medicine, Suleyman Demirel Univer-

sity, Isparta, Turkey.

Correspondence: Dr M Saygin, Department of Physiology, Faculty of Medicine, Suleyman Demirel University, 32260 Isparta, Turkey. Fax: +90 246 237 11 65; e-mail: fizyolog@gmail.com

ción, sólo EMR, EMR + L-carnitina (1.5 mg de L-carnitina/kg/día) y EMR + Se (1.5 mg Se/kg/día).

Resultados: Se compararon los niveles de Bcl-2, Bax, caspasa-3 y -8, y se halló una diferencia significativa entre los grupos de simulación y EMR sólo ($p < 0.05$), y las expresiones de Bcl-2, Bax, caspasa-3 y -8 aumentaron en el grupo de sólo EMR. Se compararon los niveles de Bcl-2, Bax, el factor de necrosis tumoral-alfa (TNF- α), y la caspasa-3 y -8, y se halló una diferencia significativa entre los grupos de simulación y EMR + L-carnitina ($p < 0.05$); las expresiones de Bcl-2, Bax, TNF- α , caspasa-3 y -8 aumentaron en el grupo de EMR + L-carnitina. Se compararon los niveles de Bcl-2, Bax, TNF- α , caspasa-3 y -8, y se halló una diferencia significativa entre los grupos de simulación y EMR + Se ($p < 0.05$), y las expresiones de Bcl-2, Bax, TNF- α , caspasa-3 y -8 aumentaron en el grupo EMR + Se. Cuando se comparó la expresión de caspasa-8, se halló una diferencia significativa entre los grupos de EMR sólo y EMR + Se ($p < 0.05$). La expresión de caspasa-8 disminuyó en el grupo EMR + Se en comparación con el grupo de EMR sólo.

Conclusión: La exposición a la radiación electromagnética dio lugar a la apoptosis testicular en ratas, principalmente por las vías intrínsecas mediante la expresión subregulada de caspasa-8. Se halló que la reducción de la activación de la vía intrínseca de apoptosis fue mayor con la administración de selenio en comparación con la administración de L-carnitina.

Palabras claves: Apoptosis, L-carnitina, selenio, espermatogénesis, testículos, Wi-Fi

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INTRODUCTION

Changes in biological parameters after exposure to electromagnetic radiation (EMR) in living organisms have been demonstrated by several studies. The electromagnetic fields caused by several sources, such as wireless network, microwave ovens and mobile phones, have been shown to influence the biological systems (1–3). Many sources of non-ionizing radiation may affect people silently and continuously on a daily basis. However, some people may be more vulnerable to the adverse effects of EMR due to individual susceptibility or work exposure. Testes are more sensitive to a variety of stresses, such as hyperthermia, inflammation, radiation and exposure, which may result in germ cell apoptosis (4, 5). Electromagnetic exposure damages the seminiferous tubules and decreases the number of Leydig cell and testosterone concentration in rats (6, 7). It has been well established that testosterone is essential for spermatogenesis and the formation of spermatozoa (8, 9).

Apoptosis can be described as a cell death process that is characterized by blebbing, the loss of the cell membrane, asymmetry, cell detachment, cell shrinkage, nuclear fragmentation, chromatin condensation and chromosomal DNA fragmentations (3, 10). During spermatogenesis, apoptosis plays a significant role in regulating the appropriate number of proliferating germ cells associated with the surrounding Sertoli cells. The regulation of apoptosis is established by various proteins that induce or inhibit apoptosis, such as Bax, Bcl, caspase-3 and -8 (11). Caspases are present as inactive precursors and are activated by caspase initiators through auto-active proteolysis (12). The caspase-8 and -9 initiators and the caspase-3 effector are accepted as the main executors of apoptosis (13, 14). The caspase-3 effector shows its functions on two pathways: the mitochondrial pathway (through the caspase-9

initiator) and the death receptor pathway [through the caspase-8 initiator] (15).

Selenium (Se), in the form of glutathione peroxidase (GSH-Px), plays an important role in the protection of tissue from oxidative damage. Apart from being an integral part of GSH-Px, Se can antagonize the toxic effects of some chemical substances (16–18).

L-carnitine and acetylcarnitine are highly concentrated in the epididymis and play a crucial role in sperm metabolism and nutrition (19, 20). They are related to sperm motility. The initiation of sperm motility occurs in parallel with the increase in concentration of free L-carnitine in the epididymal lumen (21).

The aim of this study was to investigate the effects of wireless device use on testes tissue function. We investigated the potential damages of electromagnetic radiation to rat testes tissue and related reproductive system. Especially, comparative analysis on preventive effects of Se and L-carnitine on 2.45 GHz EMR-induced testicular apoptosis was evaluated.

SUBJECTS AND METHODS

Animal model

The experimental protocol of the study was approved by the ethical committee of the Medical Faculty of Suleyman Demirel University. 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. There was no significant difference between the average weight of the groups of twelve-week old male Wistar albino rats ($n = 24$), weighing 150–170 g. Rats were housed individually in stainless-steel cages in pathogen-free conditions in our laboratory at $+24^{\circ}\text{C} \pm 3$ with light between 8 am and 8 pm and free access to water and food. They received a

commercial diet (Korkuteli Yem, Korkuteli-Turkey). Environmental average light intensity was 4000 lux and humidity was $40 \pm 10\%$.

After one week adaptation process, the animals were randomly divided into four equal groups consisting of six rats each, as follows: Sham (n = 6): control group; EMR-only (n = 6): 2.45 GHz exposed rats; EMR+L-carnitine (n = 6): 2.45 GHz exposed rats+L-carnitine group [1.5 mg L-carnitine/kg/day] (22); EMR+Se (n = 6): 2.45 GHz exposed rats+Se group [1.5 mg Se/kg every other day] (22). Inorganic sodium selenite form of Se was used. Selenium was dissolved in isotonic saline solution, prepared as 0.15% solution prior to experiment. 2.45 GHz EMR was applied to the EMR-only, EMR+L-carnitine and EMR+Se groups for 28 days (60 m/day, between 9 am and 12 pm). The one-hour exposure to irradiation in EMR-only, EMR+L-carnitine and EMR+Se groups took place between 9 a.m. and noon each day. The first dose of Se and L-carnitine administration was given 24 hours prior to the exposure. Sham-control rats received intraperitoneal injections of isotonic saline solution at an equal volume to that of the L-carnitine and Se used in the EMR+L-carnitine and EMR+Se groups. Selenium, L-carnitine and isotonic saline solution were given by insulin syringe prior to one-hour EMR exposure.

Chemicals

Chemicals for immunopathological study were obtained from Sigma-Aldrich Chemical Inc (St Louis, Missouri, USA) and all organic solvents from Merck Chemical Inc (Germany). Phosphate buffers are stable at +4 °C for one month. All reagents except the phosphate buffers were prepared on the day of the experiment and used fresh. The reagents were equilibrated at room temperature for half an hour before starting and containers were refilled with reagents.

Antibodies

Streptavidin-biotin immunoenzymatic antigen detection system (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP, Labvision, Fremont, California, USA) was used as a second antibody. (This kit has both goat anti-mouse and goat anti-rabbit antigen specificity.) Streptavidin peroxidase (TS-125-HR Labvision Fremont, CA, USA) was used.

Exposure system and design

For experimental exposure, a radiofrequency (RF) generator, SET ELECO (Set Electronic Co, Istanbul, Turkey), giving a 2.45 GHz RF emission, pulsed with 217 Hz, was used with monopole antenna system (7). This device is able to create 0.1 V/m to 45.5 V/m electric radiation densities. The whole 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). Exposure design and methodology were adapted from a similar study (7, 22, 23). All six rats in the groups were exposed at the same time in the exposure system.

This device is organized with a special cylindrical PVC strainer, which provides appropriate exposure conditions, and 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 tube was ventilated from head to tail to decrease the stress of the rats while they are in the tube (Fig. 1). Repetition time, frequency and amplitude of spectrum of RF energy was observed and verified by satellite level meter (PROMAX, MC-877C, Barcelona, Spain). All the reflection and exposure measurements were carried out by utilizing Portable RF Survey System (HOLADAY, HI-4417, Minnesota, USA) with its standard probe. The electromagnetic dosimetry was calculated by using measured electric field intensity (V/m) and digital anatomical models based on the FDTD numerical code. Consequently, specific absorption rate (SAR) value was predicted for the same condition, orientation, and antenna power by using this method as 3.21 W/kg for whole body. The rats of the sham exposure group were placed in the restrainer individually when the RF source switched off during experiments. All exposure system was kept in the Faraday cage. Exposure procedure was always carried out in this cage. Its shielding effectiveness was 100 dB. Also, the exposure of each group did not affect the other groups. To verify this result, data were taken continuously by RF measurement apparatus in the experiment room explained above.

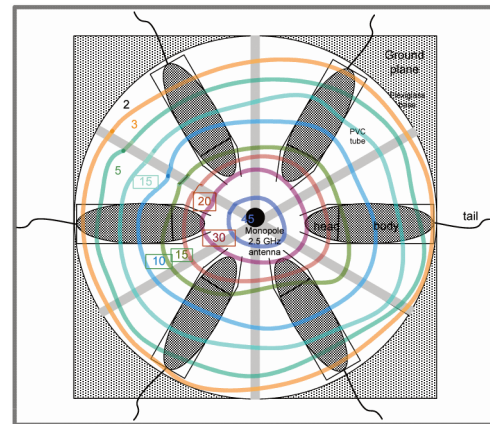


Fig. 1: Experimental set-up for irradiation of the rats (6).

Anaesthesia and tissue sampling

On day 28, rats were anaesthetised with a cocktail of ketamine hydrochloride (HCI) [90 mg/kg] and xylazine HCI (10 mg/kg) which were administered intraperitoneally before being sacrificed as described before (24). Then the testes were removed and put into 10% formaldehyde solution (25).

Immunohistochemical examination

Tissue samples from each group were fixed in neutral formalin for 72 hours and processed for paraffin embedding. Sections of 4- to 5- μ m thickness were taken onto polylysine microscope slides. Slides were stored in a microwave oven 1200 watt in 0.01 M citrate buffer (AP-9009-999 Thermo-Lab-

vision, Fremont, CA, USA; pH 6.0) for 20 minutes. Endogenous peroxidase activity (TA-060-HP Thermo-Labvision Fremont, CA, USA) was blocked by 20 minutes of incubation with 0.3% hydrogen peroxidase (TA-060-HP Thermo-Labvision, Fremont, CA, USA).

First, antibodies caspase-3 and -8 (rabbit polyclonal antibody, RB-1197-P1 Neo Marker, Labvision, Fremont, CA, USA) were prepared with 1/100 dilution and then they were incubated for 30 minutes (Neo Marker, Labvision RB-1200-P1 Labvision, Fremont, CA, USA). Bax 1/50 dilution was prepared and then was incubated for 60 minutes (sc-7480 mouse monoclonal antibody, Santa Cruz Biotechnology Inc, USA). Bcl-2 1/50 dilution was prepared and was incubated for 60 minutes (sc-783 rabbit polyclonal antibody, Santa Cruz Biotechnology Inc, USA). Tumour necrosis factor-alpha (TNF- α) 1/50 dilution was prepared and was incubated for 60 minutes (sc-1349, goat polyclonal antibody, Santa Cruz, USA). Sections were incubated with the streptavidin-biotin peroxidase labelled goat anti-rabbit immunoglobulin or goat anti-mouse immunoglobulin (Ultra Vision Large Volume Detection System Anti-polyvalent, HRP, Labvision, Fremont, CA, USA) for 20 minutes. This kit has both goat anti-mouse and goat anti-rabbit antigen specificity. After the incubation, the reaction product was detected with (freshly prepared) diaminobenzidine solution TA125-HD. Large volume DAB substrate system (Labvision Thermo, Fremont, CA, USA) was incubated for 5–10 minutes. Finally, sections were counterstained with Mayer's haematoxylin, mounted in the mounting medium, and examined using the Olympus BH2 photo-light microscope (Olympus, Lake Success, USA). Immunohistochemical results were evaluated by one of the pathologists. Two independent observers, who were blinded to the treatment, calculated the immunolabelling score twice. The rats included in this study were evaluated randomly in a blinded fashion by the researcher without knowing which rat was included into which group.

Immunoreactivity was graded semiquantitatively by considering the percentage and intensity of the staining on the whole section. A histologic score was obtained from each sample for five sections, ranging from – (no immunoreaction) to +++ (maximum immunoreactivity) [Table]. HSCORE (index) was calculated with the following equation: $HSCORE = \sum (i+1) \cdot P_i$, where i = intensity of labelling with a value of +, ++, or +++ (weak, moderate, or strong, respectively), and P_i = percentage of labelled epithelial and stromal cells for each intensity, ranging from 0% to 100%. Statistical differences were

Table: The intensity of immunohistochemical staining

Immunoreactivity	Meaning
0	No
+	weak
++	moderate
+++	strong

calculated with the Mann-Whitney U test for HSCORE (index).

Statistical analysis

The data were analysed by using a commercially available statistics software package (SPSS® for Windows v 15.0, Chicago, Illinois, USA). Kruskal-Wallis and Mann-Whitney U tests were used as statistical methods. P -values of less than 0.05 were regarded as significant. All the remaining results were expressed as mean \pm SD (standard deviation).

RESULTS

Immunohistochemical findings

Immunohistochemical staining of Bcl-2, Bax, TNF- α , caspase-3 and -8 markers were made for each of the four groups. The control group's testes tissue immunolabelling was observed as normal relating to the control group (Fig. 2A).

The levels of Bcl-2, Bax, caspase-3 and -8 were compared and a significant difference was found between the sham and EMR-only groups ($p < 0.05$), and Bcl-2, Bax, caspase-3 and -8 expressions increased in the EMR-only group (Fig. 2B–D).

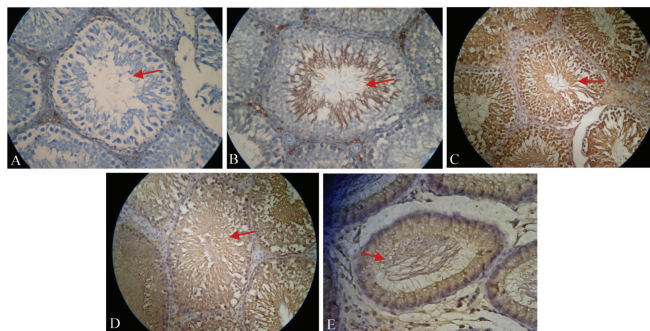


Fig. 2: Immunohistochemical findings.

A: Immunolabelling relating to sham group [$\times 40$]. Normal appearance of seminiferous tubules and Leydig cells of the testes tissue after immunolabelling. **B:** Immunolabelling of Bax activity (+++) relating to 2.45 GHz electromagnetic radiation (EMR) groups [$\times 40$]. Indicator of apoptosis Bax activity increased after 2.45 GHz EMR exposure. **C:** Immunolabelling of caspase-3 activity relating to 2.45 GHz EMR groups [$\times 40$]. Indicator of apoptosis caspase-3 activity increased after 2.45 GHz EMR exposure. **D:** Immunolabelling of caspase-8 activity relating to 2.45 GHz EMR groups [$\times 40$]. Indicator of apoptosis caspase-8 activity increased after 2.45 GHz EMR exposure. **E:** Immunolabelling of caspase-8 activity relating to 2.45 GHz EMR+Se administered group [$\times 40$]. Indicator of apoptosis caspase-8 activity decreased after 2.45 GHz EMR+Se administration group.

The levels of Bcl-2, Bax, TNF- α , caspase-3 and -8 were compared and a significant difference was found between the sham and EMR+L-carnitine groups ($p < 0.05$) and Bcl-2, Bax, TNF- α , caspase-3 and -8 expressions increased in the EMR+L-carnitine group.

The levels of Bcl-2, Bax, TNF- α , caspase-3 and -8 were compared and a significant difference was found between the sham and EMR+Se groups ($p < 0.05$); Bcl-2, Bax, TNF- α , caspase-3 and -8 expressions increased in the EMR+Se group.

When the levels of caspase-8 were compared, a significant difference was found between the groups of EMR-only and EMR+Se ($p < 0.05$). Caspase-8 expression reduced in EMR+Se group than in the EMR-only group (Fig. 2E).

Bcl-2, Bax, TNF- α , caspase-3 and -8 genes were up-regulated in the EMR group. In the EMR+L-carnitine and EMR+Se groups, these genes were down-regulated, and caspase-8 enzymes were significantly down-regulated in the EMR+Se group. Caspase-8 has protective effects that play a role in the intrinsic pathway of apoptosis through the initiation of apoptosis.

DISCUSSION

Apoptosis, which needs adenosine triphosphate (ATP), is necessary for normal spermatogenesis in mammals and sustains cellular haemostasis. This physiological process conserves the equilibrium between Sertoli cells and germ cells (26). The members of the caspase family play a key role for the regulation of the apoptosis in the seminiferous tubules (27). Previous studies indicated that apoptosis of the testicular epithelium germ cells died *via* apoptosis (28, 29).

In testicular tissue, the main cell process for damage of germ cells was apoptotic cell death (30, 31). In hipospermatogenesis and also in genetically arrested people, some authors determined that apoptotic activity was also increased (32). This is demonstrated by increased percentage of apoptosis in testicular tissue of infertile males and the authors emphasized the relationship between infertility and increased apoptosis (33). Due to increase in apoptosis, the percentage of programmed cell death increases in germ cells and this causes the formation of hipospermatogenesis (34). In those studies which try to find out what kind of apoptosis may affect the germ cells, it was shown that apoptosis could occur in primary spermatogonia, early and late spermatosis and spermatids. Beumer *et al* revealed an intermediate level of apoptosis in spermatogonia formation and an advanced level of apoptosis in early and late spermatocyte and spermatid formation (35).

There are two main pathways which are known to cause apoptosis in the cell. One is the intrinsic (mitochondrial) and the other is the extrinsic pathway. Intrinsic pathway is triggered by stress-caused reasons in the cell as irradiation, toxins and oxidative stress. These stress sources can affect the members of the Bcl-2 family which stabilizes or destabilizes the mitochondrial membrane by pro-apoptotic or anti-apoptotic (Bax and Bcl-XL) factors. The extrinsic pathway is triggered by extracellular ligands (FasL), by binding of the TNF- α on their special receptors on the membrane.

Due to activation of these receptors, intracellular caspases activate and as a result of these steps, DNA degradation is generated. Germ cell apoptosis normally occurs as an important event in spermatogenesis. Apoptosis of germ cells may be induced with both extrinsic and intrinsic pathways according to aetiological factors (36).

Apoptotic Bax gene, TNF- α enzyme, caspase-3 and -8 enzyme expression and discrepancy between the groups

showed that apoptosis is induced by means of electromagnetic radiation (37).

Between EMR and the sham group, the discrepancy between Bcl-2 genes, caspase-3 and 8 enzyme activation supports the previous findings. Furthermore, Bcl-2 gene, TNF- α , caspase-3 and -8 enzyme differences in sham, L-carnitine and Se treatment groups demonstrated the efficiency of treatment groups.

When Bcl-2 anti-apoptotic gene expression was evaluated immunohistochemically, significant differences between the sham group and electromagnetic radiation group were determined. These findings indicate that this gene may be activated in testicular tissue. When sham and electromagnetic radiation groups were compared, there was a significant difference for pro-apoptotic Bax gene expression. The extrinsic pathway of apoptosis was investigated *via* evaluating TNF- α and caspase-3 and -8. There was no significant difference for TNF- α between sham and EMR-only groups. When sham and EMR-only groups were compared, there was a significant difference for caspase-3 and -8. Kumar *et al* investigated the therapeutic effect of a pulsed electromagnetic radiation on the reproductive patterns of male Wistar rats exposed to a 2.45-GHz microwave field and the results showed significant increases in caspase in the exposed groups (38). All results indicated that apoptosis in testicular tissue in the EMR-only group might be due to the intrinsic pathway since there was no significant difference for TNF- α . Depolarization of mitochondria causes cytochrome-C release into cytoplasm and cytochrome-C binds to Apaf-1 and these steps activate cytoplasmic caspases which is called an intrinsic pathway. Activated caspases trigger DNAase and degradation of DNA, and DNA divides into 185 bp parts which is one of the best evidence of apoptosis. Activation of caspase-3 and -8 formation of Bax gene shows that EMR may trigger the apoptotic pathway. Apoptosis of these cells may be one of the amenable reasons for infertility.

Meena *et al* investigated the therapeutic approaches of melatonin in microwave radiation-induced oxidative stress-mediated toxicity on male fertility pattern of Wistar rats. The results showed that exposure to 2.45 GHz microwave-induced oxidative stress mediated DNA damage, testicular morphology and disrupted seminiferous tubules and inflammation in testicular cells, and the rate of apoptotic sperm cell in the exposed group was higher than compared to the sham group (39). Our findings were in agreement with the studies mentioned above.

Sakr *et al* investigated the effect of Se on carbimazole-induced testicular damage and oxidative stress in albino rats. They concluded that treating animals with carbimazole and Se showed an improvement in the histological structure as well as histochemical components of the testes with an increase in the number of spermatogenic cells. There was an increase in testosterone, luteinizing hormone, thyroid hormones (T3, T4) and thyroid stimulating hormone levels. Also, administration of Se led to decrease in malondialdehyde and increase

in catalase and superoxide dismutase (SOD) levels in rats. They suggested that the curative effect of Se against testicular damage induced by carbimazole may be due to its antioxidant properties (37).

In another study, Ranawat and Bansal designed an experiment to evaluate the apoptotic efficacy of Se under glutathione deprived conditions. They found out that reduction in endogenous glutathione (GSH) along with selenite treatment was associated with increased apoptosis, increased expression of p38 and JNK MAPK, decreased Bcl-2 expression and increase in caspase-3 expression. Their findings indicated that GSH participated in apoptosis in testicular cells and that depletion of this molecule might be critical in predisposing these cells to apoptotic cell death (40). Several studies were designed to investigate the critical balance between cell proliferation and apoptosis for normal spermatogenesis, and the requirement of Se for the maintenance of male fertility. Their findings implied that sodium selenite caused apoptosis and the toxicity of selenite was mediated by the increase in reactive oxygen species (ROS). Furthermore, ROS generation was associated with increased expression of p38, caspase-3 and 8, and decreased Bcl-2 expression. The results of the studies showed that p38 participated in testicular apoptosis and that was required for maintenance of the critical balance between cell death and proliferation (41).

Li *et al* investigated the toxicity of cadmium (Cd) on male reproduction in birds and the protective effects of Se against subchronic exposure to dietary Cd. According to their findings, exposure to Cd significantly lowered SOD and GPx activity, Se content in the testicular tissue, and serum testosterone levels. It increased the amount of lipid peroxidation, the numbers of apoptotic cells and Cd concentration and caused obvious histopathological changes in the testes. They recommended that concurrent treatment with Se reduced the Cd-induced histopathological changes in the testes, oxidative stress, endocrine disorder and apoptosis (42).

Our findings were in agreement with the studies mentioned above. We found significant differences in caspase-8 enzyme activity between the Se administered group and the electromagnetic radiation group. In this case, the intrinsic pathway was activated in apoptosis by activation of caspase-8, but differences with Se and the electromagnetic radiation group may help to prevent the activation of this pathway.

In conclusion, it can be said that 2.45 GHz electromagnetic radiation may have stress originated effects on rat neuroendocrine system and testicular tissue. Our previous study showed that magnetic radiation was induced by 2.45 GHz electromagnetic radiation causing testicular apoptosis (7). Occurrence of Bax gene and caspase-3 and -8 enzymes demonstrated that electromagnetic radiation might trigger apoptosis. These findings revealed that electromagnetic radiation caused degeneration of testicular cells and spermatogenesis, apoptosis, especially in spermatozoit formation, and this might be the rea-

son for infertility. Selenium may have protective effects on the testicular apoptosis. Our results supported the findings of the previous literature that Se is the most powerful and effective endogenous free radical scavenger detected to date, due to its structure of the GPx, and it increases its scavenging capabilities by increasing the antioxidant activity of this enzyme activity. Regarding L-carnitine protective effect on testicular tissue, it firstly reduces the formation of free oxygen radicals (43), and the second main mechanism is to reduce apoptosis. This affects membrane stabilization and prevents activities of caspases and Fas/FasL system inhibition (44, 45). L-carnitine affects the inhibition of the Fas/FasL system, prevents apoptosis of the extrinsic pathway, but in this experiment extrinsic pathway of apoptosis in testicular tissue was not activated. Thus, L-carnitine may have protective effects by itself to reduce the formation of free oxygen radicals in testicular tissue. Furthermore, the combined protective effects of Se and L-carnitine remain unknown and may be more efficient on testicular apoptosis impairment induced by 2.45 GHz electromagnetic radiation. However, further investigations are required to clarify the mechanism of action of the applied EMR exposure on rats' tissues, such as testes, brain and heart, as well as to establish the biological significance of the observed phenomena. Further research can be done on the effects of 2.45 GHz magnetic radiation on fertility, and the protective effects of Se and L-carnitine. Moreover, the effect of magnetic radiation on testicular function and the amount of the sperm that testes produce should be studied.

AUTHORS' NOTE

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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