Evaluation of the neuroprotective effects of electromagnetic fields and coenzyme Q 10 on hippocampal injury in mouse

Article *in* Journal of Cellular Physiology · April 2019

DOI: 10.1002/jcp.28512

CITATIONS
0

READS
14

10 authors, including:

Akram Alizadeh
Hamadan University of Medical Sciences
11 PUBLICATIONS **21** CITATIONS
SEE PROFILE

Gelareh Vahabzadeh
Iran University of Medical Sciences
9 PUBLICATIONS **10** CITATIONS
SEE PROFILE

Maryam Sarbishegi
Zahedan University of Medical Sciences
17 PUBLICATIONS **70** CITATIONS
SEE PROFILE

Fereshteh Azedi
Avicenna Research Institute
11 PUBLICATIONS **20** CITATIONS
SEE PROFILE

Received: 12 November 2018 | Revised: 23 December 2018 | Accepted: 10 January 2019

DOI: 10.1002/jcp.28512

ORIGINAL RESEARCH ARTICLE

WILEY Journal of Cellular Physiology

Evaluation of the neuroprotective effects of electromagnetic fields and coenzyme Q_{10} on hippocampal injury in mouse

Mansoureh Soleimani[1,2,3] | Fereshteh Golab[1] | Akram Alizadeh[4] | Sara Rigi[5] | Zeinab Nazarian Samani[4] | Gelareh Vahabzadeh[6] | Tahmineh Peirovi[7] | Maryam Sarbishegi[8] | Majid Katebi[9] | Fereshteh Azedi[10]

[1]Cellular and Molecular Research Center, Iran University of Medical Sciences, Tehran, Iran

[2]Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran

[3]Department of Anatomical Sciences, Iran University of Medical Sciences, Tehran, Iran

[4]Department of Tissue Engineering, School of Advanced Technologies, Shahrekord University of Medical Sciences, Shahrekord, Iran

[5]Department of Anatomy, Iranshahr University of Medical Sciences, Iranshahr, Iran

[6]Department of Pharmacology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran

[7]Department of Anatomical Sciences, Urumia University of Medical Sciences, Urumia, Iran

[8]Department of Anatomical Sciences, Zahedan University of Medical Sciences, Zahedan, Iran

[9]Department of Anatomical Sciences, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

[10]Department of Neuroscience, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Tehran, Iran

Correspondence
Majid Katebi, Department of Anatomical Sciences, Hormozgan University of Medical Sciences, Bandar Abbas 1419954161, Iran.
Email: Majidkatebi@gmail.com

Funding information
Iran University of Medical Sciences, Tehran, Iran and 9. Department of Anatomical Sciences, Hormozgan University of Medical Sciences, Bandar Abbas, Iran.

Abstract

Electromagnetic fields (EMFs) are reported to interfere with chemical reactions involving free radical production. Coenzyme Q_{10} (CoQ10) is a strong antioxidant with some neuroprotective activities. The purpose of this study was to examine and compare the neuroprotective effects of EMF and CoQ10 in a mouse model of hippocampal injury. Hippocampal injury was induced in mature female mice (25–30 g), using an intraperitoneal injection of trimethyltin hydroxide (TMT; 2.5 mg/kg). The experimental groups were exposed to EMF at a frequency of 50 Hz and intensity of 5.9 mT for 7 hr daily over 1 week or treated with CoQ10 (10 mg/kg) for 2 weeks following TMT injection. A Morris water maze apparatus was used to assess learning and spatial memory. Nissl staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) tests were also performed for the histopathological analysis of the hippocampus. Antiapoptotic genes were studied, using the Western blot technique. The water maze test showed memory improvement following treatment with CoQ10 and coadministration of CoQ10 + EMF. The Nissl staining and TUNEL tests indicated a decline in necrotic and apoptotic cell count following treatment with CoQ10 and coadministration of CoQ10 + EMF. The Western blot study indicated the upregulation of antiapoptotic genes in treatment with CoQ10, as well as coadministration. Also, treatment with EMF had no significant effects on reducing damage induced by TMT in the hippocampus. According to the results, EMF had no significant neuroprotective effects in comparison with CoQ10 on hippocampal injury in mice. Nevertheless, coadministration of EMF and CoQ10 could improve the neuroprotective effects of CoQ10.

KEYWORDS
CoQ10, electromagnetic fields (EMFs), neuroprotective effect, trimethyltin hydroxide (TMT)

1 | INTRODUCTION

Humans are exposed to a wide range of electromagnetic waves (Adey, 1993). Electromagnetic fields (EMFs) reportedly have some biological effects on different organisms. These effects can include changes in the expression of genes or changes in behavior (Lacy-Hulbert, Metcalfe, & Hesketh, 1998; Lai & Singh, 1995; Lyskov et al., 1996; McCann, Dietrich, & Rafferty, 1998; Simko, Kriehuber, Weiss, & Luben, 1998; Walleczek & Liburdy, 1990). In vitro studies have explored the potential effects of EMF on cell proliferation,

apoptosis, and differentiation (McCann et al., 1998; Simko et al., 1998). One interesting hypothesis about the biological effects of EMFs is that they interfere with chemical reactions involving free radical production (Guerriero & Ricevuti, 2016).

Free radicals can cause damage to cells, proteins, and DNA. In fact, excessive amounts of free radicals can lead to oxidative damage, which interferes with regular cell functioning and is known to cause various health problems (Navarro-Yepes et al., 2014). Therefore, free radicals are associated with many diseases in humans, including cancer, atherosclerosis, Alzheimer's disease, and Parkinson's disease. On the other hand, antioxidants play a major role in protecting tissues against oxidative damage.

Since the brain is very susceptible to oxidative damage due to its high fatty acid content and high demand for oxygen, antioxidants have been reported to improve poor neurological outcomes in the central nervous system after trauma or ischemia owing to their therapeutic effects (Ates et al., 2007; Cassarino & Bennett, 1999). Coenzyme Q_{10} (CoQ10) or ubiquinone is the only known lipid-soluble antioxidant that animal cells can synthesize de novo (Folkers et al., 1990). This vitamin-like substance can be primarily found in the mitochondria of most eukaryotic cells. It is a member of the electron transport chain and participates in aerobic cellular respiration, producing energy as adenosine triphosphate (ATP; Bhagavan & Chopra, 2006).

Recently, many studies have shown the neuroprotective effects of CoQ10 in some neurodegenerative diseases, such as Parkinson's and Alzheimer's diseases. In most countries, CoQ10 has been widely used as a dietary supplement for more than 20 years. Preclinical and clinical studies indicate that CoQ10 is highly safe for use as a dietary supplement (Hidaka, Fujii, Funahashi, Fukutomi, & Hosoe, 2008). Considering the antioxidant properties of EMFs and their neuroprotective effects, the purpose of this study was to assay and compare the neuroprotective effects of EMF and CoQ10 in a mouse model of hippocampal injury.

2 | MATERIALS AND METHODS

2.1 | Animals and experimental groups

In this experimental study, we used 36 male Balb/c mice, weighing 25–30 g (Pasture Institute, Tehran, Iran). All experimental procedures were performed in accordance with the Guidelines of the ethical Committee of Iran University of Medical Sciences. The animals were divided to the following six groups randomly: Control group (intact); with any intervention, vehicle group; received sesame oil (solvent of CoQ10) for 2 weeks and then placed in switch off device (7 hr daily during 1 week) following treated with intraperitoneal (ip) injection of trimethyltin hydroxide (TMT) 2.5 mg/kg, TMT group; received only TMT, EMF group; received EMF with frequency of 50 Hz and intensity of 5.9 mT 1 week after TMT injection (7 hr daily during 1 week), CoQ10 group; treated with CoQ10 (10 mg/kg) for 2 weeks following TMT injection, CoQ10 + EMF group; treated with co-administrated of CoQ10 (for 2 weeks) and EMF (for 1 week) after TMT injection.

2.2 | Hippocampal injury modeling

2.5 mg/kg TMT (originally obtained from Alfa Products, Danvers, MA) injected intraperitoneally to induce hippocampal injury in animals (Gunasekar et al., 2001).

2.3 | The magnetic field exposure system

Electromagnetic field generator was used to exposure EMF with frequency of 50 Hz and intensity of 5.9 mT (Ahmadian, Zarchi, & Bolouri, 2006).

2.4 | Morris water maze (MWM) training

MWM apparatus (was used to assess learning and spatial memory of mice (Barnhart, Yang, & Lein, 2015; Vorhees & Williams, 2006). The animals were trained to escape from water by swimming to the hidden platform. An animal could find the platform, which was under the water and served as a "rescue" from the stress situation, by using visual extra-maze cues. The animals were subjected to 5 days of hidden platform trials (four trials of 60 s). We trained the animals for 4 days at approximately the same time daily (10:00–12:00 a.m.). Each training day included two blocks, with four trials. Each trial was 90 s and there was a 30-s period between two trials, which was spent on the platform. Animals were allowed to rest for 5 min between two consecutive blocks. On 5th day, the time spent and latency to reach the platform was compared among all groups across the trial days. A video camera (Nikon, Melville, NY) linked to a computer directly located above the MWM was used to record the time taken to reach the hidden platform (escape latency), the length of the swim path (traveled distance) and percentage of time spent in the target quadrant. The day after the last learning trial, each animal was given a single 60-s probe trial and a visible test. The probe trials were conducted without a platform. In the visible trials, the platform was covered with aluminum foil. All trials were analyzed with EthoVision (Noldus Information Technology, Leesbury, VA) software (Asi et al., 2011; Shariati et al., 2014).

2.5 | Histopathological studies

Histopathological studies were down with Nissl staining and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) kit. The Mice were anesthetized deeply with over dosage of ketamine (150 mg/kg ip) and xylazine (15 mg/kg ip). Ribs and heart was exposed with a midline skin incision and then a catheter was inserted into left ventricle to access ascending part of aorta to perfuse solutions and right atrium was perforated for blood existion. At the first 50–75 ml of normal saline during 5–10 then 75–100 ml of paraformaldehyde 4% in 0.1 mol/L phosphate buffer (pH 7.4) during 10–15 min was perfused. Finally the brains were removed and postfixed with 4% of paraformaldehyde in 0.1 mol/L phosphate buffer (pH 7.4) at room temperature for 24 hr and processed to embed in paraffin. Embedded samples sectioned serially with a manual microtome apparatus (7 μm

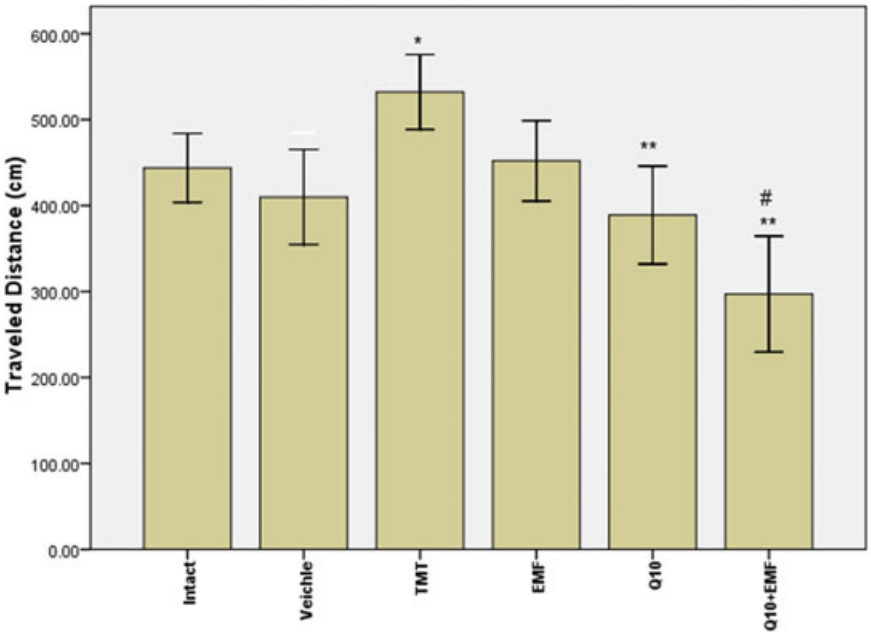

FIGURE 1 Escape latency in MWM test. *Significant increase in escape of latency in the TMT group compared with the control group ($p = 0.001$), **significant decrease in escape of latency in the CoQ10 + EMF group compared with the TMT group ($p = 0.015$). #significant decrease in escape of latency in CoQ10 + EMF in comparison with the EMF group. CoQ10: coenzyme Q10; EMF: electromagnetic fields; MWM: Morris water maze; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

thickness). Histology grade xylene used to deparaffinize paraffin-embedded tissue sections then sections hydrated and rinsed in distilled water and stained in 0.1% cresyl violet solution (Nisssl staining) for 3–10 min followed by a quick rinse in distilled water and dehydration in alcohol and clearing in xylene. Stained sections finally mounted with permanent mounting medium and covered with coverslip (Hossein Hassanshahi, Hassanshahi, Zamani, Hakimizadeh, & Soleimani, 2012; Zamani, Hassanshahi, Soleimani, & Zamani, 2013). TUNEL staining was performed according to manufacturer's instructions for some of these serial sections. Stained sections were assayed with bright field microscope and TUNEL positive cells with brownish color, were detected and counted in five fields of each section (Hossein Hassanshahi et al., 2012; Zamani, Soleimani, et al., 2013).

2.6 | Western blot analysis

Western blot analysis was performed with the hippocampi dissected from mice brains of experimental groups. The frozen hippocampi were homogenized using cold lysis buffer (containing radioimmunoprecipitation assay buffer with protease inhibitor cocktail, 1:10) for 1 hr and centrifuged (Eppendrof, Hamburg, Germany) at 12,000*g* for 20 min at 4°C temperature. Supernatant was removed and conserved. Protein concentration was determined with a Bio-Rad assay system (Bio-Rad, San Francisco, CA). Aliquots of 100 μg of proteins from each sample were denatured with a sample buffer (6.205 mM Tris–HCl, 10% glycerol, 2% sodium dodecyl sulfate (SDS), 0.01% bromophenol blue, and 50 mM 2-ME) at 95°C for 5 min and separated on 10% sodium dodecyl sulfate polyacrylamide gel

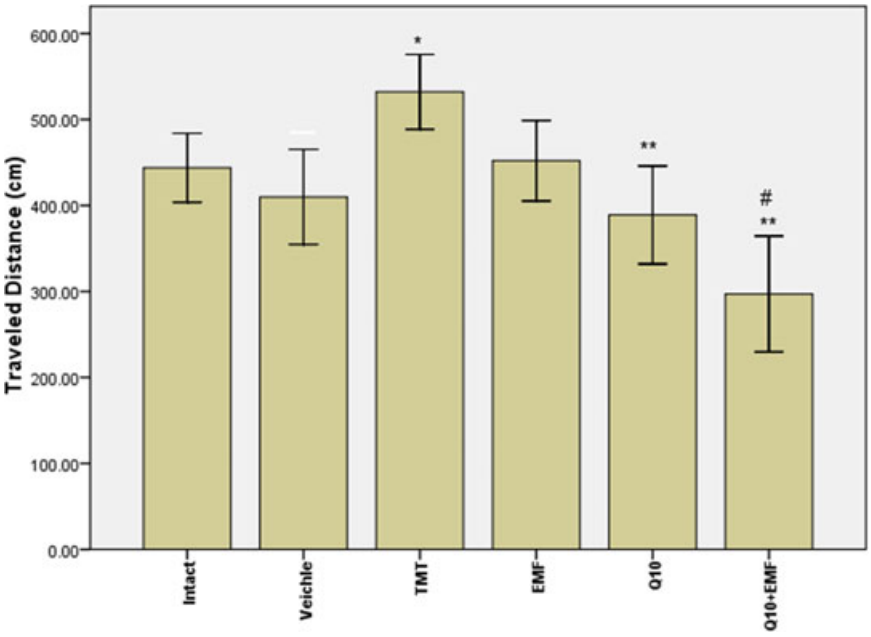

FIGURE 2 Traveled distance in MWM test. *Significant increase in the distance traveled in the TMT group in comparison with the control group ($p = 0.013$). **Significant decrease in the distance traveled in the CoQ10 + EMF group in comparison with the TMT group ($p = 0.001$). In the CoQ10 treatment group, there was a significant decrease in the distance traveled compared with the TMT group ($p = 0.001$). #Significant decrease in the traveled distance in CoQ10 + EMF in comparison with the EMF group ($p = 0.001$). CoQ10: coenzyme Q10; EMF: electromagnetic fields; MWM: Morris water maze; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

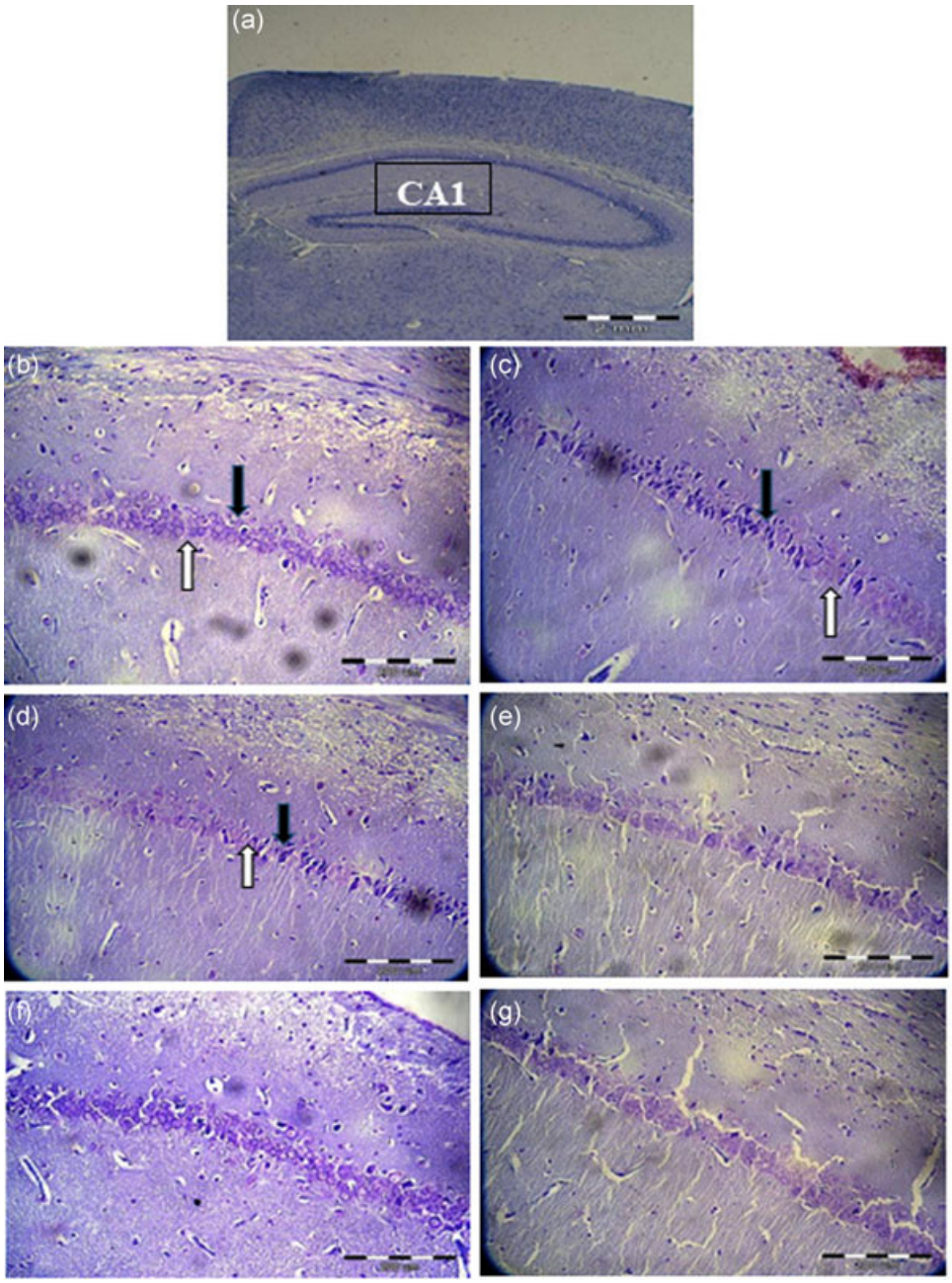

FIGURE 3 (a) Hippocampal CA1 region (× 40), (b) control group, (c) TMT group, (d) vehicle group, (e) CoQ10 group, (f) EMF group, and (g) CoQ10 + EMF (× 400). Dark arrow shows necrotic cell and white arrow shows normal cell. CoQ10: coenzyme Q10; EMF: electromagnetic fields; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

electrophoresis (90 min, 120 V). SDS page proteins were transferred to a hybond-PTM membrane (Amersham Pharmacia Biotech, Piscataway, NJ). Membranes were blocked for 1 hr with 5% nonfat milk dissolved in TTBS buffer (Tris 50 mM, NaCl 1.5%, and Tween 20 0.05%, pH 7.5). Nitrocellulose membranes were stained with anti-Bcl-2 and anti-Bax monoclonal antibodies for 2 hr (Sigma Aldrich, St. Louis, MO; 1:1,000), followed by secondary antibody alkaline phosphatase-conjugated anti-mouse antibodies for 1 hr (Sigma Aldrich; 1:10,000). Bands were detected using chromogenic substrate, 5-bromo-4-chloro-3-indolyl phosphate, in the presence of nitroblue tetrazolium. β-Actin antibody (Sigma Aldrich; 1:1,000) was used as control. Finally the bands were assayed and analyzed densitometrically using an image analysis system (UVIdoc, Houston, TX) (Zamani, Soleimani, et al., 2013; Zamani, Katebi, Mehdizadeh, Mohamadzadeh, & Soleimani, 2012).

2.7 | Statistical analysis

All data were expressed as mean ± *SE*. Statistical analyses were down with SPSS software version 20 (SPSS Inc., Chicago, Il). The groups were compared using one-way analysis of variance and posttest Tukey. A value of $p < 0.05$ was considered statistically significant.

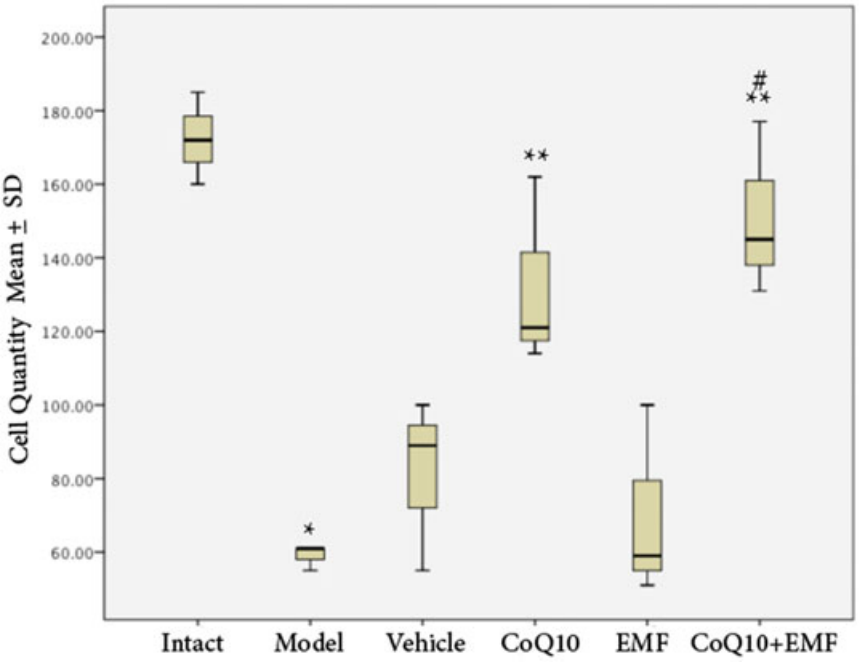

FIGURE 4 The graph shows number of normal cells in CA1 region of hippocampus after Nissl staining. *Significant decrease of normal cells compared with the control group ($p = 0.001$). **Significant increase in normal cells in the CoQ10 and CoQ10 + EMF groups in comparison with TMT group ($p = 0.011$ and $p = 0.002$). #Significant increase of normal cells in the CoQ10 + EMF group in comparison with EMF group ($p = 0.005$). CoQ10: coenzyme Q10; EMF: electromagnetic fields; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

3 | RESULTS

3.1 | Morris water maze

Analysis of data from Morris water maze task showed that a significant increase in escape of latency in the TMT group compared with the control group ($p = 0.001$). The same study showed a significant decrease in escape of latency in the CoQ10 + EMF group compared with the TMT group ($p = 0.015$; Figure 1).

Also a significant increase in the distance traveled in the TMT group was observed in comparison with the control group ($p = 0.013$). There was a significant decrease in the distance traveled in the CoQ10 + EMF group in comparison with the TMT group. $p = 0.00$). In the CoQ10 group, there was a significant decrease in the traveled distance compared with the TMT group ($p = 0.001$). There was a significant decrease in the traveled distance in CoQ10 + EMF when compared with the EMF group ($p = 0.001$; Figure 2).

3.2 | Nissl staining

We used the Nissl staining to count the necrotic cells. Coronal sections from CA1 region of hippocampus with a thickness of 5 microns stained with crystal violet. Cell counting was performed in the area of 53,500 μm and in three sections with 2.7 mm distance from Bergma and 30 μm distance from each other. The necrotic cells indicated with dark and compact nucleus. A significant decrease in the number of normal cells was observed in the TMT group (CA1) compared with the control group ($p = 0.05$). Cell counts in CoQ10 and CoQ10 + EMF groups have revealed significant increase of normal cells in comparison with TMT group ($p = 0.011$ and $p = 0.002$). But at the group treated with EMF, there was no significant increase of normal cells (Figures 3 and 4).

3.3 | TUNEL test

TUNEL kit was used to detect the apoptotic cells in the CA1 region of hippocampus. Brown colored cells indicate apoptotic cells. A significant increase of apoptotic cells was observed in TMT group in comparison with control group. In the group treated with CoQ10 and in CoQ10 + EMF group, apoptotic cells decreased significantly in the CA1 region in comparison with TMT group (Figures 5 and 6). ($p = 0.023$, $p = 0.003$).

3.4 | Western blot analysis

Apoptotic Bax protein expression that was evaluated by western blot showed a significant increase in the expression of this protein in TMT group when compared with control group. Bax protein expression in all treated groups decreased significantly in comparison with TMT group and in the CoQ10 + EMF group was less than CoQ10 treated group.

Also antiapoptotic Bcl-2 protein expression in the study groups showed a significant increase of Bcl-2 expression in the vehicle group compared with the control group. Also a significant increase of it in all treatment groups was observed in comparison with TMT group. Bcl-2 expression in the CoQ10 + EMF group showed a significant increase of it compared with the EMF group.

Procaspase 3 significantly increased in both TMT and vehicle groups in comparison with control group. A significant decrease in procaspase 3 expression was observed in all treatment groups compared with the TMT group. Procaspase 3 expression in the CoQ10 + EMF group was significantly decreased when compared with EMF group (Figures 7-10).

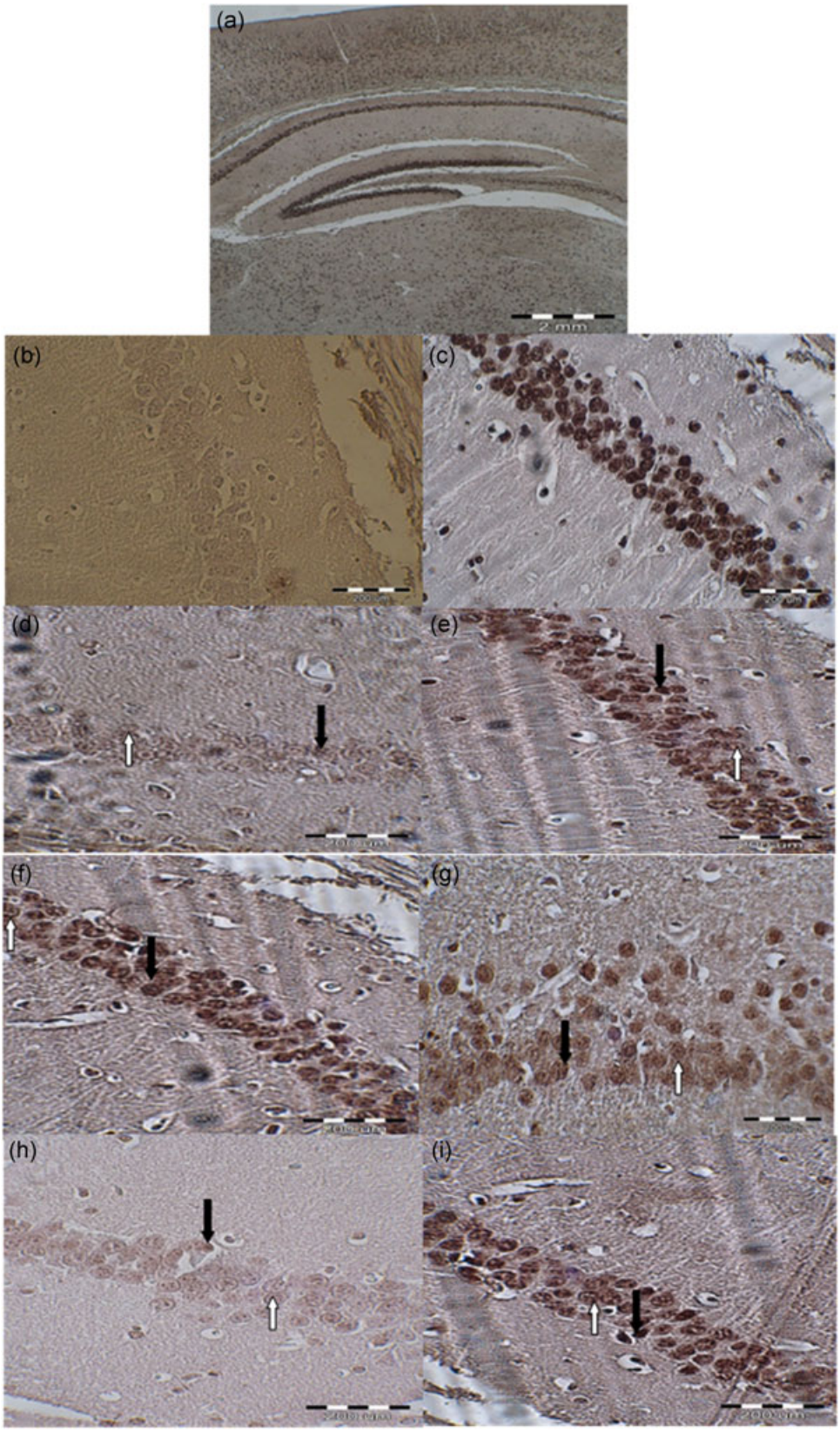

FIGURE 5 TUNEL staining: (a) CA1 region, (b) negative control for TUNEL staining, (c) positive control for TUNEL staining, (d) control, (e) TMT, (f) vehicle, (g) CoQ10 treatment, (h) combination treatment, and (i) EMF, (400 ×). In this technique apoptotic cells stain dark. Dark marker shows apoptotic cell and white marker shows normal cell. CoQ10: coenzyme Q10; EMF: electromagnetic fields; TMT: trimethyltin hydroxide; TUNEL: terminal deoxynucleotidyl transferase dUTP nick end labeling [Color figure can be viewed at wileyonlinelibrary.com]

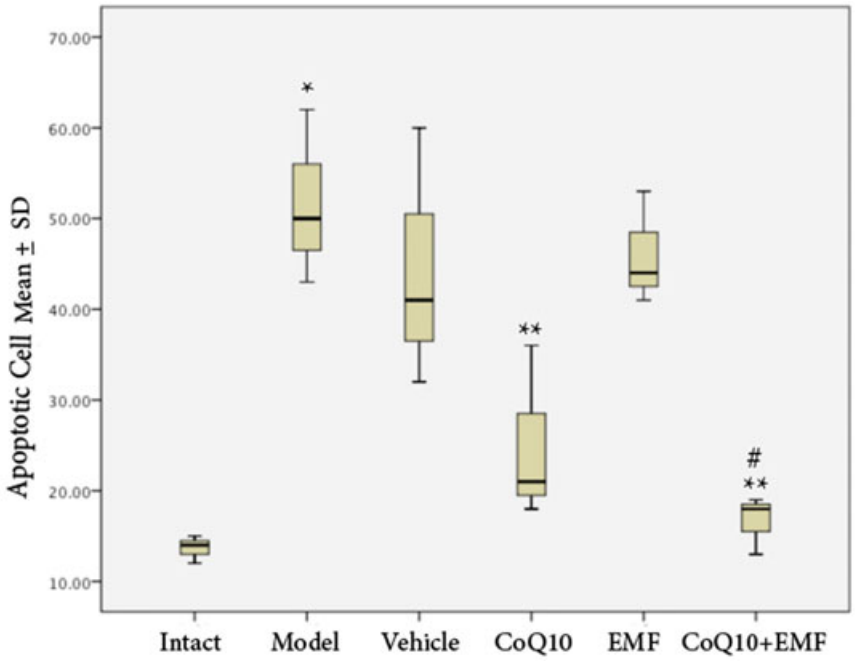

FIGURE 6 The graph shows apoptotic cell count. *Significant increase of apoptotic cells in TMT group in comparison with control group ($p = 0.002$). **Significant decrease of apoptotic cells in CoQ10 and CoQ10 + EMF groups in comparison with TMT group. ($p = 0.023$, $p = 0.003$). #Significant decrease of apoptotic cells in CoQ10 + EMF group in comparison with EMF group (0.012). CoQ10: coenzyme Q10; EMF: electromagnetic fields; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In this study, the protective effects of EMF and CoQ10 were examined and compared in a mouse model of hippocampal injury induced by the intraperitoneal injection of TMT. Our results showed that TMT induced neuronal death (necrosis/apoptosis) in the CA1 region of the hippocampus (a key region in memory organization and learning) and caused learning and memory impairments in mice. In addition, TMT increased apoptotic Bax and procaspase-3 proteins and decreased the level of antiapoptotic Bcl-2 protein in the hippocampus of mice.

TMT is a neurotoxic agent, which selectively destroys neurons in the central nervous system of humans and rodents after its administration. TMT, through binding to mitochondria, inhibits phosphorylation and damages neurons due to reversible/irreversible interference in the production of ATP (Fechter, Young, & Nuttall, 1986). Therefore, oxidative stress is reported as a common factor involved in neuronal death (necrosis/apoptosis) caused by TMT. Oxidative stress is an initiating event, associated with a number of neurodegenerative conditions (Gunasekar et al., 2001). Also, oxidative stress free radicals contribute to either apoptotic or necrotic cell death both in vivo and in vitro (Fechter et al., 1986).

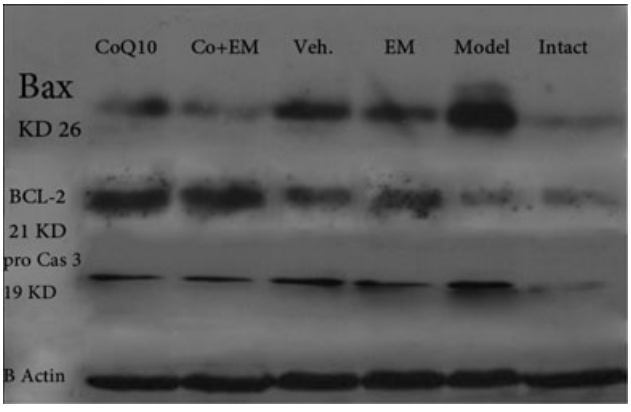

FIGURE 7 Western blot analysis bands in six groups under investigation. Bcl-2: B-cell lymphoma 2; CoQ10: coenzyme Q10

Anna Fiedorowicz et al. observed damaged granular neurons in the dentate gyrus within 3 days after TMT (2.5 mg/kg) injection in BALB/c mice. On TUNEL and Nissl staining tests, these damaged cells had characteristics of apoptotic cells (Balaban, Callaghan, & Billingsle, 1988; Fiedorowicz et al., 2001). In our study, exposure to EMF had no significant effects on cell death following TMT injection, whereas treatment with CoQ10 decreased apoptotic cells and increased normal cells in the CA1 region.

On the other hand, coadministration of EMF and CoQ10 caused a further increase in normal cells and a reduction in apoptotic cells. EMF exposure and CoQ10 treatment independently decreased the level of Bax and apoptotic procaspase-3 proteins in the hippocampal lysate and increased the level of antiapoptotic Bcl-2 proteins. Coadministration of EMF and CoQ10 also improved spacial memory of mice following TMT injection; however, coadministration was more effective than use of EMF alone.

Yang and colleagues concluded that EMF (60 HZ and 0.7 mT) could decrease neurodegeneration and oxidative stress. Additionally, in another study, Yang and colleagues showed that transcranial magnetic stimulation (TMS), as a noninvasive procedure, resulted in the survival of dopaminergic neurons in the frontal segment of the brain (Yang, Song, & Liu, 2010). Funke et al. also found that EMF-induced behavioral changes are attributed to changes in the level of neurotrophic factors, oxidative stress, and cellular density (Funke & Benali, 2011; Tasset et al., 2010).

Previously, improvements were reported on behavioral tests in animals exposed to magnetic stimulation (Kanno, Matsumoto, Togashi,

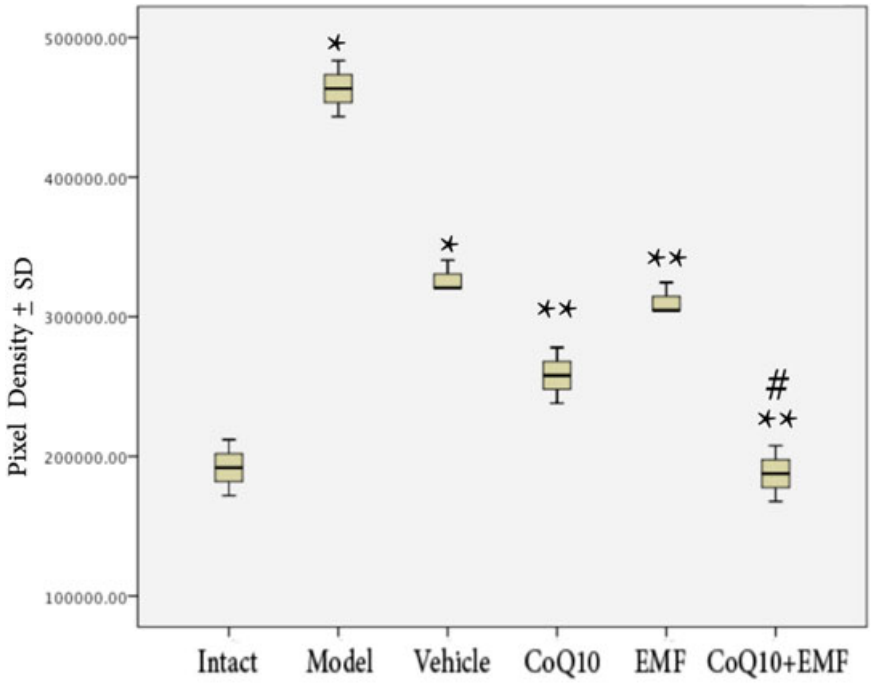

FIGURE 8 The count of the number of pixels associated with the BAX protein bands. *Significant increase in expression of the BAX in the hippocampus of the TMT group compared with the control group ($p = 0.0001$). **Significant decrease in all treatment groups compared with the control group. #Significant decrease in the CoQ10 + EMF group compared with the CoQ10 or EMF ($p \leq 0.05$). CoQ10: coenzyme Q10; EMF: electromagnetic fields; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

Yoshioka, & Mano, 2004). In another study, the antioxidant and anti-inflammatory effects of TMS were confirmed (Funamizu, Ogiue-Ikeda, Mukai, Kawato, & Ueno, 2005). Moreover, Tasset et al. (2010) showed that EMF enhances mitochondrial survival and activity. They also concluded that EMF improves behavioral patterns and increases BDNF and GDNF levels. Túnezl and colleagues found that EMF, in addition to the enhancement of mitochondrial activity, changes the expression of some proteins at the nuclear level via Nrf2 transcription factor and exerts protective effects against oxidative damage. In fact, Nrf2 is involved in the regulation of antioxidant enzyme expression (Túnez, Montilla, del Carmen Munoz, Medina, & Drucker-Colín, 2006).

A previous study concluded that EMF (50 Hz, 0.1–1 mT) had positive effects on cell survival in the primary cultures of cortical neurons from mature rats. They also claimed that EMF decreases apoptosis, which is probably related to the reduced levels of reactive oxygen species and glutathione (Di Loreto et al., 2009). In general, the findings suggest that EMF regulates the antioxidant pathways, protects the cells against oxidative damage, increases cell survival

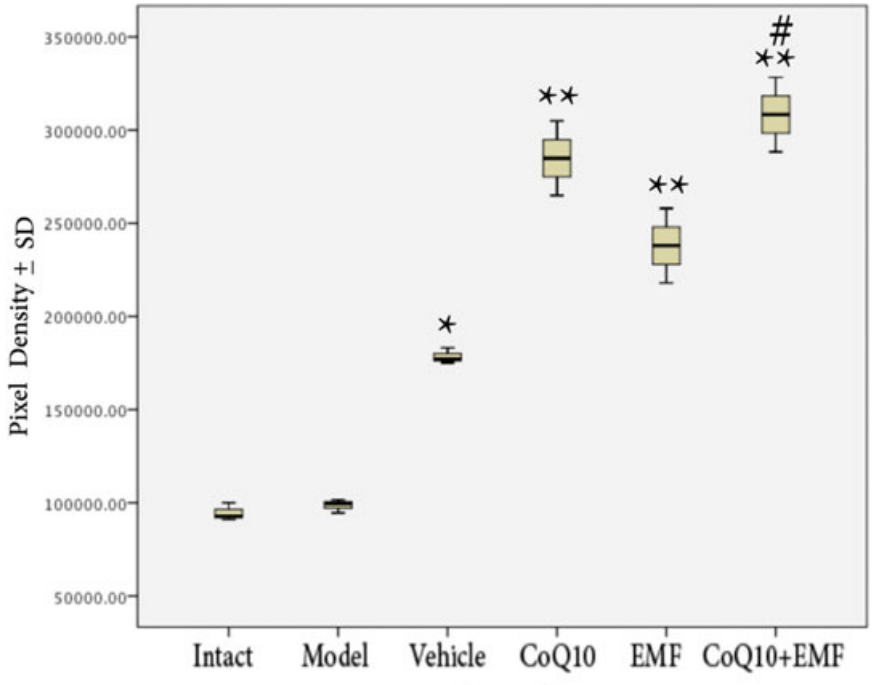

FIGURE 9 The count of the number of pixels associated with the Bcl2 protein bands. *Significant increase of Bcl-2 in the vehicle group compared with the control group ($p = 0.0001$). **Significant increase of Bcl-2 in all treatment groups, compared with the TMT group. ($p = 0.0001$). #Significant increase of Bcl-2 in the CoQ10 + EMF group compared with the EMF group ($p = 0.001$). Bcl-2: B-cell lymphoma 2; CoQ10: coenzyme Q10; EMF: electromagnetic fields [Color figure can be viewed at wileyonlinelibrary.com]

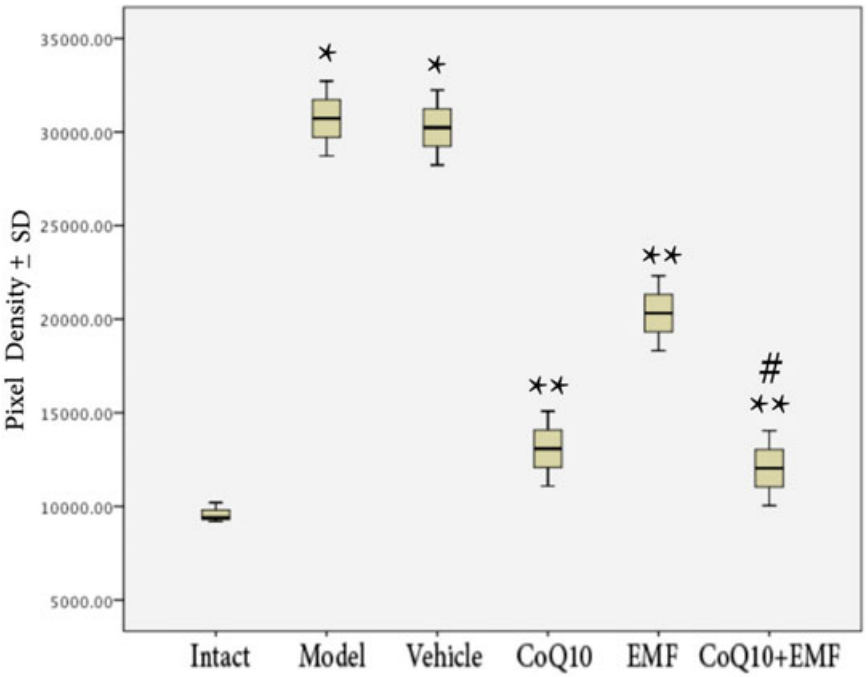

FIGURE 10 The count of the number of pixels associated with the procaspase 3 bands. *Significant increase of procaspase 3 in TMT and vehicle groups in comparison with the control group ($p = 0.0001$). **Significant decrease of procaspase 3 in all therapeutic groups compared with the TMT group ($p = 0.0001$). #Significant decrease of procaspase 3 in CoQ10 + EMF group compared with the EMF group ($p = 0.001$). CoQ10: coenzyme Q10; EMF: electromagnetic fields; TMT: trimethyltin hydroxide [Color figure can be viewed at wileyonlinelibrary.com]

and density, and decreases apoptosis (Tasset et al., 2010). Nonetheless, our study showed that the antioxidant effects are not as promising as CoQ10. In addition, continuous EMF exposure was reported to have beneficial behavioral effects in a Huntington disease model, as confirmed in various tests on rats (Berlim, McGirr, Beaulieu, & Turecki, 2011; Hargreaves, McGregor, & Sachdev, 2005; Lisanby, Kinnunen, & Crupain, 2002; Rodriguez-Martin et al., 2001; Simons & Dierick, 2005; Tasset et al., 2012; Vieyra-Reyes et al., 2008; Zwanzger, Fallgatter, Zavorotnyy, & Padberg, 2009).

The findings suggest that behavioral changes in animals following EMF exposure depend on several factors, such as the stimulus location and neurophysiological conditions. Overall, the findings show that magnetic stimulation promotes neuronal survival and neuronal density, which in turn improves the function of the nervous system and behaviors (Simons & Dierick, 2005). Cuccurazzu et al. (2010) showed that EMF exposure increases the number of neurons in the dentate gyrus of mice. Blaschke and colleagues also showed that the number of new cells in the dentate gyrus (with a primary neuron marker) significantly increased in mice affected by EMF; later, these new cells became both mature and functional (Biebl, Cooper, Winkler, & Kuhn, 2000; Blaschke, Staley, & Chun, 1996).

Concerning the effects of EMF on memory, there are controversial and contradictory reports. It has been proposed that EMF causes spatial memory impairment, depending on the duration of exposure and field intensity (Morris, Garrud, Rawlins, & O'Keefe, 1982). On the other hand, Majid Jadidi and colleagues continuously studied the effects of EMF (50 Hz, 10 mT) on adult Wistar rats during 4 weeks and concluded that EMF had no cognitive or anxiety-like effects (Akhtary, Rashidy-Pour, Vafaei, & Jadidi, 2011). Our results on the use of electromagnetism were in accordance with the results of other researchers and showed the effects of these waves on the prevention of cellular apoptosis.

CoQ10 plays an important role in cellular defense against oxidative damage. Degenerative diseases and aging may be the manifestations of decreased capacity to maintain adequate CoQ10 levels (Ernster & Forsmark-Andree, 1993). According to the literature, CoQ10 can modulate apoptosis (Doimo et al., 2014). Ostrowski and colleagues concluded that use of CoQ10 (10 mg/kg) immediately after trauma had protective effects against ischemic injury in experimental models of ischemia in the brain hemispheres and spinal cord (Erol et al., 2010; Kerimoğlu et al., 2007; Ostrowski, 1999, 2000). In our results we concluded CoQ10 alone could have protective effects on hippocampal injury.

5 | CONCLUSION

In conclusion, EMF (50 Hz, 5.9 mT) exerted neuroprotective effects on hippocampal neurons and improved spacial memory impairments in mice. However, CoQ10 had more effects (10 mg/kg, ip injection daily for 2 weeks) on hippocampal damage in mice. Although coadministration of EMF and CoQ10 improved the neuroprotective effects of CoQ10, antioxidant activity should be accurately determined through further research at molecular levels.

ACKNOWLEDGMENTS

This study was supported by Cellular and Molecular Research Center and Razi Drug Research Center, Iran University of Medical Sciences.

AUTHOR CONTRIBUTIONS

M. S., M. K., S. R. conceived and designed the study. All authors contributed to performing the relevant experiments. F. G., A. A., Z. N.

S. analyzed the data. G. V., T. P., M. S., F. A. wrote the paper. All authors contributed to reviewing and editing the manuscript and also read and approved the manuscript.

ORCID

Majid Katebi http://orcid.org/0000-0003-4957-9941
Fereshteh Azedi http://orcid.org/0000-0001-9776-7686

REFERENCES

Adey, W. R. (1993). Biological effects of electromagnetic fields. *Journal of Cellular Biochemistry*, *51*(4), 410–416.

Ahmadian, S., Zarchi, S. R., & Bolouri, B. (2006). Effects of extremely-low-frequency pulsed electromagnetic fields on collagen synthesis in rat skin. *Biotechnology and Applied Biochemistry*, *43*(2), 71–75.

Akhtary, Z., Rashidy-Pour, A., Vafaei, A. A., & Jadidi, M. (2011). Effects of extremely low-frequency electromagnetic fields on learning and memory and anxiety-like behaviors in rats. *Koomesh*, *12*(4), 435–446.

Asi, S., Farhadi, H., Naghdi, N., Choopani, S., Samzadeh-Kermani, A., & Mehdizadeh, M. (2011). Non-acute effects of different doses of 3, 4-methylenedioxymethamphetamine on spatial memory in the Morris water maze in Sprague–Dawley male rats. *Neural Regeneration Research*, *6*(22), 1715–1719.

Ates, O., Cayli, S., Altinoz, E., Gurses, I., Yucel, N., Sener, M., … Yologlu, S. (2007). Neuroprotection by resveratrol against traumatic brain injury in rats. *Molecular and Cellular Biochemistry*, *294*(1-2), 137–144.

Balaban, C., Callaghan, J., & Billingsle, M. (1988). Trimethyltin-induced neuronal damage in the rat brain: Comparative studies using silver degeneration stains, immunocytochemistry and immunoassay for neuronotypic and gliotypic proteins. *Neuroscience*, *26*(1), 337–361.

Barnhart, C. D., Yang, D., & Lein, P. J. (2015). Using the Morris water maze to assess spatial learning and memory in weanling mice. *PLOS One*, *10*(4), e0124521.

Berlim, M. T., McGirr, A., Beaulieu, M.-M., & Turecki, G. (2011). High frequency repetitive transcranial magnetic stimulation as an augmenting strategy in severe treatment-resistant major depression: A prospective 4-week naturalistic trial. *Journal of Affective Disorders*, *130*(1), 312–317.

Bhagavan, H. N., & Chopra, R. K. (2006). Coenzyme Q10: Absorption, tissue uptake, metabolism and pharmacokinetics. *Free Radical Research*, *40*(5), 445–453.

Biebl, M., Cooper, C. M., Winkler, J., & Kuhn, H. G. (2000). Analysis of neurogenesis and programmed cell death reveals a self-renewing capacity in the adult rat brain. *Neuroscience Letters*, *291*(1), 17–20.

Blaschke, A. J., Staley, K., & Chun, J. (1996). Widespread programmed cell death in proliferative and postmitotic regions of the fetal cerebral cortex. *Development*, *122*(4), 1165–1174.

Cassarino, D. S., & Bennett, J. P., Jr (1999). An evaluation of the role of mitochondria in neurodegenerative diseases: Mitochondrial mutations and oxidative pathology, protective nuclear responses, and cell death in neurodegeneration. *Brain Research Reviews*, *29*(1), 1–25.

Cuccurazzu, B., Leone, L., Podda, M. V., Piacentini, R., Riccardi, E., Ripoli, C., … Grassi, C. (2010). Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. *Experimental Neurology*, *226*(1), 173–182.

Doimo, M., Desbats, M. A., Cerqua, C., Cassina, M., Trevisson, E., & Salviati, L. (2014). Genetics of coenzyme q10 deficiency. *Molecular Syndromology*, *5*(3-4), 156–162.

Ernster, L., & Forsmark-Andree, P. (1993). Ubiquinol: An endogenous antioxidant in aerobic organisms. *The Clinical Investigator*, *71*(8), S60–S65.

Erol, B., Bozlu, M., Hanci, V., Tokgoz, H., Bektas, S., & Mungan, G. (2010). Coenzyme Q10 treatment reduces lipid peroxidation, inducible and endothelial nitric oxide synthases, and germ cell–specific apoptosis in a rat model of testicular ischemia/reperfusion injury. *Fertility and Sterility*, *93*(1), 280–282.

Fechter, L. D., Young, J. S., & Nuttall, A. L. (1986). Trimethyltin ototoxicity: Evidence for a cochlear site of injury. *Hearing Research*, *23*(3), 275–282.

Fiedorowicz, A., Figiel, I., Kamińska, B., Zaremba, M., Wilk, S., & Oderfeld-Nowak, B. (2001). Dentate granule neuron apoptosis and glia activation in murine hippocampus induced by trimethyltin exposure. *Brain Research*, *912*(2), 116–127.

Folkers, K., Langsjoen, P., Willis, R., Richardson, P., Xia, L.-J., Ye, C.-Q., & Tamagawa, H. (1990). Lovastatin decreases coenzyme Q levels in humans. *Proceedings of the National Academy of Sciences*, *87*(22), 8931–8934.

Funamizu, H., Ogiue-Ikeda, M., Mukai, H., Kawato, S., & Ueno, S. (2005). Acute repetitive transcranial magnetic stimulation reactivates dopaminergic system in lesion rats. *Neuroscience Letters*, *383*(1-2), 77–81.

Funke, K., & Benali, A. (2011). Modulation of cortical inhibition by rTMS—Findings obtained from animal models. *The Journal of Physiology*, *589*(18), 4423–4435.

Guerriero, F., & Ricevuti, G. (2016). Extremely low frequency electromagnetic fields stimulation modulates autoimmunity and immune responses: A possible immuno-modulatory therapeutic effect in neurodegenerative diseases. *Neural Regeneration Research*, *11*(12), 1888.

Gunasekar, P., Li, L., Prabhakaran, K., Eybl, V., Borowitz, J. L., & Isom, G. E. (2001). Mechanisms of the apoptotic and necrotic actions of trimethyltin in cerebellar granule cells. *Toxicological Sciences*, *64*(1), 83–89.

Hargreaves, G. A., McGregor, I. S., & Sachdev, P. S. (2005). Chronic repetitive transcranial magnetic stimulation is antidepressant but not anxiolytic in rat models of anxiety and depression. *Psychiatry Research*, *137*(1), 113–121.

Hidaka, T., Fujii, K., Funahashi, I., Fukutomi, N., & Hosoe, K. (2008). Safety assessment of coenzyme Q10 (CoQ10). *Biofactors*, *32*(1-4), 199–208.

Hossein Hassanshahi, G., Hassanshahi, J., Zamani, M., Hakimizadeh, E., & Soleimani, M. (2012). Comparison of therapeutic effects of vitamin C and CoQ10 in reducing of damaged cells in mice hippocampus following ischemia-reperfusion. *Journal of Mazandaran University of Medical Sciences*, *22*(94), 2–13.

Kanno, M., Matsumoto, M., Togashi, H., Yoshioka, M., & Mano, Y. (2004). Effects of acute repetitive transcranial magnetic stimulation on dopamine release in the rat dorsolateral striatum. *Journal of the Neurological Sciences*, *217*(1), 73–81.

Kerimoğlu, A., Paşaoğlu, O., Kanbak, G., Hanci, V., Ozdemir, F., & Atasoy, M. A. (2007). Efficiency of coenzyme Q (10) at experimental spinal cord injury. *Turkish Journal of Trauma & Emergency Surgery*, *13*(2), 85–93.

Lacy-Hulbert, A., Metcalfe, J. C., & Hesketh, R. (1998). Biological responses to electromagnetic fields. *The FASEB Journal*, *12*(6), 395–420.

Lai, H., & Singh, N. P. (1995). Acute low-intensity microwave exposure increases DNA single-strand breaks in rat brain cells. *Bioelectromagnetics*, *16*(3), 207–210.

Lisanby, S. H., Kinnunen, L. H., & Crupain, M. J. (2002). Applications of TMS to therapy in psychiatry. *Journal of Clinical Neurophysiology*, *19*(4), 344–360.

Di Loreto, S., Falone, S., Caracciolo, V., Sebastiani, P., D'Alessandro, A., Mirabilio, A., … Amicarelli, F. (2009). Fifty hertz extremely low-frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons. *Journal of Cellular Physiology*, *219*(2), 334–343.

Lyskov, Y. B., Chernysev, M., Mikhailov, V., Kozlov, A., Makarova, T., Vasil'yeva, Y. V., … Vishnevskii, A. (1996). The effect of a magnetic field with the frequency of 50 Hz on behaviour in rats depends on the value of the constant magnetic field. *Biophysics*, *4*(41), 881–886.

McCann, J., Dietrich, F., & Rafferty, C. (1998). The genotoxic potential of electric and magnetic fields: An update. *Mutation Research/Reviews in Mutation Research*, *411*(1), 45–86.

Morris, R., Garrud, P., Rawlins, Ja, & O'Keefe, J. (1982). Place navigation impaired in rats with hippocampal lesions. *Nature*, *297*(5868), 681–683.

Navarro-Yepes, J., Burns, M., Anandhan, A., Khalimonchuk, O., Del Razo, L. M., Quintanilla-Vega, B., … Franco, R. (2014). Oxidative stress, redox signaling, and autophagy: Cell death versus survival. *Antioxidants & Redox Signaling*, *21*(1), 66–85.

Ostrowski, R. (1999). Effect of coenzyme Q10 (CoQ10) on superoxide dismutase activity in ET-1 and ET-3 experimental models of cerebral ischemia in the rat. *Folia Neuropathologica*, *37*(4), 247–251.

Ostrowski, R. P. (2000). Effect of coenzyme Q10 on biochemical and morphological changes in experimental ischemia in the rat brain. *Brain Research Bulletin*, *53*(4), 399–407.

Rodriguez-Martin, J. L., Barbanoj, J. M., Schlaepfer, T. E., Clos, S. S., Perez, V., Kulisevsky, J., & Gironelli, A. (2001). Transcranial magnetic stimulation for treating depression. *Cochrane Database Syst Rev*, *2*, CD003493.

Shariati, M. B. H., Sohrabi, M., Shahidi, S., Nikkhah, A., Mirzaei, F., Medizadeh, M., & Asl, S. S. (2014). Acute effects of ecstasy on memory are more extensive than chronic effects. *Basic and Clinical Neuroscience*, *5*(3), 225.

Simko, M., Kriehuber, R., Weiss, D., & Luben, R. (1998). Effects of 50 Hz EMF exposure on micronucleus formation and apoptosis in transformed and nontransformed human cell lines. *Bioelectromagnetics*, *19*(2), 85–91.

Simons, W., & Dierick, M. (2005). Transcranial magnetic stimulation as a therapeutic tool in psychiatry. *The World Journal of Biological Psychiatry*, *6*(1), 6–25.

Tasset, I., Drucker-Colín, R., Peña, J., Jimena, I., Montilla, P., Medina, F. J., & Túnez, I. (2010). Antioxidant-like effects and protective action of transcranial magnetic stimulation in depression caused by olfactory bulbectomy. *Neurochemical Research*, *35*(8), 1182–1187.

Tasset, I., Medina, F., Jimena, I., Agüera, E., Gascón, F., Feijóo, M., … Drucker-Colín, R. (2012). Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: Effects on neurotrophic factors and neuronal density. *Neuroscience*, *209*, 54–63.

Túnez, I., Montilla, P., del Carmen Munoz, M., Medina, F. J., & Drucker-Colín, R. (2006). Effect of transcranial magnetic stimulation on oxidative stress induced by 3-nitropropionic acid in cortical synaptosomes. *Neuroscience Research*, *56*(1), 91–95.

Vieyra-Reyes, P., Mineur, Y. S., Picciotto, M. R., Túnez, I., Vidaltamayo, R., & Drucker-Colín, R. (2008). Antidepressant-like effects of nicotine and transcranial magnetic stimulation in the olfactory bulbectomy rat model of depression. *Brain Research Bulletin*, *77*(1), 13–18.

Vorhees, C. V., & Williams, M. T. (2006). Morris water maze: Procedures for assessing spatial and related forms of learning and memory. *Nature Protocols*, *1*(2), 848–858.

Walleczek, J., & Liburdy, R. P. (1990). Nonthermal 60 Hz sinusoidal magnetic-field exposure enhances 45Ca2 + uptake in rat thymocytes: Dependence on mitogen activation. *FEBS Letters*, *271*(1-2), 157–160.

Yang, X., Song, L., & Liu, Z. (2010). The effect of repetitive transcranial magnetic stimulation on a model rat of Parkinson's disease. *Neuroreport*, *21*(4), 268–272.

Zamani, M., Hassanshahi, J., Soleimani, M., & Zamani, F. (2013). Neuroprotective effect of olive oil in the hippocampus CA1 neurons following ischemia: Reperfusion in mice. *Journal of Neurosciences in Rural Practice*, *4*(2), 164.

Zamani, M., Katebi, M., Mehdizadeh, M., Mohamadzadeh, F., & Soleimani, M. (2012). Coenzyme Q10 protects hippocampal neurons against ischemia/reperfusion injury via modulation of BAX/Bcl-2 expression. *Basic and Clinical Neuroscience*, *3*(5), 5–10.

Zamani, M., Soleimani, M., Golab, F., Mohamadzadeh, F., Mehdizadeh, M., & Katebi, M. (2013). NeuroProtective effects of adenosine receptor agonist coadministration with ascorbic acid on CA1 hippocampus in a mouse model of ischemia reperfusion injury. *Metabolic Brain Disease*, *28*(3), 367–374.

Zwanzger, P., Fallgatter, A. J., Zavorotnyy, M., & Padberg, F. (2009). Anxiolytic effects of transcranial magnetic stimulation--an alternative treatment option in anxiety disorders? *Journal of Neural Transmission*, *116*(6), 767–775.

How to cite this article: Soleimani M, Golab F, Alizadeh A, et al. Evaluation of the neuroprotective effects of electromagnetic fields and coenzyme Q_{10} on hippocampal injury in mouse. *J Cell Physiol*. 2019;1–11. https://doi.org/10.1002/jcp.28512