

RESEARCH ARTICLE

An Evaluation of Neuronal PARP-1 and Caspase-3 Levels in the Brain Tissue of Female Rats Exposed to Electromagnetic Fields at Different Gestational Stages

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ABSTRACT

Foetal exposure to electromagnetic fields (EMFs) may cause marked neurocognitive impairment, although the mechanisms involved are still unclear. EMF induces region-specific neuronal and astroglial death in the rat hippocampus. Poly (ADP-ribose) polymerase-1 (PARP-1) regulates necrosis, apoptosis and other cellular processes occurring following injury. This study, therefore, investigated whether PARP-1 also regulates neuronal responses in the hippocampus of rats subjected to EMF radiation during different developmental periods. Male and female rats were first allowed to mate in separate cages. Rats identified as pregnant were then divided into four groups. A 900-MHz EMF was applied for 2 h daily on gestational days (GD) 1–7, GD 8–14 and GD 15–21. The female offspring were sacrificed at the end of the 28th postnatal day, and PARP-1 and Caspase-3 expressions in the hippocampus were then evaluated. No special treatment was applied to the control group. In the EMF-exposed group, pyramidal neurons in the cornu ammonis (CA) region appeared normal after exposure on GD 1–7 but were darkly stained and shrunken after exposure on GD 15–21, while the majority of granular cells exhibited a normal appearance during all GDs. The group exposed to EMF on GD 15–21 exhibited strong PARP-1 and Caspase-3 immune reactivity in CA and dentate gyrus (DG) cells. Higher H-scores were also observed in the EMF-exposed group following GD 15–21 irradiation. As a result, a 900-MHz EMF application at GD 15–21, which coincides with hippocampal neurogenesis, triggered hippocampal neuron cell death by activating PARP-1 and Caspase-3.

1 | Introduction

Global mobile phone use raises the possibility of harmful impacts on human health, particularly the brain (Bertagna et al. 2021). The increasing use of such devices means that most people are either deliberately or inadvertently exposed to electromagnetic fields (EMFs) generated by them. Recent years have seen 3G mobile communications operating in the 1900- to

2100-MHz frequency range (Singh et al. 2020). Many researches have taken place into the potential detrimental effects of EMF on the hippocampus (Elamin, Deniz and Kaplan 2022; Keles, Nyengaard and Odaci 2019; Kivrak et al. 2017). Investigation has therefore focused on how different conditions affect the structure of hippocampus. One such area involves how learning is affected by cellular alterations in the hippocampus caused by different factors. Research indicates that the impact of EMF may

be potentially linked to memory, recall, motor skills, learning and locomotor activities (Keles et al. 2018). Various pathways can cause cell death. Apoptotic processes continually remove numerous cells in tissues that regenerate throughout life and during development (Fuchs and Steller 2015). Two distinct pathways are primarily responsible for apoptotic cell death, the mitochondrial or intrinsic pathway (affecting type II cells) and the death receptor pathway (affecting type I cells). Caspase-3, which causes cell death, is activated by both intrinsic and extrinsic mechanisms (Lorente et al. 2015). Numerous signals can cause apoptosis through the mitochondrial or intrinsic pathway, including intrinsic stress signals resulting from exposure to harmful radiation or chemicals, oxidative stress, viral infections, hypoxia and hyperthermia. This is the case, for example, in cancer patients who undergo radiation therapy or chemotherapy (Julien and Wells 2017). Apoptosis can be promoted by extrinsic stimuli (including steroid hormones and several ligands of the tumour necrosis factor receptor) and intracellular molecules (Aram, Yacobi-Sharon and Arama 2017). The abundantly expressed PARP-1 nucleoprotein is essential for DNA base excision repair in eukaryotic cells (Demin et al. 2021). PARP-1 has been the most intensively studied of the nuclear enzymes in the PARP superfamily (D.S. Kim et al. 2020). Several studies have also described PARP-1 as essential for apoptosis, autophagy and programmed necrosis. Caspase-independent programmed cell death mostly occurs through programmed necrosis (Ying 2013). In addition to its function as an intermediary for DNA repair, PARP-1 also controls transcription, inflammatory responses, gene expression and cell death (Rajawat, Shukla and Mishra 2017). PARP-1-mediated cell death resembles apoptosis in many respects, and the apoptosis-inducing factor (AIF) from mitochondrial AIF-1 will therefore inevitably migrate to the nuclear compartment (Alano, Ying and Swanson 2004). Cell death triggered through PARP-1 activation is a key mechanism involved in AIF-mediated cell death, in which AIF is essential to the cytotoxic death pathway (Yu et al. 2002). Overactivation of PARP-1 can deplete available nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP) energy stores after severe, widespread DNA damage, which leads to nuclear AIF translocation and facilitates non-Caspase-dependent apoptosis (Arruri et al. 2021; Koh, Dawson and Dawson 2005). PARP-1 activity increases dramatically within a minute when exposed to ionising radiation, and repairs of DNA damage may occur much more slowly when PARP-1 activity is inhibited (Ryabokon, Cieslar-Pobuda and Rzeszowska-Wolny 2009). However, PARP-1 overactivation causes substantial fragmentation of chromatin DNA. It initiates parthanatos, an inherent PARP-1-dependent process of cell death that impacts several organ systems and is closely associated with a variety of illnesses, both neurological and non-neurological (Wang et al. 2016). PARP-1 serves as an indicator of DNA damage (Ray Chaudhuri and Nussenzweig 2017; Wang, Luo and Wang 2019). Excessive activation of PARP-1 in response to severe DNA damage leads to ATP and NAD⁺ depletion, and eventually to cell death (Ponce et al. 2014; Regier et al. 2019). Activation of PARP-1 has been shown to lead to cell death via c-Jun NH₂-terminal kinase-initiated signalling cascades initiated by the mitogen-activated protein kinase (Gerace et al. 2014). PARP-1 is triggered in aging and neurodegenerative diseases, disrupting autophagy, neuroinflammation and mitochondrial malfunction (Narne et al. 2017). ROS-derived DNA damage directly activates PARP-1, which eventually contributes

to cell death (Cole and Perez-Polo 2002). PARP-1 activation triggers the production of numerous pro-inflammatory mediators, including high mobility group box 1 (HMGB1) protein and metalloproteinases. These data indicate that PARP-1 performs a unique function in proinflammatory molecules relocating to cells capable of potentially damaging DNA (Zong et al. 2004).

Caspase-3 is a crucial element of programmed cell death because it is responsible for deactivating proteins that prevent healthy cells undergoing apoptosis (Comim et al. 2013). Caspase-3 activation following traumatic brain injury plays a role in loss of brain tissue and subsequent biochemical processes involved in programmed cell death (Clark et al. 2000). Microwave radiation may cause nerve cell apoptosis because elevated Caspase-3 indicates pre-apoptotic activation of the Caspase-dependent mitochondrial (intrinsic) pathway (Zuo et al. 2014). Caspase-3 activity is required for the majority of morphological and biochemical alterations related to apoptosis, and Caspase-3 cleavage is typically regarded as a universal hallmark of apoptosis (Poreba et al. 2013). Little is currently known about PARP-1 and Caspase-3 in the hippocampal region, and no information is available on PARP-1 and Caspase-3 expression or function in pyramidal and granular cells under the harmful effects of EMF exposure.

So far as we are aware, there has been no specific investigation of the reaction of PARP-1 and Caspase-3 activation in the hippocampus of rats exposed to 900 MHz during the gestational period. The fact that the rats were exposed to 900 MHz at different stages during pregnancy represents an original aspect of this study. The activity and levels of PARP-1 and Caspase-3 in exposed pyramidal and granular neurons of the hippocampus were examined during different stages of rat pregnancy in order to examine the interaction of these enzymes with neuronal damage caused by exposure to EMF.

2 | Material and Methods

The Kastamonu University Animal Experiments Local Ethics Committee, approved the experimental procedures (32/21.09.2023). Four female Wistar albino rats (10 weeks old, weighing 250 ± 50 g) were left to mate with male rats in separate cages under suitable conditions for 1 day for each study group. Vaginal smear samples were taken on the following day. Animals whose samples contained sperm were considered to be on day 0 of pregnancy and were placed in separate cages. Sixteen pregnant rats were randomly divided into the following groups, with four rats in each group:

Control Group: To obtain and compare baseline values with the experimental groups.

GD (1–7) Group: These rats were exposed to a 900-MHz EMF for 2 h a day for 7 days (GD 1–7) from the beginning of pregnancy.

GD (8–14) Group: These rats were exposed to a 900-MHz EMF for 2 h a day between days 8 and 14 of pregnancy (GD 8–14).

GD (15–21) Group: These rats were exposed to a 900-MHz EMF for 2 hours a day between days 15 and 21 of pregnancy (GD 15–21).

The experiment was terminated at 28 days after birth in order to examine the brain tissues of the female offspring. For this purpose, eight pups ($n=8$) from each group were determined according to power analysis and their brains were dissected.

2.1 | EMF Exposure System

All groups were exposed to a 900-MHz EMF at the same time every day, for 2 h a day, using a cage mechanism consisting of 21 special compartments. The cage mechanism includes a signal generator with a 0–9-W output emitting 900-MHz radio frequency waves, and a dipole antenna capable of collecting radio frequency waves in a single source. An EMF generator (Microwave Test Transmitter, Set Electronic Ltd, Türkiye) operating at 900–1800 MHz and with a 1–2-W output (PW = pulse wave) was used as the EMF source. The signal generator was operated at 2-W power. The power density in the near field of the monopole antenna was measured to precise values using an electric field probe (Extech RF EMF strength meter) throughout the experiment. EMF measurements were recorded every 10 min, starting from minute zero. In addition, before the system was started, the EMF in the environment was measured with the help of an EMF meter.

2.2 | Routine Histological Procedures

Offspring rats from all groups aged 28 days were sacrificed under anaesthesia via the intraperitoneal injection of a ketamine (50 mg/kg)/xylazine (10 mg/kg) mixture. The brain tissues were dissected and fixed in 10% neutral buffered formalin for 3 days, washed in running water, dehydrated in gradient alcohol and finally rendered transparent in xylene. They were then kept in paraffin at 58°C. The embedding process was then performed. Briefly, 5- μ m sections to cover the hippocampal region were collected from the paraffin-embedded brain tissue for histopathological and immunohistochemical analyses. For the histopathological analysis, the sections were stained with cresyl violet (Yurt, Kaplan and Kivrak 2018), while immunohistochemical analysis involved sections stained with anti-PARP-1 and Caspase-3 antibodies. Analyses were performed blindly by capturing images of tissue sections from all study groups at 40 $\times$ magnification using a camera-equipped research microscope (Zeiss Axiolab 5, Jena, Germany).

2.3 | Immunohistochemistry Procedure

Serial sections 5 μ m in thickness taken from paraffin blocks prepared from brain tissues were placed onto poly-lysine slides and stained in compliance with the Mouse and Rabbit Specific HRP/AEC IHC Detection Kit-Micro polymer (ab236467) kit procedure. The antigen retrieval process commenced once the sections had been deparaffinised in xylols and dehydrated in alcohol series. For antigen retrieval, the slides were immersed in a 10-fold diluted citrate buffer (Citrate Buffer Heat-Induced Epitope Retrieval pH: 6 Thermo Scientific:AP-9003-999) solution and heated in a microwave oven at 800 W for 20 min. After adding hydrogen peroxide to the sections, they were left to cool for 20–30 min. These were next washed in a phosphate-buffered saline (PBS, Sigma:

P4417) solution for 15 min prior to incubation in a 3% hydrogen peroxide (Merck: 108597) solution in the dark for 20 min to block endogenous peroxidase activity (Tufekci and Tatar 2023).

When the incubation was completed, the sections were once again washed with a PBS solution for 15 min and then treated with a 0.2% Triton X 100-PBS solution for 15 min to help the primary antibody pass through the cell membrane. A protein block solution was dropped onto them and left at room temperature for 10 min before washing with PBS. They were then treated with anti-PARP-1 (1:200; catalogue no. 13371-1-AP, Proteintech, RRID: AB_2160459) and anti-Caspase-3 (1:100; catalogue no. 19677-1-AP, Proteintech, RRID: AB_10733244) primary antibodies at +4°C overnight. After primary antibody incubation, goat anti-rabbit HRP-conjugate was added to the sections washed with PBS for 15 min and incubated for 15 min. Repeat washing was then performed, and AEC chromogen was applied. Sections counterstained with Gill's haematoxylin were covered with a water-compatible covering medium and studied under a microscope. PBS was used rather than a primary antibody after protein blocking in the negative control (Tatar and Tufekci 2024). The sections were examined under a light microscope, and photographs were taken.

2.4 | Semiquantitative Scoring Method

The prepared slides were examined using $\times 40$ magnification lenses under a light microscope. A dark red precipitate was interpreted as representing a positive reaction. Staining intensities in sections from all experimental animals were scored in a semiquantitative manner (Tufekci and Tatar 2023). The intensity of immunohistochemical staining as scored as follows: (–), no staining; (+), weak; (++) , medium and (+++) strong. Scoring was applied to the CA1, CA2, CA3 and DG regions of the hippocampus (Tufekci, Bakirhan and Terzi 2023). Average scores were determined for the intensities of immune reactions observed in the cells. Histo scoring (H-scores), representing a semiquantitative immunohistochemical method of calculation, involving staining intensity and percentages, was also conducted. H-scores were calculated by multiplying the staining intensity by the percentage of positive cells (Tufekci and Tatar 2023).

2.5 | Statistical Analysis

The IBM SPSS 25.0 software was used for all group comparisons. The ANOVA test (post hoc Tukey test) was applied in the analysis of normally distributed data, while the Kruskal–Wallis test (post hoc Tamhane test) was used for non-normally distributed data. The results are given as mean $\pm$ SD. A p -value of ≤ 0.05 was considered significant.

3 | Results

3.1 | Cresyl Violet Staining Results

Cresyl violet staining in the control group revealed intact pyramidal and granular neurons in the CA and DG regions. The pyramidal neuron nuclei appeared euchromatic, and the nuclear

membrane was well preserved. In the light microscopic images of the GD 1–7 EMF exposure group, most pyramidal neurons in the CA region appeared normal, although a small number of neurons were stained darkly, and the cells were shrunken. The granular, subgranular and hilus cells in the DG of this group exhibited a normal structure.

Numerous dark-stained, shrunken pyramidal neurons were observed in the CA region of the group exposed to EMF on GD 8–14. Subgranular cells in the DG of this group were also dark-stained and had lost their normal morphology. Most granular cells were normal.

In the GD 15–21 EMF-exposed group, we observed numerous dark-stained, shrunken pyramidal neurons as well as healthy cells in the CA region. The subgranular cells in the DG of this group were stained strongly dark and had lost their normal shape. Most of the granular cells in the GD 15–21 group had a normal structure (Figure 1).

3.1.1 | PARP-1 Immunohistochemistry Staining Results

A small number of neurons in the CA and DG regions of the hippocampus from the control group sections exhibited a weak PARP-1 immunoreactivity. A small number of strongly stained pyramidal cells were also observed in the CA region of the group exposed to EMF on GD 1–7. Granular neurons in the DG of this group exhibited weak staining. Numerous pyramidal neurons in the CA region of the group exposed to EMF on GD 8–14 exhibited moderate PARP-1 immunoreactivity. This group possessed strongly stained cells in the granular and polymorphic cell layers of the DG. Strongly positive stained cells were found in the CA region of the group exposed to EMF on GD 15–21. Strongly positive stained neurons were also found in the DG, especially in the subgranular region and hilus (Figure 2).

3.1.2 | PARP-1 Immunohistochemistry H-Scores

Statistical analysis of the H-scores obtained from PARP-1 immunostaining in the hippocampal sections of the different study groups revealed that the PARP-1 H-scores in the hippocampi of rats exposed to EMF on GD 8–14 and GD 15–21 were significantly higher than in the control and GD (1–7) EMF exposure groups ($p \leq 0.01$) (Figure 3).

3.1.3 | Caspase-3 Immunohistochemistry Staining Results

Anti-Caspase-3 immunohistochemical staining of hippocampal sections from the control group revealed a weak Caspase-3 immunoreactivity in cells of the pyramidal neurons in the CA regions. Granular cells in the granular layer of the control group DG exhibited no Caspase-3 immunoreactivity, while a few number of neurons in the molecular layer exhibited a weak immune reactivity. In the GD 1–7 EMF exposure group, pyramidal neurons in the CA region and neurons in the subgranular region of the DG exhibited weak Caspase-3 immune reactivity. A few number of pyramidal neurons in the CA region of the group

exposed to EMF on GD 8–14 exhibited strong immune reactivity. Strong immunoreactivity was also observed in the subgranular cells of the DG in this group. In the group exposed to EMF on GD 15–21, strong diffuse Caspase-3 immune reactivity was seen in most CA cells and the neurons of the subgranular region of the DG (Figure 4).

3.1.4 | H-Score of Caspase-3 Immunohistochemistry

Statistical analysis of the H-scores obtained from Caspase-3 immunostaining in the hippocampal sections from the groups revealed that the Caspase-3 H-score in both the CA region of the hippocampus and the DG region of the GD (15–21) group was higher than in the control, GD (1–7) and GD (8–14) groups ($p \leq 0.01$) (Figure 5). In addition, the H-score for the CA region of the GD 8–14 group was higher than those in the control and GD 1–7 groups, while the score for the DG region was only higher than that of the control group ($p \leq 0.01$) (Figure 5).

4 | Discussion

This study intended to evaluate the expression of the PARP-1 and Caspase-3 in the pyramidal and granular neurons in the hippocampus of rats exposed to 900 MHz at different periods of pregnancy. Darkly stained and shrunken pyramidal neurons were observed in the exposed groups. Most granular cells were normal in morphology, while the subgranular cells were dark-stained and impaired shaped. The study revealed strong, positively stained immunoreactivity of PARP-1 and Caspase-3 in the CA area and subgranular cells of the DG in the group exposed to EMF on GD 15–21. The PARP-1, Caspase-3 and H-score in the hippocampus of rats exposed to EMF on GD 15–21 were notably higher than those in the control group.

A previous study reported that microarray analysis revealed that the systemic inflammatory response exhibits a substantial effect on the expression of numerous genes in the hippocampus. That research found that 262 genes exhibited substantially different expression levels from the control mice in 12 h after lipopolysaccharide administration. Variations in the expression of the PARP family genes were disclosed by microarray analysis. The mouse hippocampus contains PARP-1 transcripts (Czapski et al. 2013). The hippocampal sections were exposed to varying doses of (N-methyl-N'-nitro-N'-nitrosoguanidine (MNNG) for different periods, and the hippocampal sections exhibited dose-dependent neurotoxicity caused by MNNG. Preferential degradation of the CA1 subfield was detected after 5-min incubation with MNNG at 100 μ M. As determined by morphological parameters, the amount of damaged CA1 pyramidal cells exhibited a linear correlation with CA1 propidium iodide (PI) fluorescence. The initial effects of MNNG exposure included a sharp drop in NAD⁺ and ATP concentrations and a rapid onset of PAR production. The experiment showed that excessive PARP-1 activation is the mediating factor for the MNNG-triggered specific toxicity in CA1 pyramidal cells (Gerace et al. 2014). Particulate matter (PM) has been reported to activate PARP-1, raising β -amyloid levels, and activate glial cells in mouse ex vivo hippocampal cells. These results point to the role of PARP-1 in mediating

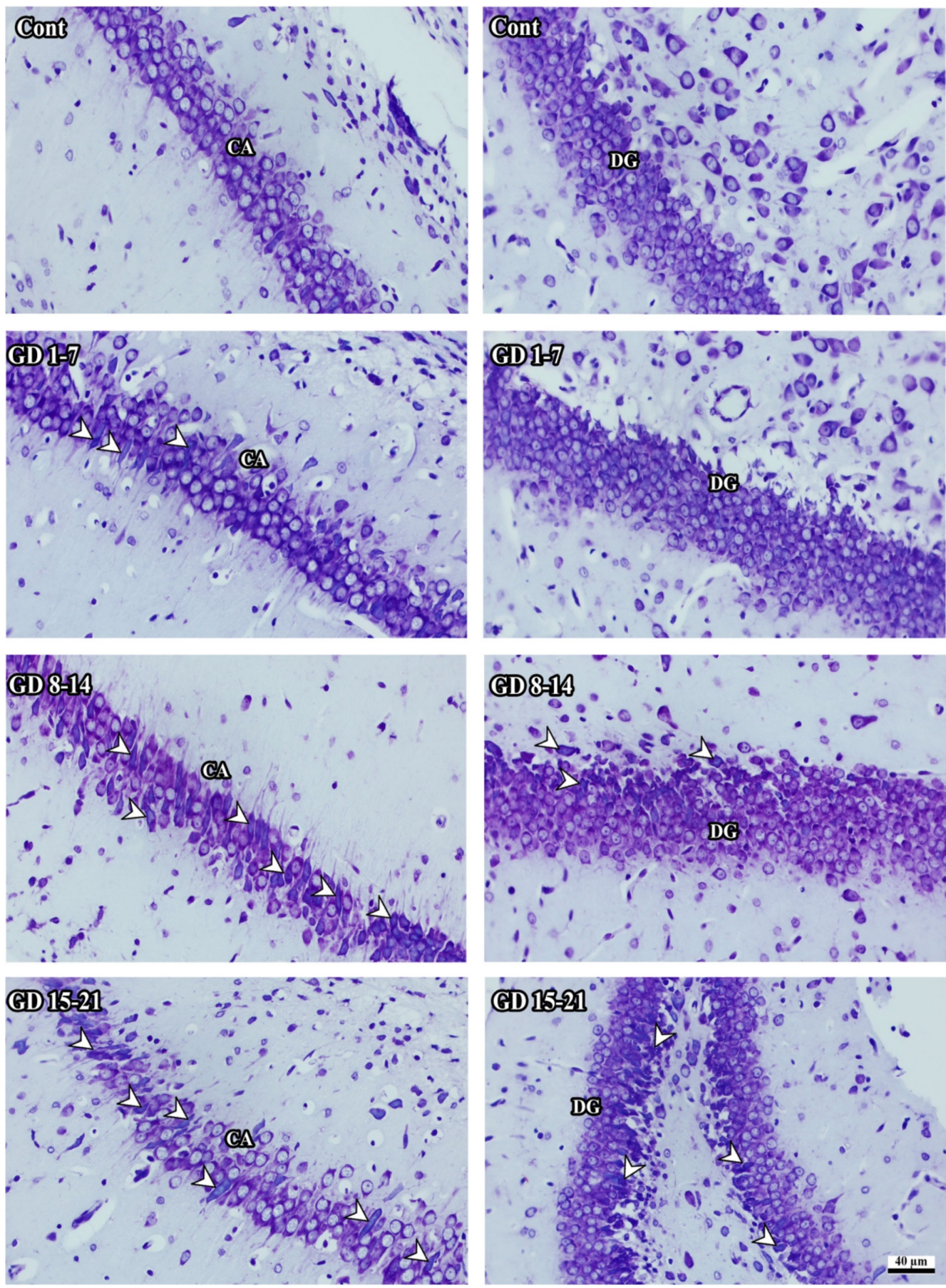


FIGURE 1 | Legend on next page.

FIGURE 1 | Light microscopic images of the cresyl violet staining of the hippocampus. In the control group (Cont), the pyramidal and granular neuron cell membranes, nuclei and nucleolus structures were all intact. A small number of dark-stained and shrunken pyramidal neurons (arrow-heads) were observed in the GD 1–7 group, whereas the neurons in the DG region seen normal. In the GD 8–14 group, the majority of the pyramidal neurons in the CA region were dark-stained, and their cytoplasmic integrity was impaired. The subgranular neurons in the DG from this group similarly lost their normal structure. In the GD 15–21 group, numerous dark-stained neurons that had lost their normal structures were observed in both the CA region and the subgranular region of the DG.

an association between PM exposure and an elevated risk of Alzheimer's disease (Jang et al. 2018). Another study investigated PARP-1 activation under oxidative stress by detecting PAR production. PARP-1 is activated in reaction to oxidative stress generated by hydrogen peroxide (H_2O_2) and is involved in autophagy and cell death. H_2O_2 rapidly causes the production of large PAR polymers, and PARP-1 activation may trigger autophagy during the response to oxidative stress. In order to determine whether PARP-1 activation contributes to H_2O_2 -generated cell autophagy, gene silencing technology was used to inhibit PARP-1 activity to observe any changes in PARP-1 after H_2O_2 treatment. PAR production was found to be significantly raised by H_2O_2 treatment relative to an untreated group (Meng et al. 2018). Another study investigated PARP-1 activity using PAR immunohistochemistry to elucidate how cellular reactions to status epilepticus are correlated with PARP-1 activity. PAR positivity was rare in the piriform cortex and hippocampal regions in non-epileptic animals. Granular cells in the dentate gyrus and CA1–3 neurons exhibited PAR positivity in 12 h after status epilepticus. Conversely, only a few neurons in the piriform cortex exhibited PAR positivity. PAR immunoreactivity decreased gradually in CA1–CA3 neurons, granular cells and piriform cortical neurons from 1 day to 4 weeks after status epilepticus. Specific astrocytes in the CA1 region stratum radiatum exhibited signs of PAR induction. These findings appear to indicate that PARP-1 activation may include CA1-reactive astrocytes and neuronal death in the hippocampus following status epilepticus (J. E. Kim et al. 2014).

Human neurons in the core area of ischemic infarcts have been observed to exhibit PARP-1 activation and PAR generation, mainly in the first 18 to 24 h. A small amount of PAR also infiltrates macrophages and glia in the surrounding area after 24 h (Love, Barber and Wilcock 2000). On the other hand, excessive PARP-1 activation results in a cell death mechanism known as parthanatos, a novel kind of programmed necrosis distinct from necrosis and apoptosis (Wang et al. 2016, 2011). The important characteristics of parthanatos include nuclear shrinkage, chromatin condensation and massive DNA fragmentations ranging from 15 to 50 kb. Parthanatos affects a number of different organ systems and plays a vital role in the pathological processes of various neurological and non-neurological diseases, such as ROS-derived injury, neurodegenerative diseases, myocardial infarction, ischemia-reperfusion injury following stroke, glutamate-excitotoxicity and inflammatory injury (Wang et al. 2016; Wang, Dawson and Dawson 2009; Wang et al. 2011).

A previous research exposed to the rat astrocyte to a 1950-MHz EMF for 12, 24 and 48 h. Following a 48-h exposure, the authors reported a substantial rise of Caspase-3 protein expression in astrocytes. However, no changes in Caspase-3 protein expression

were determined between the experimental and control groups after 12- or 24-h exposure (Liu et al. 2012). In another study, chronic stress was shown to induce neuronal death in experimental animals by downregulation of neurotrophic factors such as brain-derived neurotrophic factor (BDNF) and upregulating Caspase activity by raising Caspase-3 activity or lowering Bcl-2 activity. The hippocampi of chronically mildly stressed mice contained more Caspase-3 and Bax-positive cells than those of the control mice (Dionisie et al. 2021).

The Wistar albino rats were exposed to EMF emitted from a cell phone for 2 h each day for 45 days. Caspase-3 and creatine kinase levels in the brain were notably higher in the EMF group than in the sham-exposure group (Kesari, Kumar and Behari 2011). Research using Sprague–Dawley rats subjected to a 900-MHz radio frequency EMF (RF-EMF) 24 h a day for 20 days revealed high levels of Caspase-3 expression in Purkinje cells and granular cells of the cerebellum exposed to EMF. Cerebellar tissue from the EMF group contained significantly high numbers of Caspase-3-positive cells than the control group (Mercantepe et al. 2018). A study on the mouse hippocampus and examining the neurodegeneration that was caused by kainic acid (KA) showed that Caspase-3 was activated in glial cells and neurons between the first and third days after KA injection. Additionally, Caspase-3 was activated on the pyramidal neuron cell bodies in CA1 and CA3 regions. On the third day after KA injection into the DG, activated Caspase-3 was observed in the hilar interneurons. In the DG subgranular zone (SGZ), active Caspase-3 was detected in radial glial cells and astrocytes on days 5 to 7 following KA injection (Tzeng et al. 2013). In a study of pregnant Sprague–Dawley rats involving the ventral cochlear nucleus, the animals were exposed to a 900-MHz EMF daily throughout pregnancy. The EMF exposed groups exhibited elevated Caspase-3 activity levels in the postnatal period (Tumkaya et al. 2022).

In another study, Wistar albino rats were exposed to a 1800-MHz RF-EMF for 2 h a day for 8 weeks. Eye tissues were examined at the end of that period. A high Caspase-3 expression levels were determined in the rat eyes from the exposed group (Eker et al. 2018).

The mentioned research findings led us to identify several factors capable of inducing the expression of PARP-1 and Caspase-3. Our findings demonstrate that the activation and expression of these enzymes by a 900 MHz EMF increase their activity in the hippocampus, ultimately leading to the death of hippocampal neurons.

It is well known that extremely low-frequency EMFs can increase the generation of free radicals, particularly hydroxyl free radicals, capable of inducing the destruction of DNA double strands (Consales et al. 2012). DNA has been the subject of particular

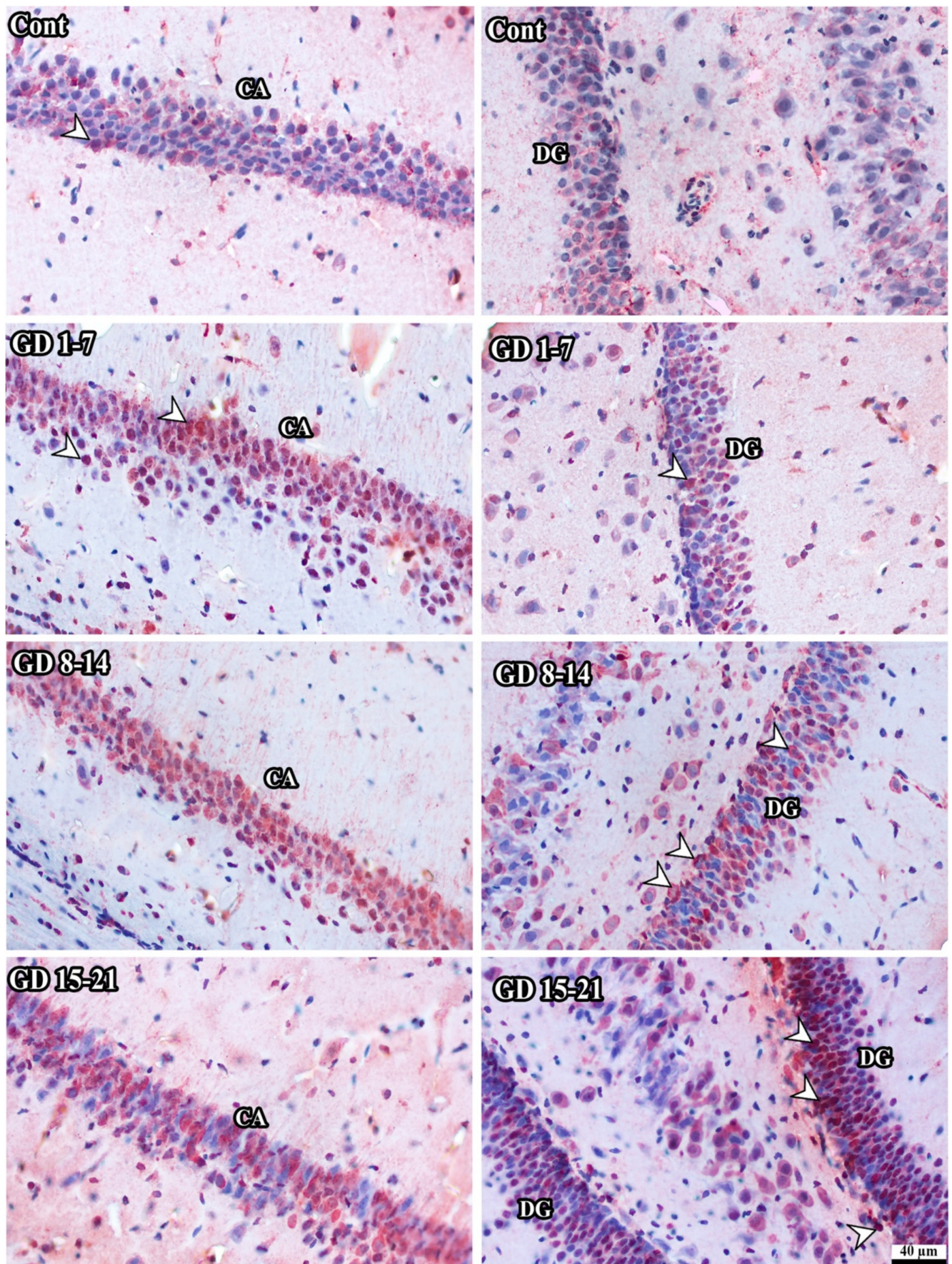


FIGURE 2 | Legend on next page.

FIGURE 2 | Anti-PARP-1 positive stained cells (arrowhead) in the cornu ammonis (CA) and dentate gyrus (DG) regions of the hippocampus from all groups. In the control group, weakly positive neurons were observed in the CA region and DG granular layer. Strong positive staining was found in CA region pyramidal neurons in all groups exposed to EMF. In the group exposed to EMF on GD 15–21, strong immunoreactivity was seen in the subgranular region and polymorphic neurons in the hilus.

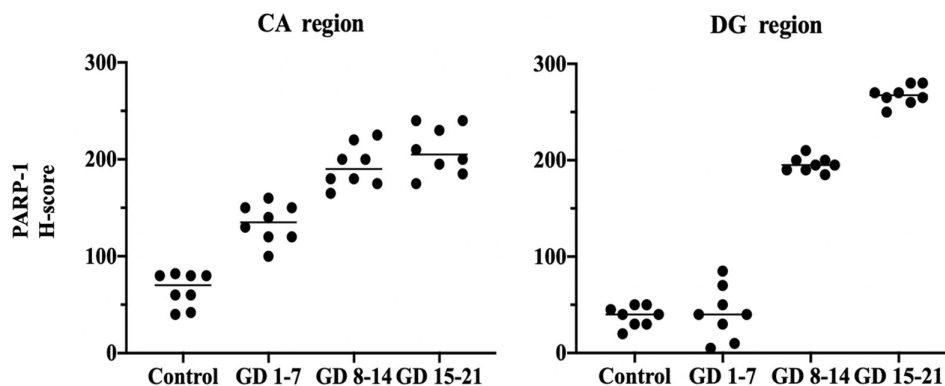


FIGURE 3 | Statistical analysis of PARP-1 immunostaining H-scores. CA, cornu ammonis; DG, dentate gyrus. Values are expressed as mean ± SD. ($n = 8$).

attention in terms of the harm that EMFs may cause to various molecules because of its importance in cell cancer, mutations, function, survival and proliferation (Franzellitti et al. 2010). In a previous study, immature (2 week-old) and mature (10 week-old) Wistar albino rats were exposed to a 1800-MHz RF-EMF for 2 h a day for 45 days. Analysis of bone marrow cells retrieved from both mature and immature rats demonstrated pronounced cytogenotoxic damage in both exposed groups. Cells from the immature exhibited irreversible damage during the 15-day recovery period in comparison to cells from the mature animals (Sekeroglu, Akar and Sekeroglu 2012).

In another study, adult Sprague Dawley rats were randomly assigned to three groups exposed to a 900-, 1800- and 2100-MHz radiofrequency radiation (RFR) for 2 h a day for 6 months. An analysis of brain tissue sections revealed that the exposed groups exhibited high levels of DNA damage and oxidative stress than the sham-control group. DNA damage to single strands was observed in the group subjected to 2100 MHz RFR (Alkis et al. 2019).

Specific findings of the present study is that a pronounced effect of EMF exposure on the hippocampus of the GD 15–21 was seen. In addition to dark-stained and shrunken pyramidal neurons, normal pyramidal neurons were also seen in the CA region of the hippocampus. However, granular cells in the GD 15–21 group were normal in morphology. Conversely, the subgranular cells of the GD group 15–21 were lost their normal architecture and were darkly stained. These results suggest that the late stages of pregnancy are especially susceptible to the deleterious effects of EMF on hippocampal neurons, because of formation of neurons in this brain region are seen peaking at GD 14–20 (Rice and Barone 2000) and postnatal 15 days (Altman and Das 1965). This is supported by the observation that the group exposed to EMF on GD 15–21 exhibited higher PARP-1 and Caspase-3 H-scores in CA and DG. Additionally, the group exposed to EMF on GD 15–21 exhibited strongly positive immunoreactivity-stained

cells in the CA and DG, particularly in the subgranular region and hilus.

Exposure to EMFs during the formative phases of the nervous system may have a particularly significant impact on development. Previous research subjected pregnant Wistar albino rats to 2.4-GHz EMF radiation in order to track the pups' physical development and BDNF levels using anthropometric measurements and a BDNF ELISA kit, respectively. The results showed that EMF exposure inhibited body development. It also reduced BDNF levels in the group exposed to EMF, suggesting that EMF exposure during pregnancy may affect BDNF levels and delay body growth in rat offspring (DastAmooz, Broujeni and Sarahian 2023). Another study evaluated the impact of RF-EMF on synapse formation and neurological development in mice exposed to a 1850-MHz RF-EMF for 5 h per day over 4 weeks during the early postnatal period. Dendritic spines (DSs) originating from neuron neurites were analysed in hippocampal sections. The findings revealed a significant diminution in the total number of DSs in the DG, but not in CA1. Further examination of DS subtypes revealed that while the number of the thin and stubby types remained unchanged, statistically significant decreases in CA1 and DG were noted in the mushroom type (J. H. Kim et al. 2021).

Exposure to a 900-MHz EMF is capable of altering the morphological appearance of cell groups affected by it. Previous research involving the hippocampus of Sprague–Dawley rats exposed to a 900-MHz EMF for 1 h throughout adolescence reported that the neuron cytoplasm from the exposed group appeared dark-stained with numerous pyknotic neurons (Kerimoglu et al. 2016). In another study, pregnant Wistar albino rats were exposed to a 900-MHz EMF for 2 h daily throughout pregnancy. Histological analysis revealed dark-stained granule cells in the offspring of EMF exposed group rat. These findings suggested that a 900-MHz EMF exposure during pregnancy may have acted as an impediment barrier to DG neurogenesis

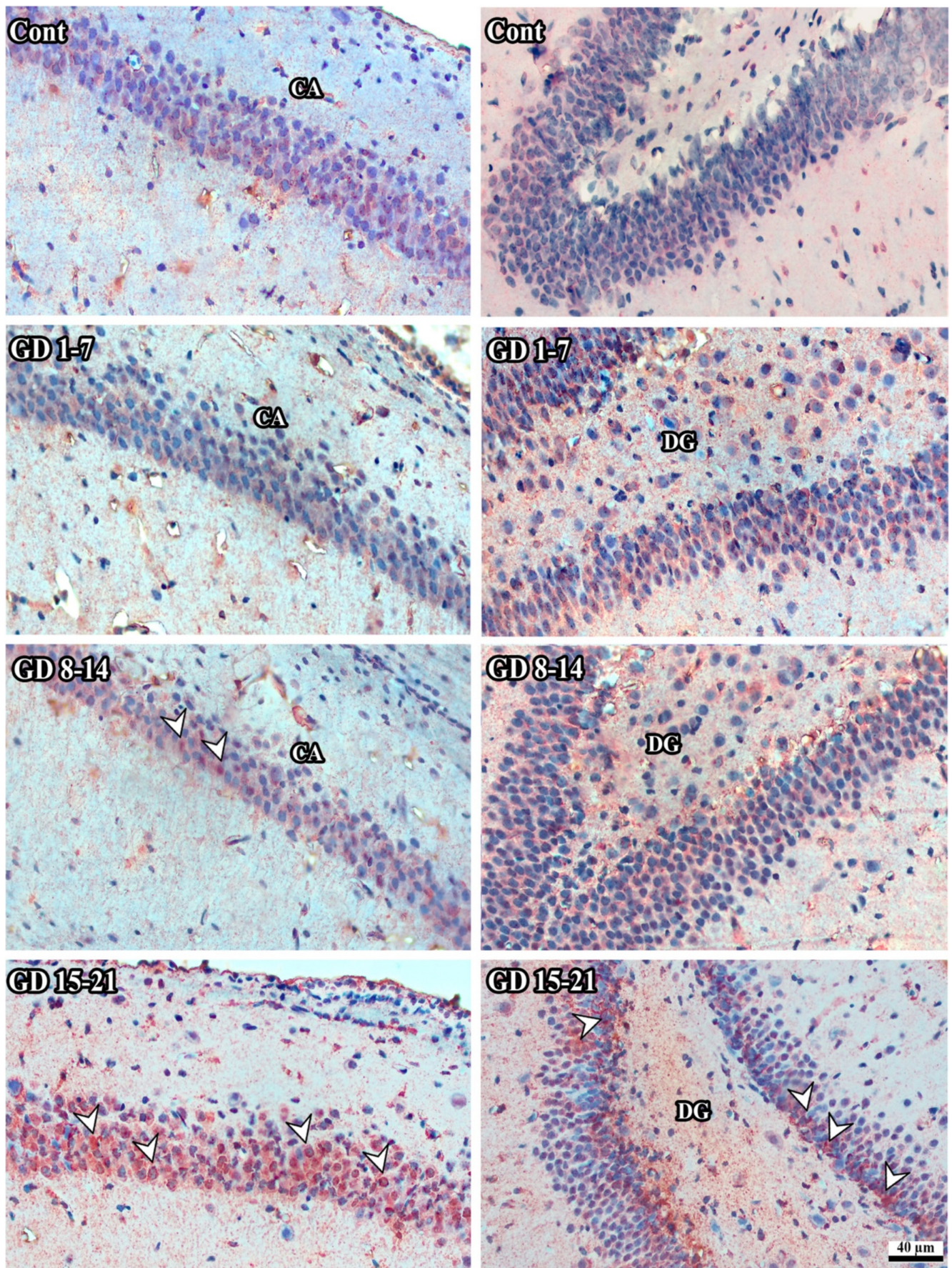


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FIGURE 4 | Anti-Caspase-3 positive-stained cells (arrowhead) in the cornu ammonis (CA) and dentate gyrus (DG) regions of the hippocampus from all groups. In the control group, weakly positive stained neurons were observed in the CA region and DG granular layer. A strong positive staining was seen in pyramidal neurons of CA region and in the subgranular region and polymorphic neurons of the hilus in the GD 8–14 and GD 15–21 EMF exposure groups.

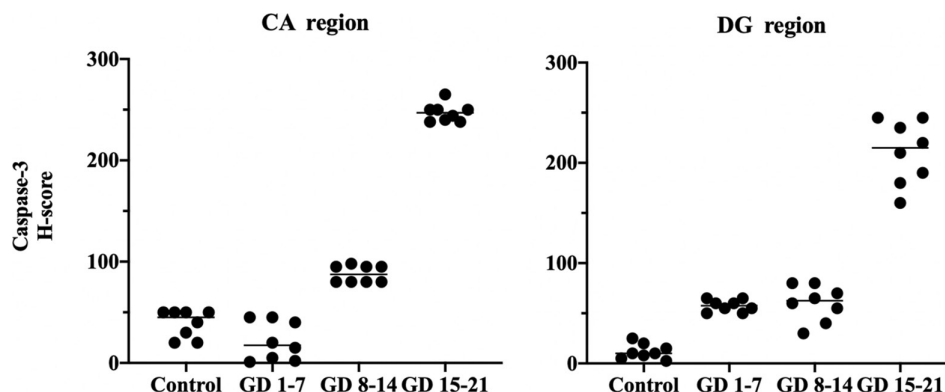


FIGURE 5 | Statistical analysis of Caspase-3 immunostaining H-scores. CA, cornu ammonis; DG, dentate gyrus. Results were expressed as mean $\pm$ SD. ($n = 8$).

and granule cell development (Odaci, Bas and Kaplan 2008). In other research on the Wistar albino rats exposed to a 900-MHz EMF for 1 h a day over 4 weeks, light microscopic evaluation showed an indistinct cell boundary and dark blue-stained neurons in pyknotic neurons from the EMF exposed group (Elamin, Deniz and Kaplan 2022). In another study, Sprague–Dawley rats were exposed to a 900-MHz EMF for 1 h a day for 25 days. Histopathological analysis showed artefacts and excessively dark-stained pyramidal and granule cells in the hippocampi from that group (Keles, Nyengaard and Odaci 2019).

In another study, Sprague–Dawley rats were exposed to a 900-MHz RF-EMF for 24 h a day for 20 days. Pyknotic nuclei and a significant decrease of cytoplasmic components were observed in granular and Purkinje cells. Decreased granular, Purkinje and molecular cell layer thicknesses were determined in the EMF exposed group compared with the control group. Cerebellar tissue from the EMF group contained significantly more Caspase-3-positive cells than the control group (Mercantepe et al. 2018). In another study, rat astrocytes were exposed to a 1950-MHz EMF for 12, 24 and 48 h. Astrocytes were observed to exhibit considerable induction of apoptosis after 48-h exposure. The percentage of apoptotic cells increased to approximately 50%, exceeding that of the other groups more than three-fold. Morphological analysis showed that numerous cells exhibited varying degrees of degeneration and altered lengths and thicknesses after a 48-h exposure. No discernible difference was found in these cells' morphology compared with the control group following a 12- or 24-h exposure (Liu et al. 2012). The results of these studies are compatible with the findings of the current research in terms of histopathological analysis of GD (15–21), the abundance of dark-stained and shrunken pyramidal neurons and the abnormal architecture of subgranular cells observed. The number of studies that have addressed on the effects of EMFs exposure on PARP-1 and Catalase-3 enzymes is limited. This research may therefore be considered a high value in terms of investigating the effect of EMF exposure on these enzymes.

In conclusion, exposure to a 900-MHz EMF for 2 h daily during pregnancy resulted in a notable induction of neuronal death in hippocampal pyramidal and granular cells. This was especially pronounced in the late stage of gestation, as demonstrated by elevated Caspase and PARP-1 activation expression. In the hippocampal regions, apoptotic pyramidal and granular cells were indicated by high levels of Caspase-3 production. Degenerating neurons are pyknotic with dark-stained cytoplasmic contents. These findings suggest that PARP-1 and Caspase-3 detection accurately indicates the death of pyramidal and granular cells. Further research are needed to elucidate the mechanism of hippocampal neuron loss caused by a 900-MHz EMF exposure and to examine nucleus components, including DNA.

This study has several limitations that should be considered when interpreting the results. First, the duration and frequency of the 900-MHz EMF exposure were standardised at 2 h daily, which may not accurately reflect real-world exposure patterns. Longer or varied exposure durations may yield different outcomes. Second, the study was conducted in a controlled animal model, and caution must be exercised when extrapolating these findings to humans, as the physiological and neurological responses to EMF may differ across species. Additionally, while PARP-1 and Caspase-3 activation were used as markers for neuronal death, other pathways of neurodegeneration were not explored, which may play a role in hippocampal damage. Lastly, the study did not investigate long-term behavioural or cognitive outcomes resulting from hippocampal neuronal death, which would provide further insight into the functional consequences of the observed structural changes.

Future research should aim to elucidate the underlying mechanisms of EMF-induced neurodegeneration. This includes exploring whether oxidative stress, mitochondrial dysfunction or disruptions in DNA repair pathways contribute to neuronal death, alongside Caspase and PARP-1 activation. Investigating

the protective potential of neuroprotective agents, such as antioxidants or inhibitors of the apoptotic pathway, may offer therapeutic strategies to mitigate EMF-induced damage during critical stages of brain development.

Finally, studies exploring the cumulative and dose-dependent effects of EMF exposure, combined with other environmental factors, will help in better understanding the risk profile for neuronal damage and provide a clearer framework for establishing safety guidelines for EMF exposure, particularly in pregnant individuals.

Author Contributions

Conceptualisation, methodology: K.K.T.; experimental procedures: K.K.T, M.T.; supervision: S.K.; writing – original draft: A.A.E.E.; writing – review and editing: A.A.E.E, K.K.T., S.K.

Ethics Statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Kastamonu University (32/21.09. 2023).

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data are available on request from the authors. The data that support the findings of this study are available from the corresponding author, upon request.

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