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Experimental Study

Effects of 900-MHz radiation on the hippocampus and cerebellum of adult rats and attenuation of such effects by folic acid and *Boswellia sacra*

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ABSTRACT

The radiation emitted from mobile phones has various deleterious effects on human health. This study was conducted to evaluate the effects of exposure to the 900-MHz radiation electromagnetic fields (EMF) emitted by mobile phones on Ammon's horn and the dentate gyrus (DG) in the hippocampus and cerebellum of male *Wistar* albino rats. We also investigated the neuroprotective effects of the antioxidants *Boswellia sacra* (BS) and folic acid (FA) against exposure to EMF. Twenty-four adult male rats were randomly divided into four groups of six animals each, an EMF group, an EMF + FA exposure group (EFA), an EMF + BS exposure group (EBS) and a control group (Cont). The EMF, EFA and EBS groups were exposed to 900-MHz EMF radiation inside a tube once daily over 21 days (60 min/day). The Cont group was not exposed to 900-MHz EMF. The results showed that EMF caused a significant decrease in total pyramidal and granular cell numbers in the hippocampus, and DG and in Purkinje cell numbers in the cerebellum in the EMF group compared to the other groups ($p < 0.05$). BS and FA attenuated the neurodegenerative effects of EMF in the hippocampus and cerebellum. Significant differences were also determined between the numbers of neurons in the EFA and EMF groups, and between the EBS and EMF groups ($p < 0.05$). However, there were no significant differences among Cont, EFA and EBS ($p > 0.05$). Our results may contribute to ongoing research into the effects of 900-MHz EMF exposure.

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1. Introduction

Technological devices now occupy a very important place in daily life. However, while making life easier, they may also cause a number of health problems [1–3], since electronic devices emit a surrounding electromagnetic field (EMF). This has been investigated in many previous studies, and EMF has been shown to have deleterious effects on various tissues in living organisms [4–7]. A

wide spectrum of electromagnetic waves is emitted from radar equipment, communication devices, mobile phone base stations, high voltage power lines, radio and television transmitters and substations, and particularly from electrical appliances at home and in the office, and other electrical systems [8]. Some studies have suggested that these devices have adverse effects on human health [9,10].

Numerous experimental and clinical studies have been performed in order to determine the effects of EMF on the central and peripheral nervous systems, and important findings have been obtained [11,12]. Mobile phone use has been shown to exacerbate headaches [1,5]. Insomnia and significant changes in electroencephalography (EEG) findings have been also reported [13–15]. Even low-frequency exposure significantly changes nervous system activity, and modifications may be observed in synaptic plasticity and neurotransmitter release, together with functional changes in hearing perception, balance, learning and memory [6,16].

Abbreviations: BS, *Boswellia sacra*; CA, cornu ammonis; CAT, catalase; CE, coefficient of error; CV, coefficient of variation; DG, dentate gyrus; DNA, deoxyribonucleic acid; EMF, electromagnetic field; EBS, the group that is exposed to EMF and received a single daily gavage of BS (500 mg/kg/day) during 21 days; EEG, electroencephalogram; EFA, the group that is exposed to EMF and received a single daily gavage of folic acid (50 mg/kg/day) during 21 days; FA, folic acid; gr, granular layer; H₂O₂, hydrogen peroxide; MHz, Megahertz; ml, molecular layer; RF, radiofrequency; ROS, reactive oxygen specimens; SEM, standard error of the mean.

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In the present study, we used folic acid (FA) and *Boswellia sacra* (BS) as antioxidants. FA is a water-soluble group B vitamin. Females planning a pregnancy are advised to take a daily dose of 0.4 mg FA for a month during gestation to prevent neural tube defects [17]. FA is particularly essential during pregnancy in order to stimulate infant brain development, since it is a basic substance necessary for the formation of new cells in the central nervous system. BS has also important effects in the prevention of deoxyribonucleic acid (DNA) damage, forgetfulness, memory loss and cancer [18].

The purpose of this study was to use stereological analysis to investigate the effects of BS and FA on the hippocampus and cerebellum of adult male rats exposed to 900-MHz EMF. Additionally, biochemical changes in oxidative stress parameters in blood samples were evaluated to support the stereological results.

There was not previous study of antioxidant effects of folic acid and especially *Boswellia sacra* on EMF exposure in literature and support of study results with biochemical analyses has given a different perspective to the study.

2. Material and methods

2.1. Animals and groups

Twenty four adult 12-week old male *Wistar albino* rats weighing 250–280 g were randomly divided into four groups; an EMF group, an EMF+FA exposure group (EFA), an EMF+BS exposure group (EBS) and a control group (Cont), each consisting of six rats. Animals were obtained from the Ondokuz Mayıs University Experimental Animals Surgical Research and Application Center, Turkey. The Ondokuz Mayıs University Animal Care and Ethics Committee, Samsun approved the experiments and procedures (date 28.08.2013, No. 2013/23). Rats were housed in standard transparent polycarbonate cage (Radon Comp., Ankara, Turkey) equipped with wood-shaving bedding at a temperature of 22 ± 1 °C under controlled lighting (12 h light/12 h dark cycle) and humidity ($50\% \pm 10$). Rats were allowed access to food and water ad libitum. The study groups were established as follows:

- 1 Cont:** This group was not exposed to EMF or any substances.
- 2. EMF:** This group was exposed to an EMF of 900 MHz for 60 min/day over 21 days. The exposure period was from 13:30 p.m. to 14:30 p.m. each day (Fig. 1).
- 3. EFA:** This group was exposed to EMF and received a single daily gavage of FA (50 mg/kg/day, for 21 days) during the experimental period, between 12:00 and 12:30 p.m.
- 4. EBS:** This group was exposed to EMF and received a single daily gavage of BS (500 mg/kg/day, for 21 days) during the experimental period, between 12:00 and 12:30 p.m.

2.2. EMF exposure

In our previous prenatal study, we used an exposure period of 20 days, since gestation in rats lasts 21 days. That study determined a substantial cell loss. The present study was performed in order to determine whether that period of exposure would cause side-effects in adult rats. The main basis for this experimental design was our previous study. An exclusive exposure device was produced; including a round plastic cage is divided into triangles with a diameter of 5.5 cm and a length of 12 cm [19]. EMF meter and a monopole antenna belonging to the exposure system [20]. The generator connected to a one-monopole antenna was used in this study and the rats were placed in the Plexiglas restrainer and at an equal distance from the monopole antenna (Fig. 1). We have used Exttech 480836 3.5 GHz RF EMF Strength Meter that has frequency range of 50 MHz to 3.5 GHz in the study. Exttech 480836 3.5 GHz RF EMF Strength Meter Exttech 480836 3.5 GHz RF EMF Strength Meter which has one

probe 900 MHz was used in the study A radiofrequency generator (SET ELEC. CO. 900/1800 Lab Test Transmitter, Model GHz 2011X, Istanbul, Turkey), emitting an EMF at a frequency of 900 MHz. A 900-MHz continuously modulated wave electromagnetic energy generator [CW=Continuous Wave and GSM pulsed wave signal (CDMA 217 Hz pulse)] was constructed at the Süleyman Demirel University Electromagnetic Compatibility Laboratory [20,21]. The power density measurements were performed using an EMF meter. The monopole antenna of the exposure system was inserted into the center of the round plastic cage to ensure an even distribution of electrical field. All rats were placed in close contact with the monopole antenna. In order to reduce the stress on these rats, an air hole, with a diameter of approximately 1 cm, was made in the plastic cage [20–22]. Rats were exposed to 900-MHz EMF for 60 min/day over 21 days between 13.30 p.m. and 14.30 p.m. The rats were positioned within 1 cm of each other, and the monopole antenna was placed in the center of the round plastic cage in a perpendicular position. The rats' heads were positioned facing the antenna, which was fixed within 1 cm of their heads. The long axis of the antenna was perpendicular to the long axis of the rats in order to ensure that all rats were equally exposed to EMF between the two ends of the antenna [23]. The power of the signal generator was fixed at 2 W during exposure. Additionally, the rats' positions were changed daily throughout the exposure period. In the many studies, similar systems have been used [23–26] (Fig. 1).

2.3. Tissue procedures and analysis

At the end of the 21st day, all rats were sacrificed by general anesthesia with intramuscular ketamine (10 mg/100 g body weight; Sigma Chemical Comp., St. Louis, MO, USA) and prilocaine hydrochloride (0.25 mg/100 g body weight; Sigma Chemical Comp., St. Louis, MO, USA). Brain and cerebellum tissues were removed for histological, stereological and biochemical analysis and were fixed in 10% formalin. Fresh tissue samples were used for biochemical study. For histomorphometric assessment, tissue samples were processed through graded alcohol (70% to 100%) (Sigma Chemical Comp., St. Louis, MO, USA), and xylene series (Sigma Chemical Comp., St. Louis, MO, USA), and embedded in paraffin (Merck, Darmstadt, Germany) for sectioning. Sections 20 μm in thickness in the sagittal plane were taken from the tissue blocks based on systematically random sampling strategies using a rotary microtome (Leica RM 2135, Leica Instruments, Nussloch, Germany). Each slide was stained with cresyl violet for light microscopic examination (Leica RM 2135, Leica Instruments, Nussloch, Germany) and stereological analysis. For analysis of the number of the granular cells in the dentate gyrus (DG), the counting frame size was 100 μm^2 and the sampling grid area was 7225 μm^2 . For analysis of the number of the pyramidal cells in the hippocampus, the counting frame was 1600 μm^2 and the sampling grid area was 22.500 μm^2 . Finally, from analysis of the number of Purkinje cells in the cerebellum, the counting frame was 1600 μm^2 and the grid sampling area was 22.500 μm^2 .

2.4. Stereological analysis

The numbers of pyramidal and granular neurons in the hippocampus and of cells in the cerebellum were estimated using the optical fractionator method. We used the Stereo Investigator Workstation system (Stereo Investigator 9.0 Micro Bright Field Inc., Colchester, VT, USA) for this purpose [27,28]. At the beginning of the analysis, we performed a pilot study to define the sampling strategy and counting schedule. A sampling ratio was first determined based on previous studies. The section series obtained from the groups were then systematically and randomly selected based on the ratio determined. A section-sampling fraction of 1/6 was

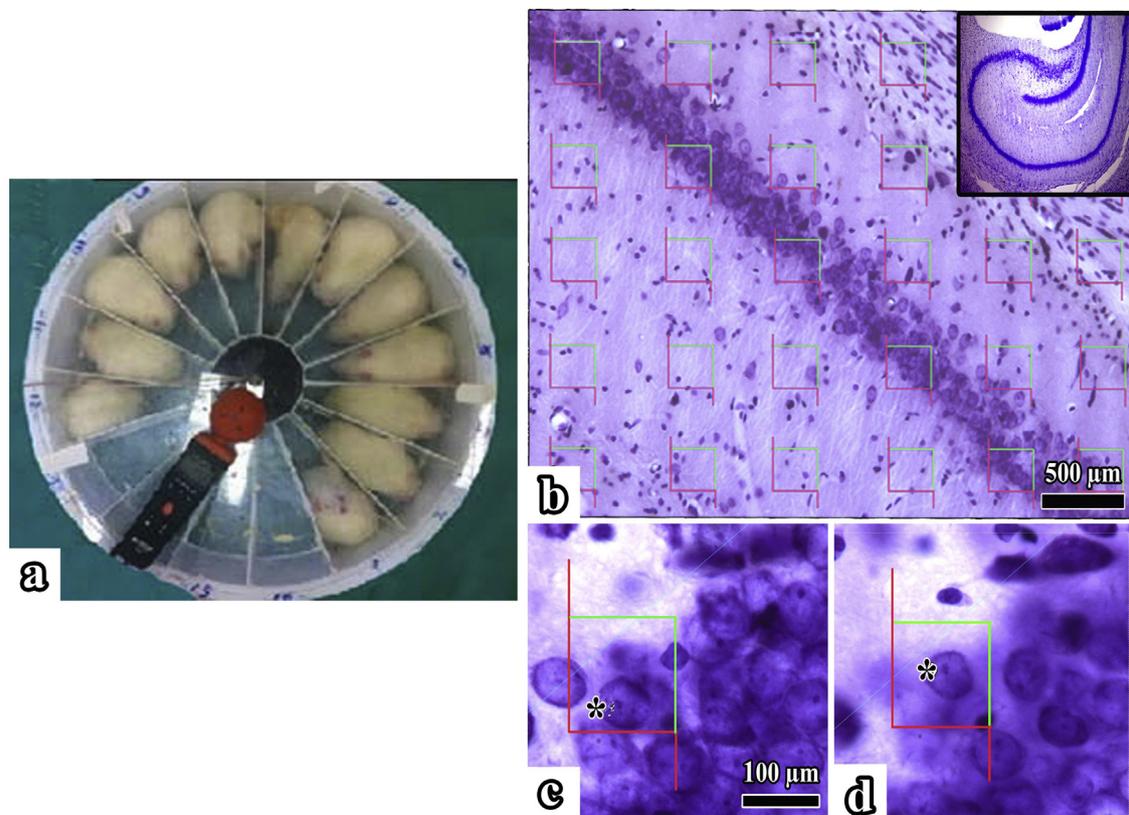


Fig. 1. In the image on the left (a), the application of 900-MHz EMF is shown. In the image on the right, the optical fractionator (b) and disector (c, d) is shown. The counting frame consists of including and excluding edges. Asterisks indicate countable cells in c and d.

determined. Every 6th section was taken from the section series, giving a 1/6 section-sampling fraction. Approximately 12–18 sections from each hippocampus and cerebellum are known to be sufficient for estimating total neuron numbers in rodents (i.e. rats) using the optical disector method [29]. The estimates of neuron number described here are based on optical disector samples made according to a “fractionator” sampling scheme [28]. This combination, the optical fractionator, involves counting neuronal nuclei with optical disectors in a uniform and systematic random sampling that constitutes a known fraction of the region to be analyzed [27]. Optical disector probes were placed on the marked area with random angles and evenly spaced. The Purkinje and granular cells in counting frames and the cells on countable sides were counted [30]. During this process the section was viewed on a monitor and a 100x oil objective was used. The microscopic fields were sampled by moving the microscope stage in an equivalent interval using a stage micrometer. Using a microcator connected on the stage, the Z-axis movement of the microscope stage was measured. Guard zones need to be big enough to cover up any artifact at the top and bottom of the section. Typically, tissue sections between 15–30 μm thick post-processing allows for upper and lower guard zones [29]. In our study, the section thickness for each animal was measured as approximately between 18 and 20 μm after post-processing. The efficiency of the sampling strategy and the number of sampled cells for total neuron number estimation has previously been tested using CE and CV formulae [31]. CE and CV values were calculated based on Gundersen and Jensen’s formula [30]. Once the strategy had been verified, neuron numbers in the hippocampi and cerebella of the study groups were estimated. Neurons were counted if the nuclear profile of the largest neuron came into focus within the unbiased counting frames spaced randomly and systematically throughout the delineated regions and total neuron numbers were determined by multiplying the number of neurons by the sampling

fraction [32]. A sample illustration designed for the application of the optical fractionator method is shown in Fig. 1.

2.5. Biochemical analysis

2.5.1. Determination of catalase activity

Blood samples were collected into heparinized tubes. Following separation of plasma, hemolysates were prepared by adding one volume of blood to 19 vols of ice-cold distilled water, and the hemolysates were diluted (1:500) with distilled water. Next, the peroxidatic activity of CAT was determined using an assay first described by Aebi. Quantitation was performed by measuring the absorbance at 550 nm in comparison with CAT calibrators as references. This method was compared with the method referred to by the Sigma Company and described by Aebi [33]. Decomposition of H_2O_2 in the presence of CAT was performed at 240 nm. CAT activity was defined as the amount of enzyme required to decompose 1 nmol of H_2O_2 per minute at 25 °C and pH 7.8. Results were expressed as millimoles per minute per ml of blood sample (mmol/min/ml) [33].

2.6. Statistical analysis

All computational analyses were performed on SPSS (Statistical Package for the Social Sciences, version 15.0, SPSS Inc., Chicago, Illinois, USA) software. Significance was set at $p < 0.05$ for all tests. The test of normality was applied for each group and data were found to be homogeneous. Values among the groups were analyzed using One-way ANOVA based on the results of the Levene and Shapiro Wilk tests for equality of variances and normality assumption, respectively ($p > 0.05$). Bonferroni post-hoc multiple comparison tests were applied to determine any differences among the groups.

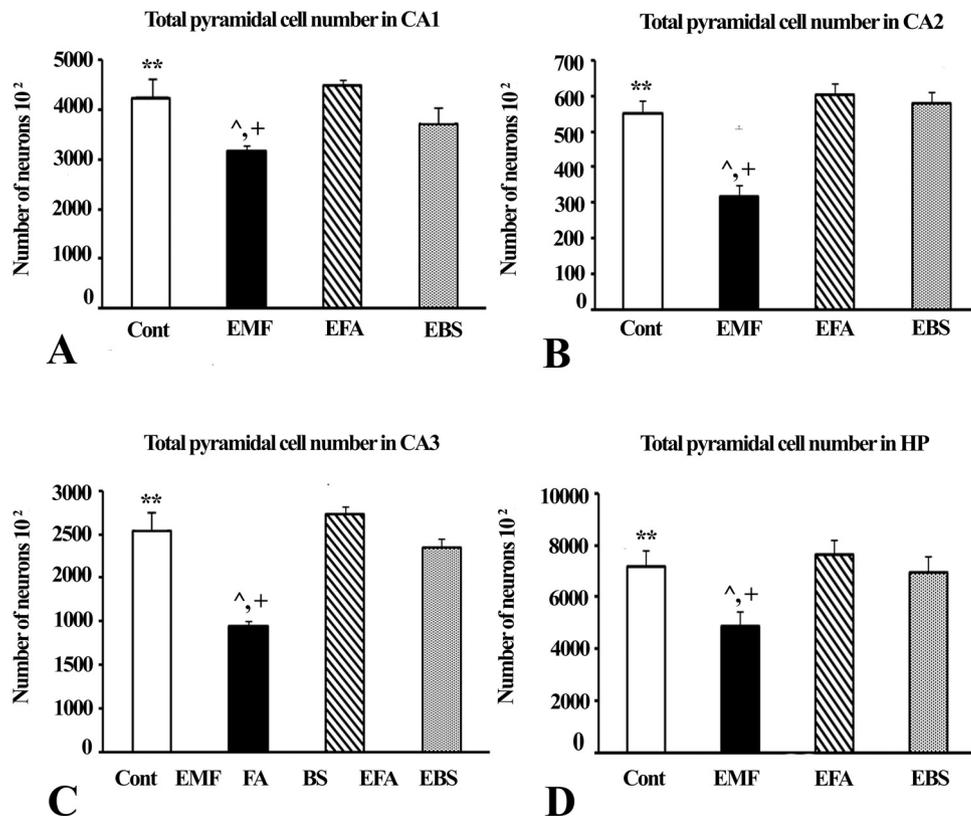


Fig. 2. Neuron numbers (\pm SEM) in the different hippocampal areas. The results of cell number in CA1, CA2, CA3 regions in (A–C) and total neuron number in hippocampus (HP) in (D) are shown by graphs for each group. (**) shows a significant difference at the $p < 0.05$ level between the Cont and EMF groups; (\wedge) a significant difference at the $p < 0.01$ level between the EMF and EFA groups; and (+) a significant difference at the $p < 0.05$ level between the EMF and EBS groups.

Table 1

The mean coefficient of error (CE) and coefficient of variation (CV) values for the data obtained in the hippocampus, cerebellum and dentate gyrus are listed for each group of animals examined.

Groups	Hippocampus			Cerebellum	Dentate gyrus
	CA1	CA2	CA3	Purkinje cell numbers	Granular cell numbers
Cont	0.02	0.05	0.03	0.07	0.02
EMF	0.03	0.06	0.02	0.07	0.04
EFA	0.04	0.03	0.05	0.07	0.03
EBS	0.03	0.06	0.03	0.07	0.02
CV values					
Cont	0.12	0.15	0.08	0.07	0.1
EMF	0.15	0.17	0.08	0.08	0.12
EFA	0.1	0.12	0.13	0.14	0.08
EBS	0.09	0.14	0.08	0.09	0.06

3. Results

3.1. Stereological results

3.1.1. Hippocampus

Stereological methods provide quantitative descriptions about three-dimensional structures using two-dimensional images [34]. The optical fractionator technique is the most commonly used method in stereological counting calculations. The mean number of neurons estimated using the optical fractionator in the hippocampal regions of the EMF group was significantly lower compared with the Cont group ($p < 0.05$, One-Way ANOVA) (Fig. 2A–D). Significant differences were also determined between the numbers of neurons in the EFA (EMF + FA exposure) and EMF groups, and between the EBS (EMF + BS exposure) and EMF groups ($p < 0.05$, One-Way

ANOVA) (Fig. 2A–D). However, there were no significant differences among Cont, EFA and EBS ($p > 0.05$, One-Way ANOVA). Coefficient of error (CE) and coefficient of variation (CV) values for all groups are shown in Table 1.

3.1.2. Dentate gyrus

Mean granular cell numbers in the DG were estimated using the optical fractionator method. Granular cell numbers in the EMF group were lower compared to the Cont group ($p < 0.05$). Neuron numbers in the EFA and EBS groups were significantly higher compared to the EMF group, while neuron numbers in the EFA and EBS groups were significantly lower compared to the Cont group ($p < 0.05$). No significant difference was determined between the EFA and EBS groups ($p > 0.05$) (Fig. 3A). CE and CV values for all groups are shown in Table 1.

3.1.3. Cerebellum

Mean Purkinje cell numbers in the cerebellum were estimated using the optical fractionator method. A significant decrease in total numbers of Purkinje cells was observed in the EMF group compared to the Cont group ($p < 0.05$). In the EFA group, a highly significant decrease in Purkinje cell numbers was observed compared to the Cont group ($p < 0.01$). The significant difference was observed between the EMF and EBS groups ($p < 0.05$). There was also a significant difference between the EMF and EFA groups ($p < 0.05$) (Fig. 3B). CE and CV values for all groups are shown in Table 1.

3.2. Biochemical results

3.2.1. Catalase activity

Catalase (CAT) activity was measured from blood samples. The results are presented in the diagram. A significant increase in CAT activity was observed in the EMF group compared to the Cont group

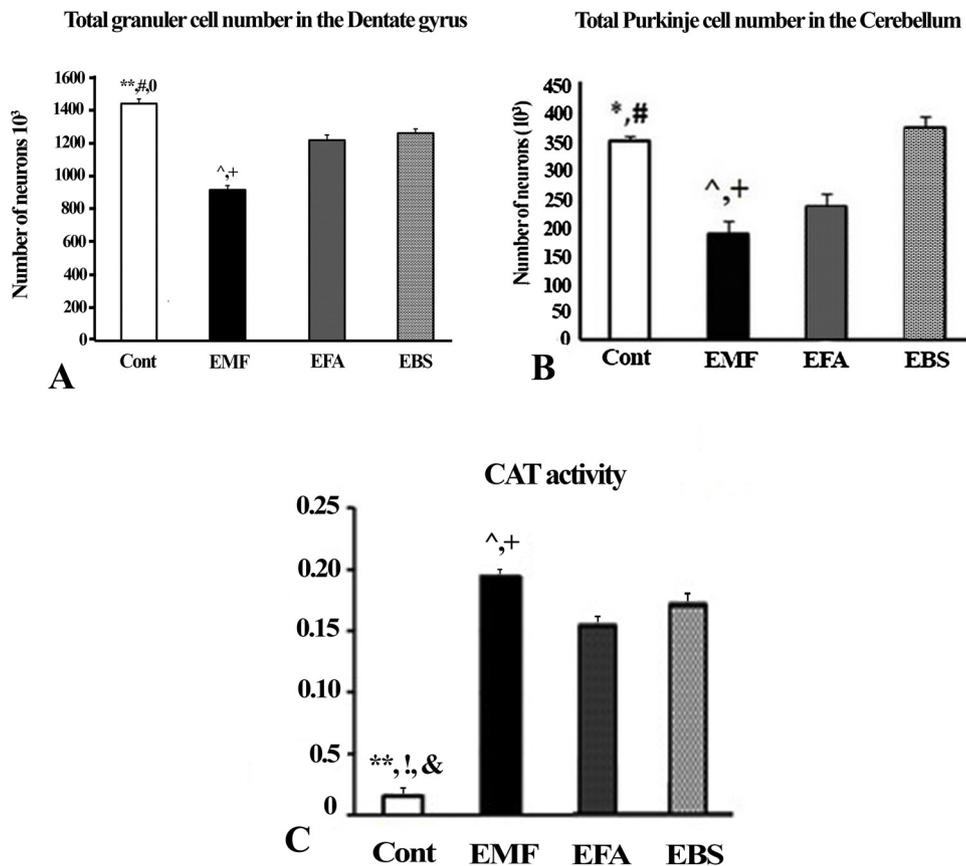


Fig. 3. The granular cell number in dentate gyrus in (A), the Purkinje cell number in cerebellum at (B), and the mean CAT activities (C) are shown by graph for each group. Mean number values for neurons (\pm SEM) in the dentate gyrus. (**) shows a significant difference at the $p < 0.01$ level between the Cont and EMF groups; (*) shows a significant difference at the $p < 0.01$ level between the Cont and EMF groups; (#) shows a significant difference at the $p < 0.05$ level between the Cont and EFA groups; (!) shows a significant difference at the $p < 0.01$ level between the Cont and EBS groups; (&) shows a significant difference at the $p < 0.05$ level between the Cont and EBS groups; (^) a significant difference at the $p < 0.05$ level between the EMF and EFA groups; and (+) a significant difference at the $p < 0.05$ level between the EMF and EBS groups.

($p < 0.01$). Also, activity was significantly lower in the EFA and EBS groups compared to the EMF group ($p < 0.01$) (Fig. 3C).

3.3. Histopathological results

Pyramidal neurons and Purkinje cells in the Cont group exhibited a normal appearance. The margins of the neuronal soma were regular, and the neurons had a healthy appearance. The nuclei of these cells were euchromatic and clear, and the dendrites and axons were of normal size. In the EMF group, most neurons were condensed in comparison to the other groups. Nuclei with reduced chromatin and narrow or dark-stained cytoplasm were observed. In some areas, the cell margins of damaged neurons could not be determined. Additionally, Nissl granules were condensed in the cytoplasm, and the nuclei were acidophilic. Occasional dark-stained basophil neurons were also observed. The hippocampal structure of the EFA and EBS groups was similar to the healthy structure observed in the Cont group. Histopathological findings are shown in Figs. 4 and 5.

4. Discussion

The increasing use of mobile phones at ever-earlier ages has increased the risk of radiation exposure. Adverse effects on the brain caused by EMF emitted by mobile phones are a subject of particular concern. This has led to many studies being performed on the subject [32]. In one case-control study, Hardell et al. compared 233 patients with brain tumors and 466 control subjects.

They reported a greater risk of tumor in the temporal and occipital regions, the areas of the brain most exposed to EMF [35]. Another case control study by Villeneuve et al., reported a 5.3-fold increased risk of one brain cancer type, glioblastoma, in personnel exposed to EMF, but no increased risk for other brain cancers [36]. An epidemiological study from Denmark determined an association between amyotrophic lateral sclerosis and exposure to high intensity EMF, but no correlation was observed with other neurodegenerative diseases [37]. A paper published in 2008 assessing the effect of EMF on health care workers reported various symptoms such as nausea; a metallic taste in the mouth, blood pressure and pulse changes, ectopic beats and recycled arrhythmias [38]. In this study, our results showed that EMF caused a significant decrease in total pyramidal and granular cell numbers in the hippocampus and DG and in Purkinje cell numbers in the cerebellum in the EMF group compared to the other groups ($p < 0.05$). A significant increase in CAT activity was also observed in the EMF group compared to the Cont group ($p < 0.01$). Also, CAT activity was significantly lower in the EFA and EBS groups compared to the EMF group ($p < 0.01$).

Heating of tissue due to EMF exposure and the resulting physiological response in the body may lead to deleterious changes, especially in brain activity, and may promote the formation of some types of cancer [39]. Some studies investigating the adverse effects of mobile phones have indicated that EMF disables the immune system, which is responsible for eliminating harmful proteins and toxins in the blood [40,41]. Symptoms such as fatigue, headaches, a burning sensation in the skin, and high blood pressure have also been observed [42,43]. Many studies have suggested that EMF may

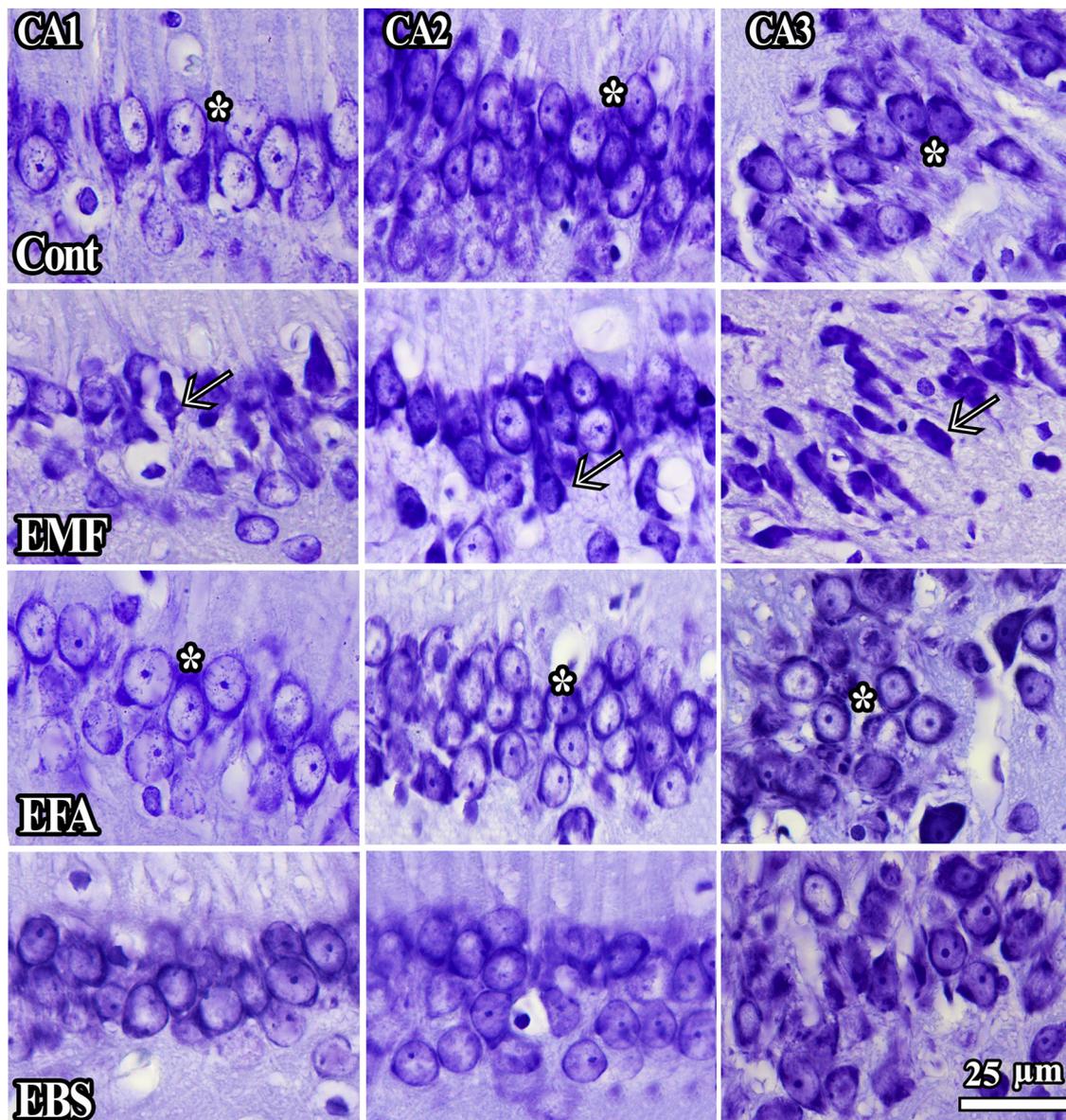


Fig. 4. CA1, CA2, and CA3 regions in hippocampus are respectively shown in the cresyl violet stained sections. Especially, neuronal cell loss of EMF groups in CA1 and CA2 regions was shown in the cresyl violet stained sections. The white star indicates healthy neurons in the Cont, EFA and EBS groups, and the white arrow shows a degenerated neuron with dark cytoplasm and shrinkage in the cell body. It should be noted that such pictures may not be informative since their appearance may be changed by the section plane involved, tissue shrinkage or swelling, or the area from which the images were taken. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

trigger the formation of reactive oxygen species (ROS) in exposed cells in vitro [44–46]. Oxidative stress is known to be essential for pathophysiological processes in the brain, and plays important roles in the DNA damage process, general and specific gene expressions and cell apoptosis. The brain has a high metabolic rate, making it more prone to damage by ROS and oxidative damage compared to other organs. Excessive amounts of ROS in tissues may lead to necrosis, the death of neurons and neuronal damage in brain tissue [47]. Lai and Singh showed that EMF damages DNA and has harmful effects on the mouse brain [48]. Schmitz et al. exposed pregnant rats to a long-term γ irradiation between embryonic day 13 and 16 or were sham-irradiated. Then, thirty months old male and female offspring were analyzed by using stereology technique for changes in hippocampal and cerebellar morphology. They reported a significant decrease of approximately 50% in numbers of hippocampal pyramidal and granule cells in the cerebrum as well as of cerebellar Purkinje and granule cells in male and female

irradiated offspring [49]. Our stereological results revealed a significant decrease in the numbers of pyramidal neurons in the groups exposed to EMF in comparison to the other groups. The qualitative histological analysis results support the quantitative stereological analysis findings. Light microscopic examination of the EMF group revealed fewer healthy neurons. Both the histological and stereological results indicate that exposure to 900-MHz EMF caused a decrease in the numbers of pyramidal neurons in the CA region and led to neuronal degeneration.

FA can prevent the adverse effects of EMF exposure, such as a decrease in the numbers of pyramidal neurons in the CA region and neuronal degeneration. In our study, FA has a protective effect against EMF exposure. Research into the effects of FA in preventing neuronal tube defects began in the early 1980s. Iskandar et al.'s experimental study showed that FA exhibits neuroprotective effects in the central nervous system of adult rats [50]. Especially folic acid and related B vitamins may play important roles in neurobe-

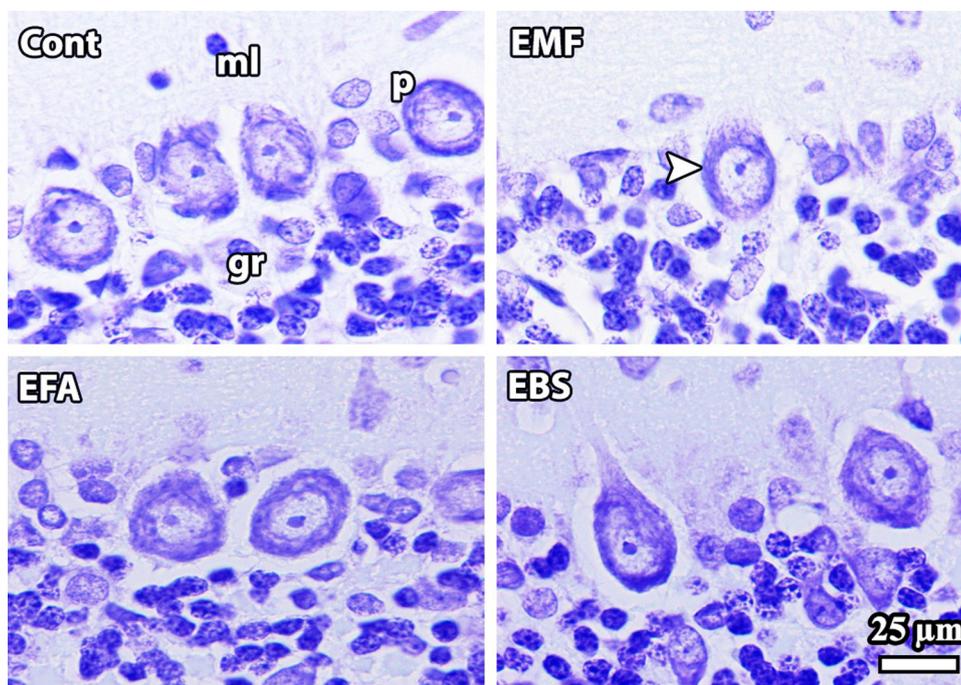


Fig. 5. Representative images from the cerebella from each group. As seen in the EMF group, Purkinje cell numbers clearly decreased after EMF exposure compared to the other groups in this experiment. The neuroprotective effects of BS and FA against EMF exposure can be seen in the images from the EBS and EFA groups. The arrowhead shows a degenerative Purkinje cell and (p) shows a healthy Purkinje cell. ml; molecular layer, gr; granular layer (Cresyl violet staining). It should be noted that such images may not be informative since their appearance may be changed by tissue shrinkage or swelling and the section plane concerned. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

havioral development of offspring [51–53]. In particular, maternal folate deficiency may impair learning and memory ability in offspring [54]. In our study, FA showed protective effect by reducing the neurodegenerative effect of EMF.

BS belongs to the Burseraceae family. The resin has significant and favorable effects on forgetfulness and memory. BS resin prevents brain cell degeneration in the hippocampus and reduces disease progression in malignant cells. Hosseini-Sharifabad et al. in their study show that hippocampal granule cells of BS-treated aged rats had more dendritic segments, larger arbors, more numerical branching density and more dendritic spines in comparison to control animals [55]. Also, they observed BS in the aged Wistar rat improves learning capability along with enhancement of dendritic arbors and dendritic spines in hippocampal granule cells. Jalali et al. observed that BS dramatically increased the number of neuronal processes in CA1 region and improves passive-avoidance learning ability [56]. In the present study, we observed various favorable effects of BS, such as rescuing neuron number in the EBS group and exhibiting neuroprotective effects against neuronal damage in the hippocampus.

Bas et al. indicated that postnatal EMF exposure gave rise to a significant decrease in pyramidal cell numbers in the CA of the EMF group [57]. Erdem et al. reported that 900 MHz EMF caused cellular losses in the total number of pyramidal cells of 4-week-old male [19]. Scalford et al. found highly significant ($p < 0.002$) evidence for neuronal damage in the cortex, hippocampus, and basal ganglia in the brains of exposed rats [58]. Altun et al. investigated protective effects of melatonin and omega-3 on the hippocampus and the cerebellum of adult *Wistar albino* rats exposed to electromagnetic fields. They observed that 900 MHz EMF lead to cell loss in the hippocampus and the cerebellum [59]. Our results showed that EMF leads to neuronal losses in CA1, CA2 and CA3 and the DG regions of the hippocampus and cerebellum in comparison to the other groups. EMF is known to cause oxidative stress by increasing free radicals. FA and BS both show antioxidant activities against

this oxidative stress. Two major functioning mechanisms have been identified for antioxidants [60]. The first is a mechanism of disrupting the chain in which the primary antioxidant releases an electron to the free radical found in the systems. The second mechanism includes elimination of the initiators of species of ROS/reactive nitrogen (secondary antioxidants) by suppressing chain-initiating catalyst. Also, antioxidants might display their impact on biological systems by various mechanisms involving electron releasing, metal ion chelation, co-antioxidants, or by maintaining the expression of genes [61].

The use of 900- and 1800-MHz mobile phones has become particularly widespread in the previous two decades. However, these frequencies may cause a number of adverse effects on biological systems. Recent studies have reported that radiation emitted by mobile phones may cause ROS to form in various tissues [62–64]. Overproduction of ROS can cause oxidative stress in the body and leads to changes in antioxidant defense systems and to oxidative stress. The other studies have demonstrated that RF radiation may cause oxidative damage in tissues, and that increasing antioxidant enzyme activity such as CAT, superoxide dismutase, malondialdehyde and some oxidative stress parameters can help to show the relationship between apoptosis and radiation [65,66]. Çelik et al. investigated the antioxidant defense mechanism in rats subjected to trauma by studying CAT levels [67]. One study by Daşdağ et al. reported higher total antioxidant capacity and CAT levels compared to a sham group [68]. Schtmiz et al. reported increased superoxide dismutase and CAT activities in a 2.0 mg/kg methylphenidate-treated group, but observed no alteration in levels of reactive species, thiobarbituric acid reactive substances levels and the sulfhydryl group in the rat cerebellum [69]. Our biochemical results indicate that EMF caused oxidative stress and increased CAT levels in rats exposed to it. FA and BS both exhibit antioxidant activities against this enzyme. These effects involve reducing CAT levels.

Some studies have also investigated the effects of EMF on the cerebellum. Sonmez et al.'s study of female rats exposed to a 900-MHz EMF determined a significant decrease in Purkinje cell numbers in the cerebellum [32]. Aslan et al. show that long-term and continuous exposure of rats to 900 MHz EMF during early and middle adolescence can produce pathological effects including a decreased number of Purkinje cells [70]. Barua et al. showed that exposure to a high-dose FA diet during gestation resulted in deregulation of the expression of several genes in the cerebella of male and female pups [71]. However, no previous studies have investigated the effect of BS on the cerebellum. Our results indicate that EMF exposure causes a decrease (approximately 50%) in numbers of Purkinje cells in the cerebella of male rats. Purkinje cells were analyzed and cell nuclei were taken as reference. Therefore, during cell counting, a cell that is darkly stained and whose nucleus is not clearly seen was not included into the counting. This large difference between the groups may be due to the fact that degenerate neurons are not counted. We therefore conclude that FA and BS may be useful in reducing damage caused by EMF exposure.

In conclusion, the stereological and histological results of this study indicate that EMF has harmful effects on the hippocampus and cerebellum. The neuroprotective and antioxidant effects of FA and BS were also demonstrated using stereological, histological and biochemical methods.

Ethics statements

The Animal Ethic Committee of Ondokuz Mayıs University approved the protocol, and appropriate measures were taken by our study group to minimize pain or discomfort to the animals involved (date 28.08.2013, No. 2013/23). The experimental part of this study and stereological examinations were performed at the Department of Histology and Embryology, Ondokuz Mayıs University.

Author contributions

BZA designed the project. EGK and KKY performed the experimental procedures. EGK, AK, IA and KKY analyzed the data and MEO contributed important revisions. All authors approved the final version of the manuscript.

Conflict of interest

The authors have no conflict of interest to declare.

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