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ORIGINAL ARTICLE

Investigating the Effects of Electromagnetic Fields on Neuronal Protection in Embryonic and Postnatal Hippocampus of Pregnant Mice Exposed to Lead Acetate

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KEYWORDS

Lead acetate;
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ABSTRACT: Lead acetate is one of the neurotoxic pollutants that can lead to a decrease in neurogenesis, learning, and memory disorders.This study aimed to determine the effectiveness of electromagnetic fields (EMF) on the expression of Postsynaptic Density 95 (PSD-95) and Synapsin II genes in the embryos and infant hippocampus of pregnant mice exposed to lead. The pregnant NMRI mice were randomly divided into 5 groups. Control, Sham, Lead acetate, the group exposed to EMF, and the group EMF and lead. Mice embryos were sacrificed on the 17th day of pregnancy and mice infants were analyzed 4-5 weeks after birth, with the hippocampus being isolated and analyzed using real-time PCR methods. Significant decreases in the relative expression of Psd-95 and synapsin II are present in embryonic and adult mice in the Pb group compared to the sham group. Additionally, the expression of the Synapsin II gene had a significant increase in Pb+EMF group compared to the Pb group during the embryonic period. There was a significant increase in the number of cells in DG, CA1 and CA3 regions in Pb+EMF group compared to the Pb group during the embryonic and adult periods. The combination of lead and EMF exposure may have a differential effect on the expression of synapsin II compared to Psd-95. EMF and lead co-treatment, by stimulating neurogenesis in the damaged area and the expression of the Synapsin II gene, causes the primary differentiation of neurons.

INTRODUCTION

Nowadays, transcranial magnetic stimulation (TMS) has a huge impact on neurogenesis, increasing the irritability, proliferation, and survival of neurons, formation of inter-neuronal synapses, and vascularization. It has a wide therapeutic application. More than 30 years ago, Barker and his colleagues showed that electromagnetic fields (EMF) can penetrate the scalp, skull, and meninges, creating an electric current in the brain and stimulating neurogenesis. Low-frequency stimulation reduces nervous activity, while high-frequency stimulation has thrilling effects [1, 2]. Recently, the clinical application of TMS has expanded due to research showing its high therapeutic potential in migraines, tinnitus, anxiety, acute depressive disorders, stroke, and the control of neurological disorders such as Parkinson's, Alzheimer's, and amyotrophic lateral sclerosis [3].

The therapeutic effects of EMF depend largely on their ability to reduce nerve irritability and inflammation,

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prevent blood-brain barrier (BBB) disruption, and increase neuronal survival. The hippocampus, an important part of the vertebrate central nervous system, plays a crucial role in memory and spatial navigation [4, 5]. Research shows that the antioxidant effects of TMS are higher than other treatments and that TMS affects oxidative stress [6]. Studies indicate that 60 Hz electromagnetic waves have many therapeutic effects [7, 8]. TMS applied to the hippocampus has been shown to reduce depression in patients [9]. Experiments on the hippocampus have demonstrated that low-frequency TMS can improve memory and cognitive impairment in mice with Alzheimer's disease [10, 11].

In another experiment, they also showed that TMS improves spatial learning by facilitating the activity of potassium and calcium channels in people with Alzheimer's disease [12]. In other experiments conducted on the hippocampus, they showed that Repetitive Transcranial Magnetic Stimulation (RTMS) reduced depression and cognitive impairment in Parkinson's patients [13]. It has been reported that TMS increases the proliferation of mature stem cells as well as neural precursor cells [14]. TMS can also affect the formation of synapses, according to a 2009 study [15].

Lead is one of the most toxic heavy metals to the body, and today its poisoning is considered one of the most important public health problems. Many researchers have shown that lead can cause neurological, hematological, digestive, reproductive, circulatory, and immune system problems. Lead can enter the body mainly through eating, drinking or inhaling and can affect many tissues such as the kidney, liver, bone, muscle and brain. Lead can quickly cross the blood-brain barrier and spread to the brain, and can also be easily passed to the foetus through the placenta in pregnant mothers [16, 17]. After that, lead passes through cerebrospinal fluid, causing deposition in the developing fetal brain, and in some cases can also replace calcium. Lead in the immature brain alters the behavior of endothelial cells and disrupts the cerebrospinal fluid, also directly affecting nerve impulses [18].

There is strong evidence that lead during pregnancy and lactation causes irreversible effects on the cognitive and behavioural functioning of the foetus. This is because the brain develops rapidly during development, and exposure to toxic metals such as lead can lead to impaired cognitive and memory function in the foetus [19]. While most of the side effects of lead on the nervous system in adults are eliminated by lead interruption, evidence shows that it is irreversible in children [20]. PSD95 (postsynaptic protein with density 95) plays a vital role in synaptic plasticity and stabilization of synaptic changes during long-term reinforcement [21]. The level of synaptic proteins such as Synaptophysin and PSD95, and the density of dendritic spines, are associated with learning and cognitive processes. PSD95 is a postsynaptic membrane protein that plays an important role in synaptic plasticity by facilitating the absorption of channel proteins and proteins into the postsynaptic membrane. The findings suggest that PSD95 expression is essential for the maturation of newly formed neurons in the hippocampus of adult mice. Previous in vitro and in vivo studies have shown that PSD95 controls the number and size of dendritic spines and synaptic maturity in the hippocampus [22, 23].

Finally, in view of the above, we studied the effects of EMF on the neutralisation and spread of inter-neuronal synapses in the hippocampus of embryonic and infant mice born to mothers who drank leaded water during pregnancy and were also exposed to electromagnetic waves. We wondered whether electromagnetic waves have the potential to increase plasticity in the hippocampus of mice born to mothers exposed to lead, or to prevent the death of brain neurons at an older age. Therefore, the aim of this study was to determine the effectiveness of electromagnetic fields (EMF) on the expression of PSD-95 and Synapsin II genes in the infant and embryonic hippocampus of pregnant mice exposed to lead acetate.

MATERIALS AND METHODS

Preparation and maintenance of laboratory animals

Adult NMRI mice were purchased from the Pasteur Institute of Tehran and transferred to the Animal House of Damgan University. Animals were maintained under suitable conditions of 12 h light, 12 h darkness, at a temperature of 20-24°C, with free access to water and food. All stages of animal maintenance and slaughter were carried out according to the guidelines of the

Damghan University Animal Ethics Committee (IR.DU.REC.1400.005). Male and female animals were placed in a cage for mating. To ensure mating, a smear test was taken from female animals after 12 hours. If vaginal plaque was observed, pregnant mice were isolated, and this day was considered the zero-day of pregnancy.

Experimental groups

Pregnant rats were randomly divided into five groups: Sham: Animals were placed in an electromagnetic device and fed with distilled water twice daily, for 30 days from the zero day of pregnancy without receiving waves. Pb group: animals that were fed lead acetate with a dose of 5mg/kg, soluble in double distilled water (1cc/ daily), from the zero day of pregnancy for 30 days. EMF group: animals exposed to electromagnetic waves with a magnetic wave generating device (Magno 915x) with a magnetic wave intensity of 2 ml Tesla and a frequency of 50 Hz (4 hours/day) from zero days of pregnancy for 30 days. The electromagnetic wave radiation time was from 8 am to 12 am. Pb + EMF group: animals exposed to electromagnetic waves similar to the previous group, and simultaneously fed lead acetate similar to the Pb group. Control group: pregnant mice that only consumed double distilled water (the distilled water and lead acetate were gavaged to animals).

At the end, in each group, male infants of pregnant mice were sacrificed on designated fetal days (17 Days of pregnancy) and adulthood (4 to 5 weeks after birth), and their brains were fixed in formalin solution for tissue studies [24].

Determination of the zero day of pregnancy based on vaginal smearing of female mice

The vote to determine the zero day of pregnancy in pregnant mice, as well as the time of sacrifice, was taken from them. To do this, adult male and female mice were put together in order to mate. After a day, smears were prepared daily from female mice, and after observing the sperm, that day was considered day zero of pregnancy.

Cresyl violet staining

After treatment, infants of pregnant mice were sacrificed on designated days. Embryos (17 days of pregnancy) and adults (4 to 5 weeks after birth) were then dissected, and their brains were removed. The hippocampi were isolated and fixed in a 4% paraformaldehyde solution for 3 days, followed by embedding in paraffin after tissue preparation. Neuronal counting was performed using the Cresyl Violet staining method.

Counting neurons in different areas of the hippocampus

The number of neurons in the dentate gyrus (DG) and the Ammon's horn of all the experimental groups was counted, and the final result of the neuron count in these areas was compared among the different groups. To count neurons, several areas of 250 micrometers were randomly selected. A microscope with a magnification of 40 was used for this purpose.

Cell counting method

According to the Paxinos Atlas, the range of measurements for the hippocampus is from 8.2 mm behind the bregma to 52.4 mm (in this range, the regions of DG, CA1, and CA3 are visible). Coronal microscopic sections with a thickness of 7 microns were prepared [25, 26]. Then, from each group, 5 to 7 sections with a distance of 200 µm were removed (one cut was removed from every 20 cuts). The sections were stained using Cresyl Violet and then counted using a graticule and 400 X magnification. The number of neurons in the DG regions (in full) was counted in five fields of view with an area of 400 μm^2, and the average was calculated. The cell count was repeated 7 times. The sections were then studied and photographed using a fluorescent microscope (Japan, E 600, Nikon Eclipse) with 400 X magnification and a digital camera 120 (Nikon, USA DXM). The Nissl bodies appeared purple, the nucleus and some cytoplasmic organelles of the neuron appeared purple, and the background was colorless. Neurons with a rounded nucleus and chromatin in densely basophilic masses were considered apoptotic neurons and were not counted.

Evaluation of the expression of genes

Using the real-time PCR technique, the expression rate of the synapsin II and PSD-95 genes was investigated. The TBP gene is also used as an internal control gene. The specifications of the primers used in this study are given in Table 1.

Table 1. The primer sequences of PSD-95, Synapsin II, and TBP genes were used in the Real-time q-PCR

Gene	Accession number	Forward (5' to 3')	Reverse (5' to 3')	Ampliconsize (bp)
PSD-95	NM-007864.3	ATCCTGTCGGTCAATGGT	ATCGGCTATACTTCTGGTT	124
SynapsinII	NM-013681.3	GGTAGATGCCTGCTCTGA	GTTGTCTGTCCTCCACTTG	146
TBP	NM-013684.3	GGAGCCAAGAGTGAAGAAC	TGCCACCTGTAACTGAGT	461

Statistical analysis

Statistical analysis was conducted using SPSS software version 24. The Independent-samples t Test method was utilized to compare differences between groups after confirming the normality of the data through the Shapiro test and ensuring normal distribution. The Real-time q-PCR data was normalized using the Pfaffl method and the 2^-ΔΔCT calculation in Excel software. After verifying the normality of the results with the Shapiro test and ensuring normal distribution, the Independent-samples t Test method was applied. A significance level of $p<0.05$ was considered statistically meaningful. It is important to note that each test was repeated at least four times.

RESULTS AND DISCUSSION

Changes in the relative expression of the PSD-95 gene in the hippocampus of 17-day embryos

Evaluation of the results (Figure 1) showed a significant decrease in the relative expression of Psd-95 in the lead-to-sham treatment group (respectively: $p < 0.05$: 1.015 ± 0.185 and 0.015 ± 0.035). There was also a significant decrease in the relative expression of Psd-95 in the EMF treatment group relative to Sham (respectively: $p < 0.05$: 1.015 ± 0.185 and 0.25 ± 0.005). Whereas in the Pb + EMF treatment group, there was no significant difference in the relative expression of Psd-95 relative to the Pb group (respectively: $p > 0.05$: 0.035 ± 0.015 and 0.175 ± 0.145).

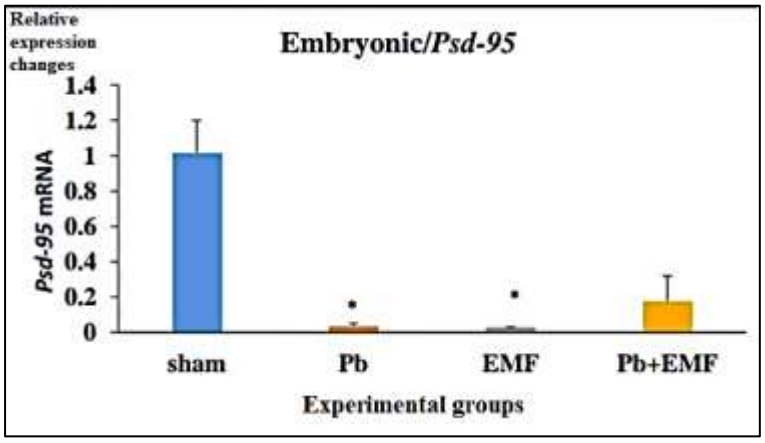

Figure 1. changes in the relative expression of Psd-95 in the hippocampus of the four groups studied in 17-day embryos. * P<0.05 compared to Sham

Changes in the relative expression of the PSD-95 gene in the hippocampus of mice 4 to 5 weeks:

The evaluation of the results (Figure 2) showed a significant decrease in the relative expression of Psd-95 in the lead-to-sham treatment group (respectively: $p < 0.05$; 1.005 ± 0.065 and 0.34 ± 0.15). There was also a significant decrease in the relative expression of Psd-95 in the EMF treatment group relative to sham

(respectively: $p < 0.05$; 1.005 ± 0.065 and 0.225 ± 0.075). Whereas in the Pb + EMF treatment group, there was no significant difference in the relative expression of Psd-95 relative to the Pb group (respectively: $p > 0.05$; 0.34 ± 0.15 and 0.025 ± 0.005).

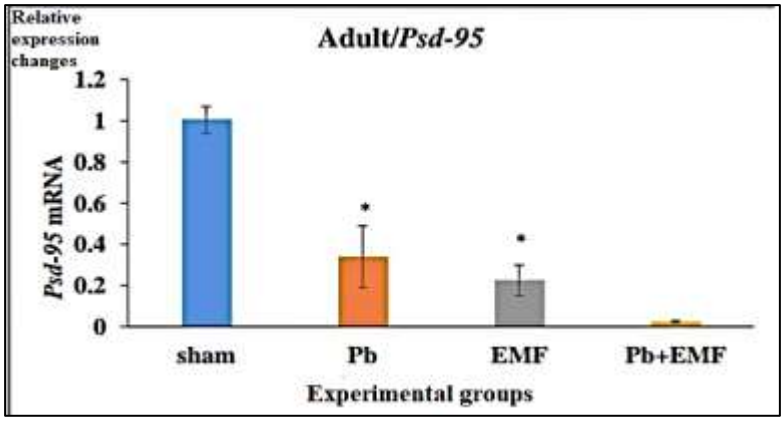

Figure 2. changes in the relative expression of Psd-95 in the hippocampus of the four groups studied in mice 4-5 weeks. * P<0.05 compared to Sham

Changes in the relative expression of the synapsin II gene in the 17-day fetal hippocampus:

Analysis of the results indicates (Figure 3) a significant decrease in the relative expression of Synapsin II in the lead treatment group compared to sham (respectively: $p < 0.01$: 1.01 ± 0.12 and 0.035 ± 0.015). There was also a significant decrease in the relative expression of Synapsin II in the treatment group with EMF compared to sham (respectively: $p < 0.01$: 1.01 ± 0.12 and 0.04 ± 0.01). While in the Pb + EMF group, there was a significant increase in the relative expression of Synapsin II relative to the Pb group (respectively: $p < 0.05$: 0.035 ± 0.015 and 0.35 ± 0.06).

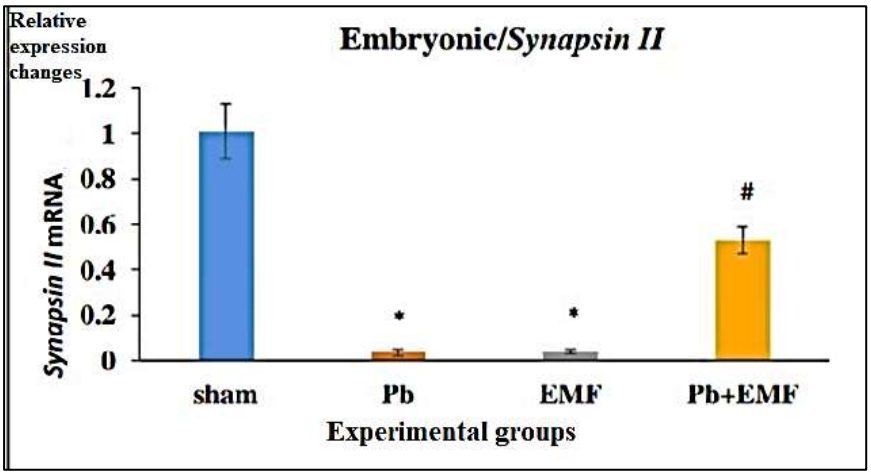

Figure 3. relative expression of Synapsin II in the hippocampus of the four groups studied on fetal day 17. # P<0.05 compared to the Pb group. * P<0.05 compared to sham.

Changes in the relative expression of Synapsin II in the hippocampus of mice 4-5 weeks

Samples extracted from the hippocampus during adulthood (4 to 5 weeks after birth) were examined to measure the expression rate of Psd-95 using the RT-PCR Real-time method (Figure 4). The evaluation of the

results showed a significant decrease in the relative expression of Synapsin II in the lead treatment group relative to sham (respectively: P < 0.01: 1 ± 0.03 and 0.345 ± 0.105). While there was no significant difference in the relative expression of Synapsin II in the treatment group with EMF relative to sham (respectively: P > 0.05: 1 ± 0.03 and 0.65 ± 0.06). There was also no significant difference in the relative expression of Synapsin II compared to the Pb group (respectively: P > 0.05: 0.345 ± 0.105 and 0.025 ± 0.005).

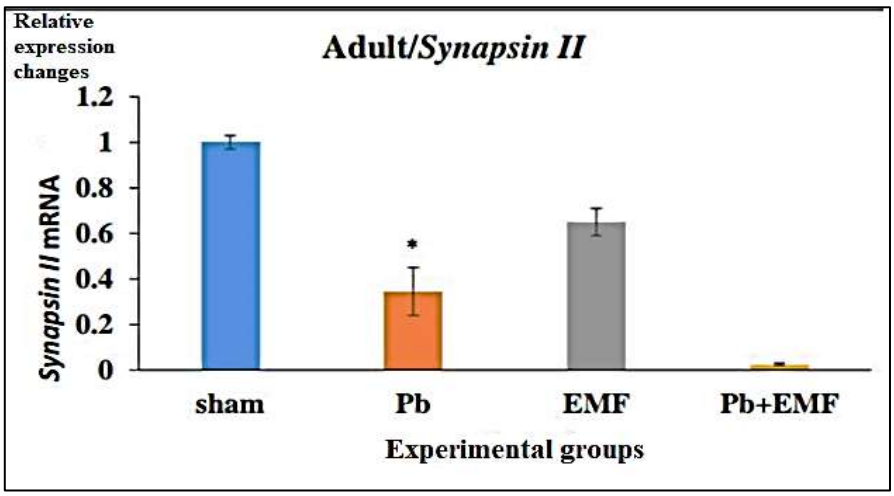

Figure 4. relative expression of Synapsin II in the hippocampus of the four groups studied in adulthood (4 to 5 weeks after birth). * P<0.05 compared to sham

Hippocampal histology results in fetal (fetal day 17) and adulthood (4 to 5 weeks after birth)

After the end of the treatment, various groups of embryonic mice (fetal day 17) and adults (4 to 5 weeks after birth) were sacrificed on specific days, and their hippocampus was extracted to provide tissue sections and cell counts.

Comparison of the number of CA1 pyramid cells in experimental groups

It was found that (Figure 5), there was a significant decrease in the number of CA1 hippocampal cells during the embryonic period in the Lead group compared to Sham (respectively: P<0.001: 49.49667±3.5 and 19.66±0.58). There was also a significant decrease in the number of cells in the EMF group compared to sham (respectively: P<0.001: 49.49667±3.5 and 26.22±1.4). In the Lead + EMF group, there was a significant increase in the number of cells compared to the Lead group (respectively: P<0.001: 19.66 ± 0.58 and 56.61 ± 1.02). Hippocampal CA1 cell counts were also conducted in different groups during adulthood (4 to 5 weeks after birth). By examining the average number of cells through unilateral variance analysis, it was found that there was a significant decrease in the number of cells in the Lead group compared to sham (respectively: P<0.001: 444.8867±6.8 and 105.4967±7). There was also a significant increase in the number of cells in the EMF group compared to sham (respectively: P<0.001: 444.8867±6.8 and 509.6667±17.8). Additionally, there was a significant increase in cell number in the Lead+EMF treatment group compared to the Lead group (respectively: P<0.001: 105.4967±7 and 329.33±3.9).

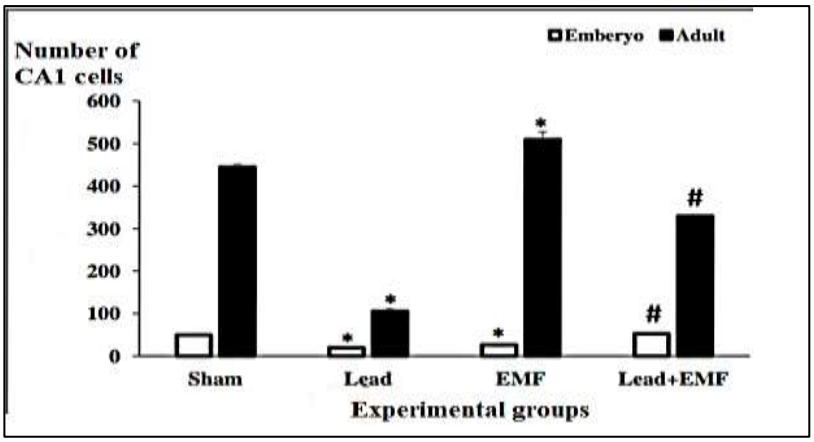

Figure 5. comparison of the number of pyramidal cells in the CA1 area of the hippocampus of the four groups studied during the fetal period (fetal day 17) and adulthood (4 to 5 weeks after birth), * P<0.05 compared to sham and #P<0.05 compared to Lead group

Comparison of the number of pyramidal cells in the CA3 region

There was a significant decrease in the number of CA3 hippocampal cells (Figure 6), during the embryonic period in the Pb group compared to sham (respectively: P<0.001: 49.05333±2.3 and 24.10667±0.8). There was also a significant decrease in the number of cells in the EMF group compared to sham (respectively: P<0.001: 49.05333±2.3 and 24.10667±0.8). In the Pb + EMF group, there was a significant increase in the number of cells compared to the Pb group (respectively: P<0.001: 15.82±0.99 and 52.55333±0.55). Hippocampal CA3 cell counts were also conducted in different groups during adulthood (4 to 5 weeks after birth). It was found that there was a significant decrease in the number of cells in the Pb group compared to sham (respectively: P<0.001: 411.3867±6.02 and 114.2767±7.5). There was also no significant difference in the number of cells in the EMF group compared to sham (respectively: P>0.05: 411.3867±6.02 and 421.94±4.9). In the Pb + EMF group, there was a significant increase in the number of cells compared to the Pb group (respectively: P<0.001: 114.2767±7.5 and 349.1667±7.3).

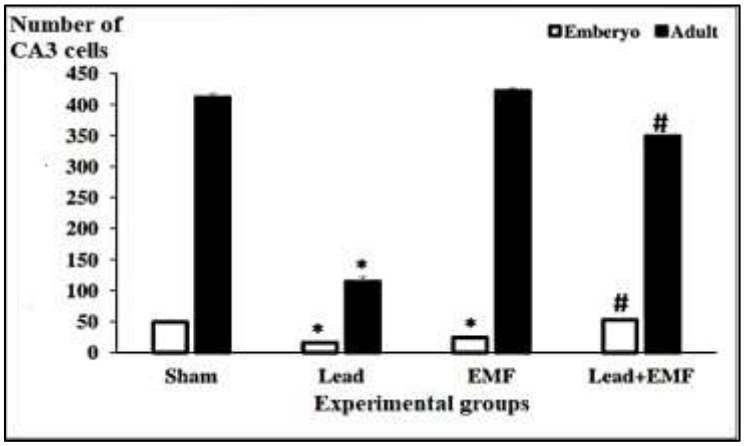

Figure 6. comparison of the number of pyramidal cells in the CA3 area of the hippocampus of the four groups studied during the fetal period (fetal day 17) and adulthood (4 to 5 weeks after birth)
* P<0.05 compared to sham and #P<0.05 compared to Lead group

Comparison of the number of granular cells in the dental torture area (DG)

It was found that there was a significant decrease in the number of cells (Figure 7) during the embryonic period in the Lead group compared to the sham group (respectively: P<0.001: 52.43±2.6 and 18.99333±0.96). Additionally, a significant decrease in the number of cells was observed in the EMF group compared to the sham group (respectively: P<0.001: 52.43±2.6 and 28.22±0.59). In the Lead + EMF group, a significant increase in the number of cells was observed relative to the Lead group (respectively: P<0.001: 18.99333±0.96 and 57.72±0.31). Furthermore, cell counting in the dentate gyrus of the hippocampus in adulthood (4 to 5 weeks after birth) was conducted in different groups. A significant decrease in the number of cells in the Lead group was shown compared to the sham group (respectively: P<0.001: 462.22±16.7 and 119.443±4.7). Similarly, there was a significant decrease in the number of cells in the EMF group compared to the sham group (respectively: P<0.001: 462.22±16.7 and 544.61±4.2). Conversely, there was a significant increase in the number of cells in the Lead + EMF group compared to the Lead group (respectively: P<0.001: 119.443±4.7 and 379.83±4.4).

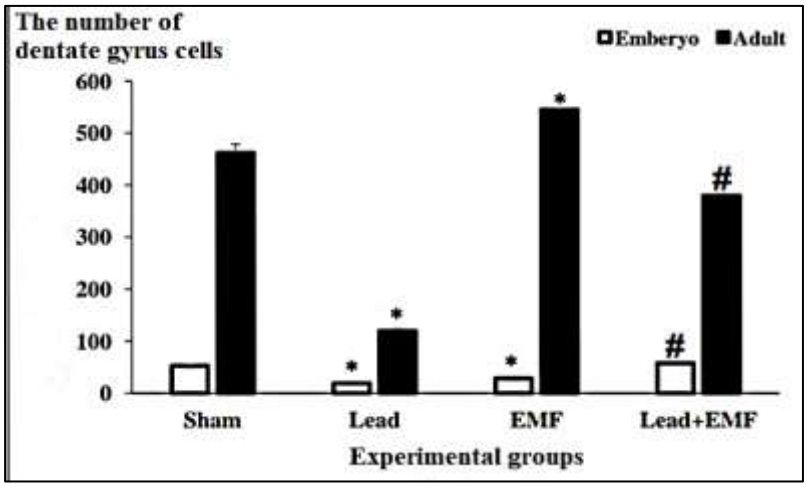

Figure 7. comparison of the number of granular cells in the hippocampal dentate gyrus of the four groups studied during the fetal period (fetal day 17) and adulthood (4 to 5 weeks after birth)
* P<0.05 compared to sham and #P<0.05 compared to Lead group

Cresyl Violet staining of the hippocampal coronal sections

In order to investigate the destructive effects of lead on neurons in the CA1, CA3, and DG regions of the hippocampus, as well as the protective effects of EMF on them during the adulthood (4 to 5 weeks after birth) (Figure 8), and embryonic period (fetal day 17) (Figure 9), Cresyl Violet staining was used. The density of neurons in the three regions of CA1, CA3, and DG in the hippocampus was quantitatively examined in the study groups after staining with Cresyl Violet.

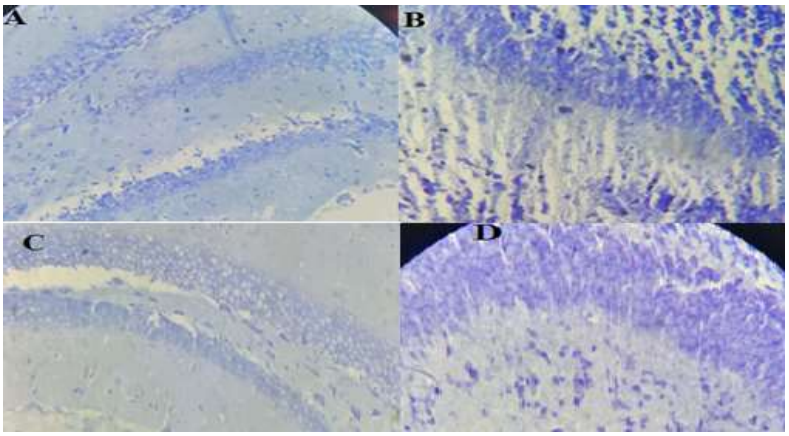

Figure 8. The images of hippocampal cells stained by Cresyl Violet in adult period (4 to 5 weeks after birth) in different groups subject of study (A) sham group, (B) Pb group, (C) EMF group, (D) Pb + EMF group. Magnification ×400

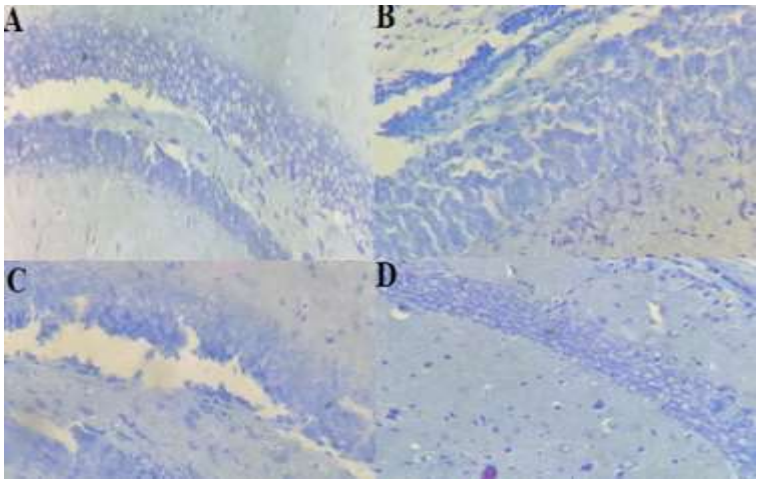

Figure 9. images of hippocampal cells with Cresyl Violet staining during the period embryos (day 11 of embryos) in different study groups (A) sham group, (B) Pb group, (C) EMF group, (D) Pb+EMF group. magnification×400

Lead and its derivatives are one of the most dangerous environmental pollutants. All ages, from children to adults, are exposed to various complications of lead. Many studies show the harmful effects of lead on different tissues of the body. For this reason, the widespread distribution of this toxic metal in the environment and its harmful effects are very important and discussed topics worldwide [27, 28].

Many studies in humans [29, 30] and animals [31, 32] have shown that lead exerts the greatest harmful effects on the central nervous system, especially during development due to excessive cell proliferation, differentiation, and synaptogenesis that occur during this period [33, 34]. Therefore, lead exposure during brain development causes irreversible adverse effects on cell differentiation, proliferation, and synaptogenesis. However, several studies have shown that the brain is much more susceptible to lead in the early stages of development due to the immaturity of the blood- brain-barrier, resulting in higher lead entry, and a greater vulnerability to lead [35, 36].

In this study, pregnant mothers were exposed to lead and EMF during pregnancy and breastfeeding (21 days after delivery). The effect on embryos and adult mice was then examined [37, 38]. Studies have shown that lead causes degeneration of neurons and atrophy of the brain, with lead damage mainly in the cerebellum, prefrontal cortex, and hippocampus [39].

In this research, to evaluate the effect of EMF, tissue examinations were performed on three regions CA1, CA3, and DG of the hippocampus. After staining the hippocampal sections with cresyl violet, the cell density in the mentioned areas was checked. Cell counting showed that exposure of embryonic mice (fetal day 17) to electromagnetic waves with a frequency of 50 Hz and an intensity of 2 mT caused a significant decrease in the

number of granular cells in the DG region, as well as the number of pyramidal cells in the CA1 and CA3 regions compared to the sham group. There was also a significant decrease in the number of granular cells in the DG region, as well as pyramidal cells in the CA1 and CA3 regions compared to sham in embryonic mice fed lead. This is a result of the Hashem study. Hashem and colleagues in (2009) showed that lead causes a significant decrease in neurogenesis in different areas of the hippocampus [40]. In 2012, Baronoska Bosiaska reported that 10% lead exposure during pre- and postnatal development decreased activity of antioxidant enzymes as well as increased MDA in the brains of rat [41]. Data analysis showed that in the Pb+EMF group, a significant increase in the number of granular cells in the DG region as well as pyramidal cells in the CA1 and CA3 regions was observed compared to the Pb group. The result is that the decrease in cell density created in the hippocampus of lead-induced embryonic mice has been completely compensated by the use of electromagnetic waves. But the electromagnetic waves themselves alone have reduced cell density and as a result have not had much effect on cell density. Parkin and colleagues reported in 2015 that electromagnetic waves have many effects on low intensity and also affect neurology [42].

In this study, the exposure of adult mice (4 to 5 weeks old) to electromagnetic waves with a frequency of 50 Hz and an intensity of 2 mT caused a significant increase in the number of granular cells in the DG region, as well as the number of pyramidal cells in the CA1 region compared to the sham group. However, in the CA3 region, there was no significant difference in the number of pyramidal cells compared to the Sham group. Additionally, in the group of adult mice fed lead, a significant decrease in the number of granular cells in the DG region, as well as pyramidal cells in the CA1 and CA3 regions, was observed compared to the Sham group. Antonio and his colleagues reported that lead significantly reduces the number of granular cells during embryology and adulthood [32, 43].

The results showed that in the Pb + EMF treatment group, there was a significant increase in the number of granular cells in the DG region, as well as pyramidal cells in the CA1 and CA3 regions compared to the lead treatment group. The result is that the decrease in cell density created in the hippocampus of adult mice, caused by lead using electromagnetic waves, has a completely compensated effect of stimulation in the hippocampus of adult mice compared to embryonic mice. It can be claimed that electromagnetic waves in the hippocampus of embryonic mice can have a contrasting effect, as Timori and colleagues reported in 2017, increasing age reduces resistance to EMF prooxidant attacks [44].

Prochnow and colleagues also reported in 2011 a decrease in PSD95 gene expression and a decrease in plasticity in the hippocampus of mice exposed to electromagnetic waves [45]. The expression of the PSD95 gene in adult and embryonic mice in the Pb group relative to sham also experienced a significant decrease. Our results suggest that decreased synaptic density may be underpinned by a decrease in glutamate receptors in hippocampal neurons of early postnatal mice following EMF exposure, given that the reduction in PDS95 expression decreased receptor stability in dendritic spines. But in the treated mice of the Pb + EMF group, there was no significant difference in the expression of the gene compared to the Pb group. So, by changing the wavelength, frequency, and duration of treatment, you can probably see an increase in the gene in the Pb + EMF group. Also, the results showed that the expression of the synapsin II gene in the treatment group with EMF showed a significant decrease compared to the sham group in embryonic mice. The expression of the gene in the Pb group relative to sham was also associated with a significant decrease. But in the Pb + EMF group, there was a significant increase over the Pb group, which showed that EMF had activated the early differentiation of neurons by stimulating neurons in the affected areas. But given that the PSD95 gene is expressed in adult neurons, and there was no significant difference between the Pb and EMF groups in this study. Therefore, EMF did not cause the ultimate differentiation of neurons, and perhaps by changing the frequency, wave intensity, and duration of the differentiation of neurons to the stage of gene expression [46].

But in adulthood of the Pb + EMF group, there was no significant difference in synapsin II gene expression compared to the Pb group. Also, the results showed that there was no significant difference in the treatment group

with EMF compared to the sham group. Additionally, the expression of the Synapsin II gene in the Pb group compared to the sham group also showed a significant decrease. These results indicate that EMF had a detrimental effect on hippocampal neurons during adulthood, and that its neuronal protection effect was not observed in lead-treated mice.

The findings of this study provide compelling evidence for the significant impact of lead exposure and electromagnetic field (EMF) on the relative expression of Psd-95 and synapsin II in mice during embryogenesis and adulthood. The results demonstrate that lead exposure alone is associated with a significant decrease in the expression of Psd-95 and synapsin II in mice during embryonic development and adulthood. This suggests that lead exposure may have a detrimental effect on synaptic plasticity and neuronal function in the developing and adult brain.

Furthermore, the study findings reveal that the combination of lead exposure and EMF during embryonic development and adulthood results in a further decrease in the expression of Psd-95 compared to lead exposure alone. This suggests that the combination of lead exposure and EMF may have a synergistic effect on the expression of Psd-95, leading to a more significant decrease in its expression. This finding is particularly concerning given the ubiquity of EMF in modern society and the widespread exposure to lead in the environment. Interestingly, the expression of the Synapsin II gene showed a significant increase over the Pb group during the fetal period in the Pb + EMF group. This finding suggests that the combination of lead exposure and EMF may have a differential effect on the expression of synapsin II compared to Psd-95. The underlying mechanism for this differential effect is unclear and requires further investigation. The findings of this study have important implications for our understanding of the impact of environmental factors on brain development and function. The study provides evidence that lead exposure and EMF can have a significant effect on the expression of Psd-95 and synapsin II, which are critical proteins involved in synaptic plasticity and neuronal function. The study also highlights the potential for a synergistic effect between lead exposure and EMF on the expression of Psd-95, which could have significant consequences for brain development and function.

CONCLUSIONS

Based on current research, it is recommended that health policy makers consider the potential benefits of EMF exposure in mitigating the adverse effects of lead exposure in pregnant mothers and developing embryos. This could include further research to determine the safest and most effective methods of EMF exposure, as well as the development of guidelines for EMF use in pregnant women and young children. Additionally, it is recommended that health policymakers continue to prioritize efforts to reduce lead exposure in the environment and in consumer products. This includes regulations on lead in paint, gasoline, and other sources, as well as education and outreach efforts to inform the public about the dangers of lead exposure and how to reduce their risk. Furthermore, it is recommended that health policymakers consider the potential impact of EMF exposure on neurodevelopment and brain function in human populations. This includes funding for further research into the effects of EMF exposure on the brain, and the development of guidelines for safe levels of EMF exposure. Finally, it is important to note that these findings are specific to lead exposure and EMF exposure during the fetal and embryonic period in mice.

The new neuron production is a phenomenon that occurs in the hippocampus of adult mice as well as in the brains of several species of mammals is directly related to neural plasticity. There is also strong evidence that neurogenesis occurs in the adult human hippocampus of human. In addition some factors disrupt this process in humans. However, how the neurogenesis process is carried out and the factors affecting neurogenesis in humans remain unclear. EMF with different radiation patterns and frequencies have contradictory effects on the process of neurogenesis and subsequently on neural plasticity and cognitive functions. However, animal experiments in recent years have shown that low-frequency EMFs, mainly improve neurogenesis and cognitive processes.

An experimental study on the adult mice model of hippocampal Injury showed that EMF stimulates new

neuron production in the dentate gyrus, and improves cognitive functions in these animals.

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Conflict of interests

There are no conflicts of interest to declare by the authors.

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Data availability

The datasets and results are available from the corresponding author on reasonable request.

Supplementary information

No applicable.

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