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Effect of Electromagnetic Filed (EMF) Equal to that Emitted form Cellular Phones on The Layers of Cerebral Cortex of The Frontal Lobe of Male Albino Rats of Different Ages

Histology

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ABSTRACT

Background: Cell phone users increased over the world and the question is whether health hazards could result from microwaves of these instruments.

Aim of the work: Studying the effect of Electromagnetic Filed (EMF) similar to that emitted from cellular phones on the histological structure of frontal cerebral cortex of different ages.

Material and Methods: Sixty male albino rats were used in this study divided into control group (I), EMF exposure group (II) and recovery group (III) .Each of the three groups of twenty rats and subdivided according to the age into 3 subgroups: adult of seven rats, young of seven rats and middle age of six rats. Control group left without interference. EMF exposure group exposed by EMF generator to electromagnetic field near the mobile phone pulsing signal of 217 Hz 3 h/day for 6 days/week for 1.5 month then sacrified. Recovery group left for 1.5 month to recover after exposure before sacrifaction At the study end, paraffin sections were prepared from cerebrum of all rats then subjected to hematoxylin and eosin and immunohistochemical stain for Glial Fibrillary Acidic Protein (GFAP). Statistical analysis for assessment of frontal cortex thickness, number of its cells and GFAP stained area

Result: EMF groups showed shrunken cells with darkly stained nuclei with pericellular haloes and congested vessels with increased immunoreactivity for GFAP. On recovery decrease number of pyknotic nuclei and decreased immunoreactivity for GFAP.

Conclusion: EMF induces histopathological and immune histochemical changes in frontal cortex and withdrawal from EMF can reverse or decrease these changes.

Keywords: *EMF; Phone; Recovery; Frontal cortex.*

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INTRODUCTION

There is an increase in cell phone users in the world. We ask could microwaves of these instruments cause health affection .Disturbance in cells metabolic function is expected with their exposure to magnetic field with very large strength relative to cells biomagnetic field leading to their death or increased division.[1]

Various cellular changes may occur on exposure to extremely low frequency electromagnetic field.[2]

Frontal lobe: is the largest lobe, has significant functions for our body as prospective memory that involves remembering the plans that you made, speech production, personality. Making decisions and movements control.[3]

Frequent using of mobile phones daily led to a concern of cancer risk possibility from frequent exposure to radiofrequency radiation. However, this relation is not raised to be a clear scientific evidence.[4]

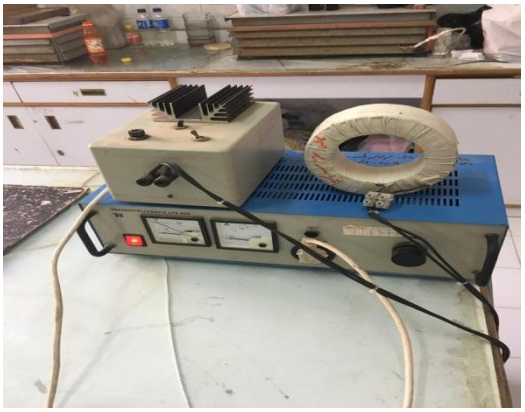
Increased lipid peroxidation and oxidative DNA damage formation in rats frontal lobe may result from exposure to 900, 1800 and 2100-MegaHz released from mobile phones.[5]

Our objective in the research To shed light on the changes induced by low frequency Electromagnetic Filed (EMF) similar to that emitted form cellular phones on the histological structure of cerebral cortex in the frontal lobe of male albino rats of different ages.

MATERIAL AND METHODS

a- EMF generator:

Electromagnetic field (EMF) generator (trannstelltrafo. LTS (602), Sewed). It was available by the international center for advanced researches (ICTAR Egypt) at Al-Azhar University, Cairo, Egypt. It generates an electromagnetic field frequency (0.035mT) and adjusted by Tesla meter digital counter near to the pulsing signal of mobile phone of 217 Hz.[6]



b) Animals

The study included sixty adult male albino rats. They were obtained and handled according to the guidelines after the approval of the ethical committee (CU III F 87 17) from the Animal House-Faculty of Medicine at Al-Azhar University. All the animals were handled for a period prior to experiment to minimize nonspecific stress on the days of the experiment, and were kept at room temperature (24-26C) with a 12:12h light: dark cycle and provided with standard crushed food and water during the experiment.

Experimental design: The experiment was done in the research unit at the histology department Faculty of Medicine at Al-Azhar University. Rats were equally dispensed into three main groups each of which subdivided into three groups according to age:

Group I Control Group

Twenty rats were used, left without any interference. Divided into three subgroups:

Adult group I (A):7 rats aged five months.
Young group I (Y):7 rats aged one month.
Middle age group I (M): 6 rats aged twelve months.

Group II (EMF exposed group)

All exposed to frequency EMF near to the emitted signal of mobile phone of 217 Hz three hour daily for six days per week for one and half month, this group divided into three subgroups.

Adult group II (A):7 rats aged five months
Young group II (Y):7 rats aged one month
Middle age group II (M):6 rats aged twelve months

Group III recovery group rats leaved for spontaneous recovery for 1.5 months after exposure to EMF as described in group II this group divided into three subgroups.

Adult group III (A): 7rats aged five months.
Young group III (Y): 7rats aged one month.
Middle age group III (M): 6 rats aged twelve months.

At the end of experiment (according to the group EMF group after 1.5 month and recovery group after 3 months from the experiment beginning) animals were euthanized by IP injection of sodium pentobarbital (100 mg/kg).[7] Then the animals were given intracardiac injection of 10% formalin. Skull of each animal was opened and cerebrum was immediately dissected out and divided into two parts:

The first part from each cerebrum was fixed in 10% buffered formalin solution, dehydrated in ascending grades of ethanol and embedded in paraffin. Serial sections of 7 µm thickness were cut and subjected to the

1. Light Microscopic study

- Haematoxylin and eosin stain.[8]

- Immunohistochemical stain for Glial Fibrillary Acidic Protein (GFAP) of the astrocyte.[9]

2. Statistical Analysis

Non overlapping fields from all groups were analyzed measuring frontal cortex thickness and counting cells number were done by image j analyzing software ,also GFAP stained area was measured by the same program [Image J 1.53e Wayne Rasband and contributors National institute of Health ,USA http://imagej.nih.gov/ijJava 1.8.0_172(64-bit) 4490K of 3000MB({1%)]. Comparison between normally distributed (parametric) quantitative data of more than two groups was conducted using ANOVA test by (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp. followed by post-hoc for multiple comparisons

NB: The P value of $\leq$ 0.05 indicates significant results at confidence interval.[10]

The measurements were done in 10 high power fields (HPF) in the experimental groups in measuring thickness and counting the cells number. Measurements were done in 200 (HPF) in measuring GFAP stained area using the binary mode.

RESULTS

Light Microscopic Results:

Hematoxylin and eosin stained sections (Figures 1, 2 and 3)

The control adult young and middle age groups revealed the frontal cortex covered with pia matter. Below it, six layers named: molecular, external granular, external pyramidal, internal granular, internal pyramidal and multiform layers.

The molecular and external granular layers formed of few neurons, granule cells and neuroglial cells in pink background of neuropil. (Figures 1A, B and C)

The external pyramidal layer had varying sizes of pyramidal cells with scattered granular cells.

The internal granular and internal pyramidal layers showed normal pyramidal cells and normal granule cells. Glial cells appeared smaller in size with small deeply stained nuclei in the intercellular area (neuropil).

The inner most multiform layer had variety of neurons of different shapes and sizes.

Neurons divided into pyramidal cells had a long apical dendrite, basophilic cytoplasm and large vesicular nucleus and granular cells had round cell bodies and large rounded open face nuclei.

Young and middle age group appeared less in thickness relative to adult. The middle age groups were similar to adult but other pyramidal cells appear with rounded dark nuclei and acidophilic cytoplasm. (Figures 1 D, E and F)

In EMF exposed group, the frontal cortex exhibited many shrunken cells with dark stained nuclei with little acidophilic cytoplasm .Cells are surrounded by empty spaces. Also congested blood vessels and vacuolated neuropil are present in some areas in neuropil. (Figure 2 A, B and C)

Frontal cortex of adult and young recovery rats revealed many pyramidal granular cells with apparently normal appearance. There are also few shrunken distorted pyramidal dark stained nuclei surrounded by empty space also the congestion in the pia matter and some neuropil vessels still. (Figure 3 A and B)

In middle age recovery group few pyramidal and granular cells had apparently normal appearance, while many darkly stained nuclei shrunken ones surrounded by empty space were still present also congestion in pia matter and neuropil still present. (Figure 3 C)

Immunohistochemically stained sections for GFAP (Figure 4, 5 and 6)

Control adult and young groups showed weak positive immunostaining in GFAP immunoreaction stained area as in cytoplasm and processes of astrocytes. They appear small (Figure 4 A and B)

The middle age control group showed moderate positive GFAP immunoreaction stained area as in astrocytes cytoplasm and processes (arrows) appearing enlarged (Figure 4 C)

EMF exposure group in all ages show strong positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes (arrows) appearing enlarged (Figure 5 A, B and C)

The adult and young rat of recovery showed moderate positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes appearing enlarged (Figure 6 A and B)

The middle age recovery group showed strong positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes appearing enlarged (Figure 6 C)

Morphometric and statistical results (Table 2 and Histograms 1-3)

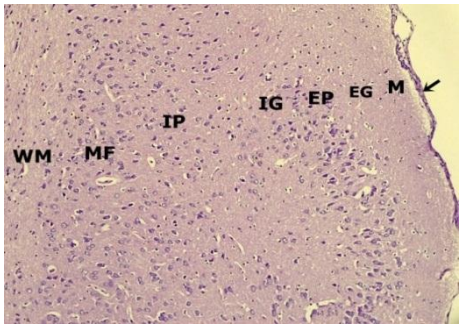
The mean thickness and number of cells of frontal cortex in control adult show highly significant increase if compared with control young and middle rats in adult and young rats (group II A) was highly significant lower as compared to control adult and non-significantly lower in thickness but significantly lower in cells number as compared to recovery adult (group III A).

(Group II M) was none significantly lower than control middle age (group I M) and (group III M).

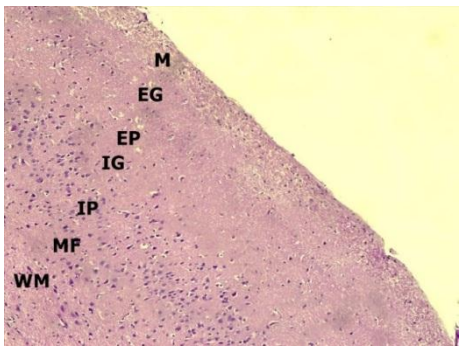
Concerning the mean of percentage area of positive GFAP in control middle age (group I M) showed highly significant increase than control adult and young groups.

(Group II A, Y) showed highly significant increase as compared to control adult and recovery adult and young (group III A) groups.

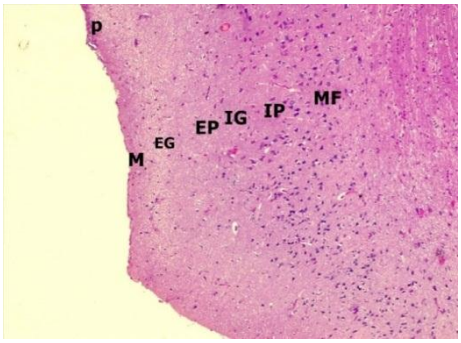
EMF middle age (group II M) show highly significant increase than control middle age (group I M) and non-significant increase as compared to recovery middle age (group III M).



1A

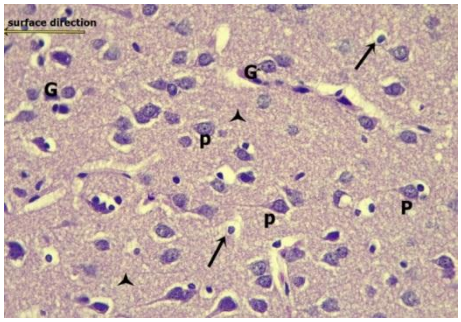


1B

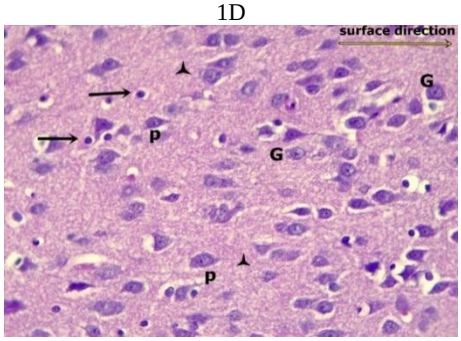


1C

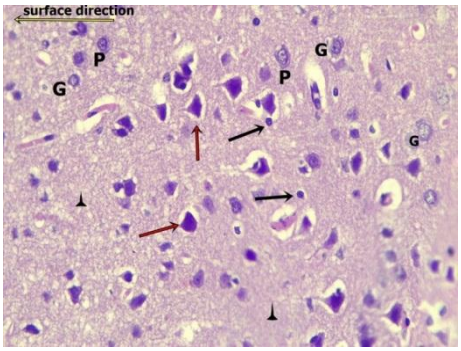
Fig. 1 A, B and C: Frontal cortex of all ages in control rats stained with H&E (x100) Molecular layer (M) followed by External granular (EG), External pyramidal (EP), Internal granular (IG), Internal pyramidal (IP) and Multiform (MF) layers. Part of white matter (WM) appears.



1D



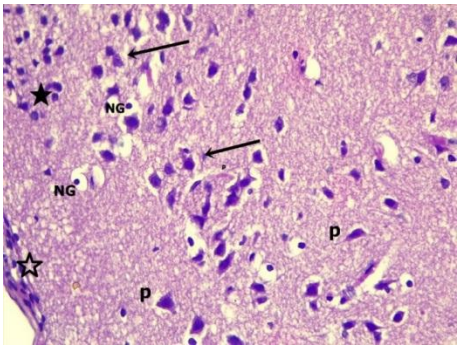
1E



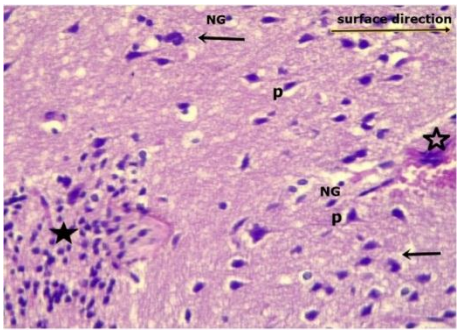
1F

Fig. 1 D and E: Frontal cortex of rats H&E (x400) control adult and young groups external pyramidal, internal granular, internal pyramidal layers showing pyramidal cells (p) with rounded vesicular nuclei and multipolar shape with basophilic cytoplasm. Granular cells (G) with rounded open face nuclei, prominent nucleolus and little cytoplasm. Pink stained background is the neuropil (arrow heads). Neuroglial cells (arrows).

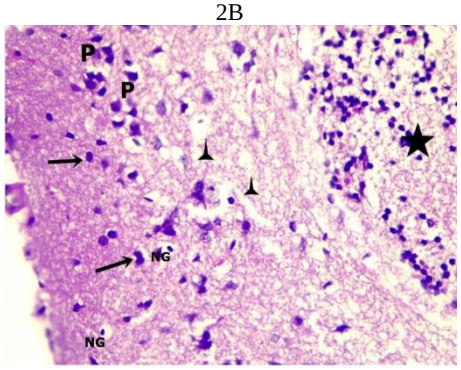
Fig. 1F: Control middle aged internal granular, internal pyramidal and multiform layers. Some pyramidal cells (p) with pale nuclei, prominent nucleoli, and multipolar shape with basophilic cytoplasm. Other pyramidal cells appear with rounded dark nuclei acidophilic cytoplasm (red arrows). Granular cells (G) had rounded open face nuclei, and little cytoplasm. Neuroglial cells (arrows) are seen.



2A

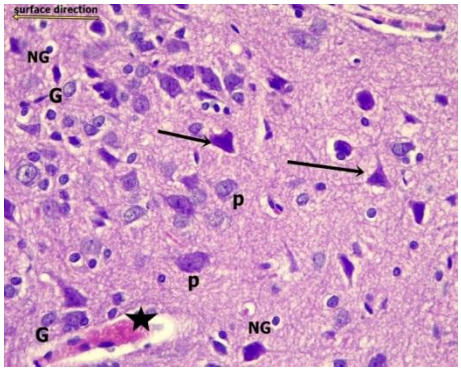


2B

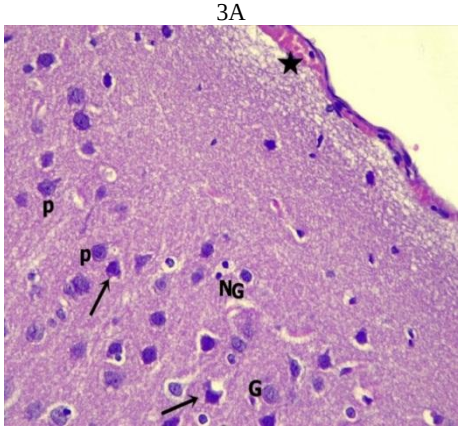


2C

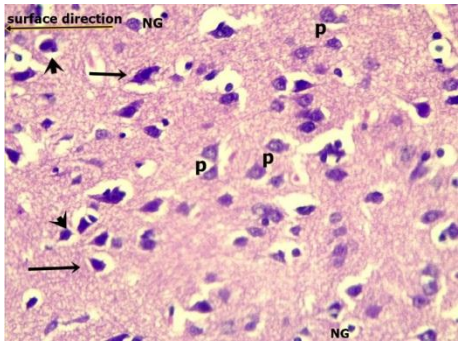
Fig. 2 A, B and C: Frontal cortex of all ages of EMF rats H&E (x400) showed many shrunken cells with small darkly stained pyknotic nuclei and little acidophilic cytoplasm with empty space around (arrows) Shrunken distorted pyramidal cells (p) are seen and inflammatory infiltration (black asterisk) neuroglial cells (NG). Congested blood vessel (empty asterisk) in EMF adult and young groups. Vacuolated neuropil in EMF middle age group (arrow heads).



3A



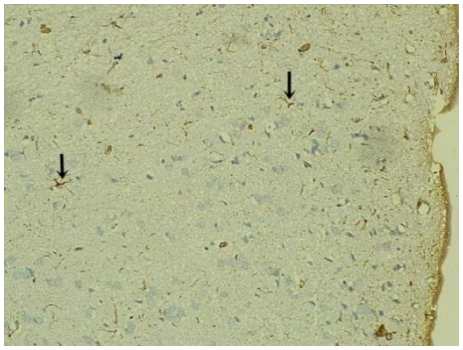
3B



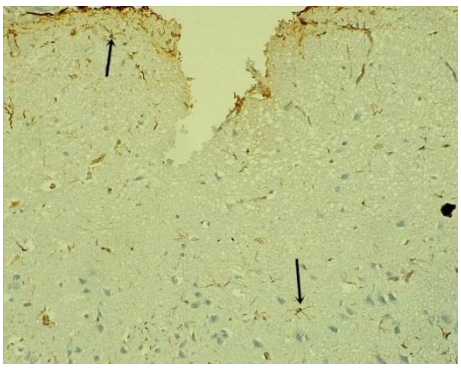
3C

Fig. 3 A and B: Frontal cortex of adult and young recovery rats H&E (x400) many pyramidal cells (p) multipolar shape with rounded nuclei and basophilic cytoplasm. Granular cells (G) with round open face nuclei, prominent nucleolus and little cytoplasm. Also few shrunken distorted pyramidal cells with dark stained nuclei (arrows). Congested blood vessel still (asterisks). Neuroglial cells (NG).

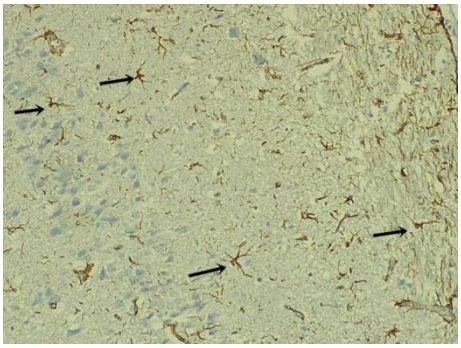
Fig. 3C: Many shrunken cells with small darkly stained nuclei and little acidophilic cytoplasm with empty space around (arrow head). Shrunken distorted pyramidal cells (arrows) surrounded by empty space are demonstrated. Few pyramidal cells (p) approximately normal .neuroglial cells (NG).



4A

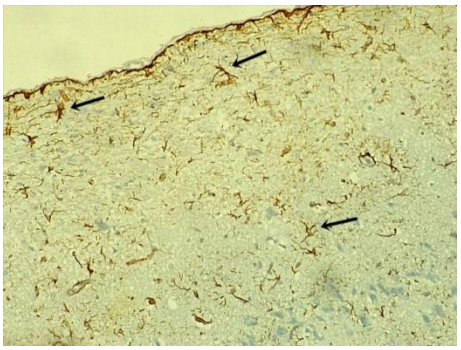


4B

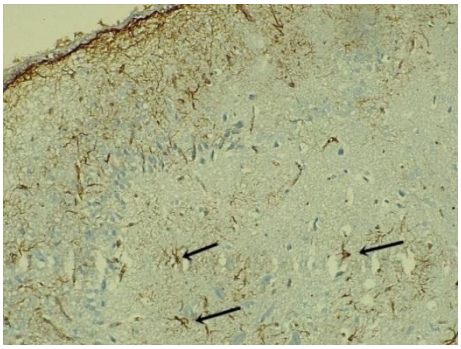


4C

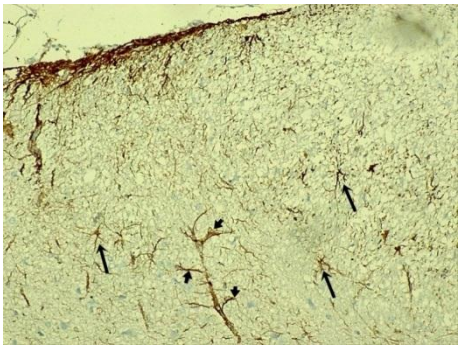
Fig. 4 A and B: Frontal cortex of adult and young control rats stained with GFAP immunostaining (x200) demonstrating the pia matter superficial half of cortex showed faint positive GFAP immunoreaction stained area as in cytoplasm and processes of astrocytes. They appear small. **Fig.4C:** in middle age control group showed moderate positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes (arrows) appearing enlarged.



5A

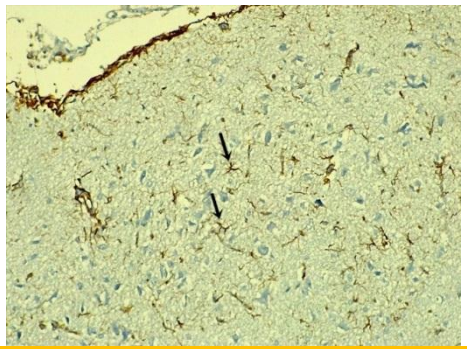


5B

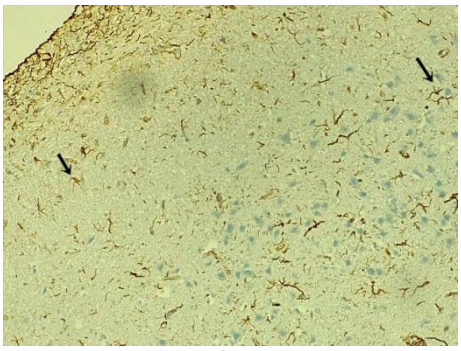


5C

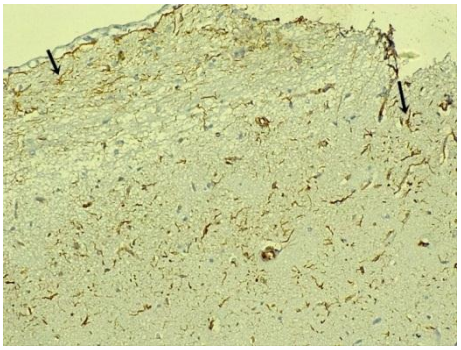
Fig. 5 A, B and C: Frontal cortex of EMF exposure rats GFAP immunostaining (x200) in all ages strong positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes (arrows) appearing enlarged.



6A



6B



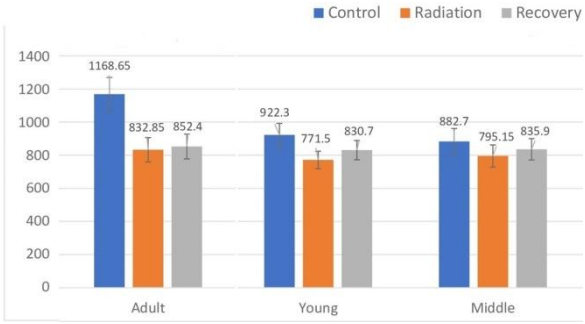
6C

Fig. 6 A and B: Frontal cortex of young and adult recovery rats GFAP immunostaining (x200) showing moderate positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes appeared enlarged **Fig. 6C:** middle age recovery group showing strong positive GFAP immunoreaction stained area as astrocytes cytoplasm and processes appearing enlarged.

Age		Thickness of frontal cortex in pixels										
		Control		Radiation		Recovery		ANOVA		Tukey's test		
		Mean	SD	Mean	SD	Mean	SD	f	P-value	P1	P2	P3
Adult		1168.65	100.55	832.85	73.57	852.4	75.08	30.221	<0.001*	<0.001*	<0.001*	0.658
Young		922.3	69.75	771.50	52.56	830.7	58.19	9.434	0.002*	0.002*	0.033*	0.094
Middle		882.7	78.60	795.15	66.84	835.9	64.4	2.335	0.131	0.065	0.286	0.307
ANOVA	f	275.403		5.070		1.754						
	P-value	<0.001**		0.007*		0.176						

P1 = Control & Radiatio n, P2 = Control & Recovery, P3 = Radiation & Recovery

Table 1: The mean thickness of frontal cortex in all groups.

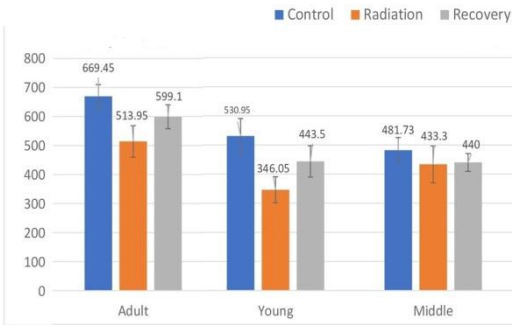


Histogram 1: The mean thickness of frontal cortex in all groups.

Age		Cells number in frontal cortex in 500x800pixel										
		Control		Radiation		Recovery		ANOVA		Tukey's test		
		Mean	SD	Mean	SD	Mean	SD	f	P-value	P1	P2	P3
Adult		669.45	40.32	513.95	54.59	599.1	41.23	17.308	<0.001*	<0.001*	0.014*	0.012*
Young		530.95	59.78	346.05	44.59	443.5	53.89	18.190	<0.001*	<0.001*	0.024*	0.007*
Middle		481.73	43.83	433.3	63.27	440	30.78	1.804	0.199	0.154	0.085	0.820
ANOVA	F	18.310		1937.852		22.026						
	P-value	<0.001*		<0.001*		<0.001*						

P1 = Control & Radiation, P2 = Control & Recovery, P3 = Radiation & Recovery

Table 2: The mean cells number in frontal cortex in all groups.

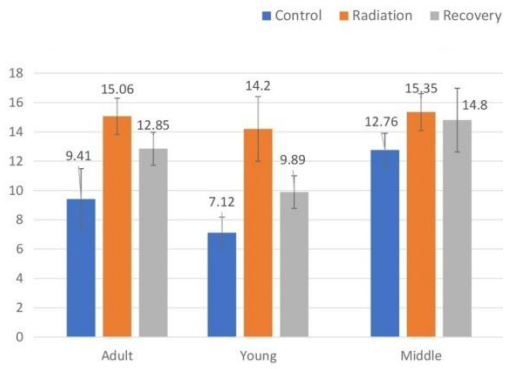


Histogram 2: The mean cells number in frontal cortex in all groups.

Age		GFAP STAINED AREA %										
		Control		Radiation		Recovery		ANOVA		Tukey's test		
		Mean	SD	Mean	SD	Mean	SD	f	P-value	P1	P2	P3
Adult		9.41	2.078	15.06	1.238	12.85	1.125	68.350	<0.001*	<0.001*	<0.001*	<0.001*
Young		7.12	1.070	14.2	2.212	9.89	1.113	104.950	<0.001*	<0.001*	<0.001*	<0.001*
Middle		12.76	1.142	15.35	1.256	14.8	2.174	14.685	<0.001*	<0.001*	0.002*	0.333
ANOVA	f	21.402		0.805		15.215						
	P-value	<0.001*		0.466		<0.001*						

P1 = Control & Radiation, P2 = Control & Recovery, P3 = Radiation & Recovery.

Table 3: The mean percentage area of positive GFAP in frontal cortex of all groups



Histogram 3: The mean percentage area of positive GFAP in frontal cortex of all groups.

DISCUSSION

Exposure to extremely low frequency electromagnetic field may cause various cellular changes.[2]

Light microscopic examination of H&E stained sections of the frontal cortex revealed decreased thickness in control young (group IY) relative to control adult (group IA). Similar to results obtained by Ryan et al.[11]

Control middle age (group IM) showed decreased thickness than adult group similar result in comparison middle to adult rats is recorded by Lemaitre et al.[12]

In the present study in the control middle age (group IM) revealed shrunken neurons had dark stained pyknotic nuclei contribute to the shrinkage and cortical thinning of the aging brain. Similar results obtained by Alexis.[13]

In our study there was decrease in cells number in control young (group IY) control middle age (group IM) relative to control adult (group I A). This can be explained by that major increase in neurons number occurs between 1 and 2–3 months of age, followed by a significant widespread and progressive neuronal loss that begins at 3 months and the reduction in numbers become evident at 12 or 22 months.[14]

In the present study, examination of H&E stained sections of the frontal cortex exposed rats group II revealed many shrunken cells having small dark pyknotic nuclei, little cytoplasmic acidophilia and empty spaces around cells. These criteria as signs of apoptosis were described by Elmore et al and Eser et al.[15, 16]

Apoptosis is one of the final outcomes during the process of MW radiation-induced brain damage.[17]

Ca2+ influx through voltage-dependent Ca channels in EMF exposed cells result in cell activation and an antioxidant response. With more activation oxidative stress causing DNA damage or cell death may follow.[18]

Lipid peroxidation previously mentioned by Mehmet etal[5] can be explained by Blessy stating that oxidative damage occurs in the form of lipid peroxidation. Products of membrane lipid peroxidation and other oxidants like H 2 O 2 may react with superoxide dismutase causing oxidative modification causing loss of enzyme activity. Reactive oxygen species (ROS) start the radical peroxidation chain reactions by attacking unsaturated fatty acids containing multiple double bonds with reactive hydrogen atoms. Oxidative stress involves complex processes and change of many cellular components by ROS including proteins, nucleic acids and lipids.[19]

Due to high needs for oxygen and high concentration of polyunsaturated fatty acids brain is a target organ for oxidative damage (free radicals).[20]

The previous causes can explain the resulted decrease in thickness and cell loss in all groups after EMF exposure. In a study on cerebellum after A 900-MHz EMF causes apoptosis (as explanation) there was decreases in the layers compared with control group.[21]

In our study there were inflammatory cell infiltrations after EMF exposure similar results by Sibghatullah et al in the brain parynchema after EMF 900MHz 1hr/day.[22]

Empty spaces or vacuolation, distorted pyramidal cells and congested blood vessel after exposure to EMF in the present study can be explained by the results in a study in H&E stained sections obtained from 900 MHz exposed animals revealed most nerve cells shrunken with loss of their processes and peri-cellular halos. The affection was more to pyramidal cells as they lost their processes with shape irregularity. With higher EMR (1800MHZ) animals showed more severe degeneration in nerve cells, vacuolated neuropil in all layers and congestion in many blood vessels.[23]

Congestion in the vessels and RBCs extravasation in a myocardial study, may be due to free radical generation with EMF.[24]

In recovery groups (III) pyramidal or granular cells showed approximately normal appearance this can be supported by persistent appearance of newborn cortical neurons after stroke.[25]

Also from adult brains multipotent neural stem cells were isolated.[26]

With exposure to media containing epidermal growth factor, stem cells proliferation and differentiation into neurons and glial cells can occur in vitro. The

ependymal cells lining lateral ventricle have been further traced as the possible origin of the stem cells residing in the subventricular zone.[27]

Neurogenesis can explain increased thickness after recovery from EMF as a cause of cerebral injury. Neurogenesis in adult humans is a controversial topic in neuroscience field. While some researchers report that a sharp drop in neurogenesis as the human brain ages.[28]

Others report that neurogenesis in the dentate gyrus of the hippocampus of human brains continues into old age.[29]

Also, some researches revealed Prefrontal cortical thickening after mild traumatic brain injury.[30]

In our study there is highly significant increase in GFAP stained area in control middle age (group IM) as compared with control young (group IY) and control adult (group IA)

This is supported by Goodall et al stated that the cortex and the cerebellum of the mouse cohort GFAP expression significantly correlated with aging in mice.[31]

Also in our study showed highly significant increase in GFAP stained area in all EMF exposure groups. Similar results revealed an apparent increase in the number of the star-shaped astrocytes with intense GFAP immunoreaction by Ammari et al.[32]

Increased GFAP-reactivity has been associated with neuronal injury.[33]

In our study highly significant decrease in GFAP stained areas in recovery adult (group IIIA) and recovery young (group IIIY) groups as compared to radiation group of the same age. This can be explained by that acute trauma to the brain cause gliosis, most often in its severe form with the development of a glial scar. Diffuse traumatic injury can cause diffuse or more moderate gliosis without scarring. In such cases, gliosis may also be reversible. In all instances of gliosis resulting from CNS trauma, the long-term clinical outcome is highly dependent on the degree of astrogliosis and scar formation.[34]

Spontaneous regression of pilocytic astrocytoma (astrocyte tumor) in children, young adults also in a unique case of a woman 64-year old shortly regressed before surgery.[35]

CONCLUSION

EMF exposure induce histopathological and immune histochemical alternation in frontal cerebral cortex and these changes can be reversed or decrease with withdrawal of EMF exposure.

Conflict of interest : none

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