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Effect of Radiofrequency Electromagnetic Radiation on the Spinal Cord of Albino Rats: A Neurohistological Study

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Abstract: Mobile phone usage has become an essential component of daily life. Several studies have raised concerns about the possible deleterious effects on the nervous system due to exposure of electromagnetic radiations. We aimed to study the effect of radiofrequency electromagnetic radiation (RF-EMR) on the spinal cord of albino rats. In the present study twenty-four adult albino rats of either sex weighing 180-200 grams each were divided into four groups: 1 control and 3 experimental and were exposed to RF-EMR via complete missed calls of 45 seconds duration each. Both the experimental and control groups were then sacrificed and spinal cord was isolated for tissue processing. The processed tissue was then stained (hematoxylin and eosin) and observed under light microscope. Light microscopy of the spinal cord showed the cellular size of the neuronal cells was reduced in dorsal and ventral horn interneurons, their nucleus was heterochromatic. Neurons were irregular, loosely arranged, darkly stained and decreased in size. Ventral horn cells of the spinal cord showed absent cytoplasm, heterochromatic nucleus with invisible nucleoli. Dose dependency with more radiation exhibit more changes in comparison to less exposed rats. From our findings, it is suggestive to protect the population living around base stations and users of mobile handsets. For this the government and regulatory bodies adopt safety standards which minimizes the hazardous effects of mobile radiation.

Keywords: Radiofrequency electromagnetic radiation (RF-EMR), Albino rats, spinal cord, Light microscopy, Hematoxylin and eosin staining, Electromagnetic field (EMF).

INTRODUCTION

The discovery of radiofrequency waves in the early 1980s in the Nordic countries and 1990s in the United States came into widespread use. The cell phone, which emits non ionizing radio waves through an antenna, is commonly held close to the head [1]. The development of mobile communications has moved rapidly. First generation mobile phones allowed transmission of sound only during the early 1980, s. Digital transmission, and the global system for mobile communication, started in 1991 and include such new developments as data and image transmission. Presently in the modern world mobile phone offer internet access, fax and emailing facility. For both analogue and digital mobile phones, the signals transmitted and received are in the form of waves in the radio frequency (RF) and microwave parts of the electromagnetic spectrum. Wavelengths of non-ionizing radiation varies in the range of 3 kHz to 300 MHz and microwaves range from 300 MHz to 300 GHz. Mobile phones and telecommunication networks operates with frequencies ranging from 900 MHz to 1.8 GHz and up to 2.1 GHz, although it the wavelength varies with different types of mobile phones. These frequencies are applicable for both mobile and their base station to send and receive Calls [2].

With the rampant use of mobile phones, people have accepted this technology without the knowledge of potential health hazard. There are 6.5 billion mobile phone users worldwide, 2.5 billion of these users are from Asia Pacific. Soon this number will cross the world population. However, alarms about the possible deleterious effects on health, as a result of the exposure to RF and microwave electromagnetic fields, have been voiced since the introduction of mobile phones as RF-EMR can penetrate deep into organic tissues and get absorbed producing many biological effects in the human body. This ubiquitous exposure to an emerging technology prompted the initiation of large-scale health studies (some started over 20 years ago) in the United States and throughout the world [1]. Spinal cord receives the long axons of upper motor neuron fibers from the motor cortex and synapses with lower motor neurons fibers which innervate the skeletal muscles.

Spinal cord receives primary afferent fibers from peripheral receptors located in widespread somatic

and visceral structures, and sends motor axons to skeletal muscle. It also contains the cell bodies of all the preganglionic neurons responsible for the sympathetic innervations of cardiac and smooth muscle and secretory glands, and for the parasympathetic innervations of smooth muscle in the erectile tissues of the external genitalia, distal part of the hind gut, the pelvic viscera. In majority of the neurons, the action potential initiates in the peripheral receptors and propagates to the posterior root of the spinal cord and synapses with other neurons and the sensation, reaching the brain. The dorsal root ganglia, which develops from the neural crest cells, and hence spinal ganglia regarded as gray matter of the spinal cord. Several studies investigated the effects of radio frequency radiation on evoked potentials in the brain. Recorded evoked potentials in the thalamus and spinal cord of cats, power density evoked potential was observed. These data were interpreted as RF-EMR affected the multisynaptic neural pathway [3]. Recorded spinal cord ventral root responses to electrical stimulation of ipsilateral gastrocnemius muscle, nerve in rats, the spinal cord was irradiated with continuous wave 2540MHz Radiofrequency radiation on the incident power of 7.7W. Decrease in latency and amplitude of the reflex response were observed during exposure of 3 minutes and the responses were returned to normal immediately [4].

In the view of the fact that the spinal cord is involved in transmission of action potentials in the neurons from the receptors to the central nervous system and serve an important function and RF-EMR might have deleterious effects on it. We attempted to study the effect of radiofrequency electromagnetic radiation (RF-EMR) on the spinal cord of albino rats under light microscopy and to evaluate such changes after exposure to graded doses of RF-EMR.

MATERIAL AND METHODS

The present study was carried out in the department of Anatomy, Jawaharlal Nehru medical college, AMU Aligarh. The ethical approval was obtained from above mentioned institution. Twenty-four adult albino rats of either sex weighing 180-200 grams each were included in the study. The rats were sheltered in plastic cages of size 36 cm × 23 cm × 21 cm (three/four rats in each cage) with controlled temperature and humidity environment & provided with a standard pellet laboratory diet (Lipton India Limited) and water ad-libitum. The animals were weighed, marked and divided into four groups based on the number of calls/day they received. The rats were exposed to RF-EMR by giving complete missed calls of 45 seconds duration each one after the other, every day for 4 weeks, keeping a GSM (0.9 GHz/1.8 GHz) mobile phone in silent mode (no ring tone & no vibration) in the cage.

Four groups were as under:

Control Group

CTRL : exposed to NIL calls/day

Experimental Groups

E80 : exposed to 80 calls /day
E120 : exposed to 120 calls /day
E160 : exposed to 160 calls /day

Tissue Processing for Neurohistology

Procurement of the tissue

After proposed experimental duration of 4 weeks, exposure the animals of both the experimental and control groups were sacrificed by giving overdose of diethyl ether vapors and the heart was exposed through the thoracic approach. The blood transfusion set needle was introduced into the left ventricle (apex) and a nick was made in the right atrium. After the saline wash, Karnovsky fixative was then infused till the body tissues showed signs of fixation.

After a couple of days, the brain was approached through the dorsal aspect of the skull. A T-shaped incision was given on the dorsal aspect of the head and the skin was reflected. The skull was incised at the midline fissure using a pair of scissors. The scissors were lifted up while cutting to avoid any damage to cerebral. Dorsal part of the skull was excised using curved forceps. The brain was removed by releasing it gently from all its attachments from below and sides. Brain was cleaned and washed in tap water. After removing the brain, Spinal cord was approached from back. Skin and other soft tissues were removed until vertebral column was exposed. Bony parts and others attachments were dissected out in cervical, thoracic and lumbar region. Spinal cord with dorsal root ganglion was removed very gently and put into karnovsky fixative.

Tissue Processing

Each tissue was then dehydrated in ascending grades of ethyl alcohol for 30 minutes each separately:

50% Alcohol → 70% alcohol → 90% alcohol → absolute alcohol → xylene I & II (clearing agent) for 1 to 1.5 hrs → impregnation was done using molten wax and xylene mixture (1:1) for 1 hr → 100% wax for 1 hr → embedding in wax, blocked, labeled & stored.

Sectioning was done using rotary microtome at 7 to 10 µm thickness with ribbon formation → 3-4 sections length ribbon selected at 10 section interval with the help of water bath warmed at 50 degree centigrade → the tissue sections were picked up on the egg albumin smeared glass slides. Slides were dried and labeled properly and stored.

Hematoxylin & Eosin Staining

Deparaffinization & Hydration

Slides with paraffin sections were deparaffinized in xylene for 15 min. Hydration done with descending grades of alcohol 5 min. each as under.

Absolute alcohol → 90% alcohol → 70% alcohol → 50% alcohol then distilled water.

Staining

Slides were kept in Hematoxylin for 10 min → Washed with tap water to remove excess hematoxylin → Bluing – section dipped in 1 % HCl for 1 second then washed with tap water for 10 - 20 min. (controlled by repeated checking under light microscope).

Counter staining

The slides were then dipped in Eosin (1%) for 5 min. Sections were then dehydrated by one time dipping in ascending grades of ethyl alcohol → 50% alcohol → 70% alcohol → 90% alcohol → Absolute alcohol → Slide dried in the air → Cleared in xylene for 15 min. → Permanent mounting in DPX → Labeled and stored.

Cresyl violet staining

Slides with paraffin sections were deparaffinized with xylene for 15 min. Hydration was done with descending grades of alcohol 5 min. Each as under- Absolute alcohol → 90% alcohol → 70% alcohol → 50% alcohol then distilled water. Then the slides were stained in 0.1% cresyl violet for 3 minutes. Then the slides were rinsed in tap water to remove excess of stain. After that slides were again dehydrated in ascending grades concentration of ethyl alcohol (50%, 70%, 90% and absolute alcohol). Then the slides were cleared in xylene for a few minutes and afterward mounted with DPX.

OBSERVATIONS

Light microscopy and paraffin sections

Spinal cord: Transverse section of spinal cord showing lighter and darker areas (Fig. 1). Darker area is H- shaped grey matter. Anterior, posterior and lateral grey columns are visible (Fig. 1 and 2). Cell bodies of neurons are of larger size in the anterior column, smaller and less prominent in the posterior column and of intermediate size in lateral grey column (Fig.3 and 4).White matter is composed of remaining lighter areas. Deep anterior fissure and shallow posterior median septum of the white matter can be observed. Dorsal grey commissure connecting two halves of the grey matter and ventral white commissure connecting two halves of the white matter can also be seen. Photomicrographs from experimental rats are showing marked morphological changes.

In fig. 5 (A) neurons in the dorsal horn are very small size and their nucleus is heterochromatic. The same thing can be observed in ventral horn interneurons as well (Fig. 2). Fig.6 represents photomicrographs from dorsal (A) and middle (B) part of dorsal horn of spinal cord from E-120 rats. Both pictures show the irregular and loosely arrangement of neurons. They are darkly stained and size is reduced. Nucleus and nucleoli cannot be differentiated. Fig. 7 from E-120 rats is showing the ventral horn of the spinal cord. Few neurons are very small and very darkly stained. Cytoplasm is totally absent. The nucleus is highly heterochromatic with invisible nucleoli. Fig. 8 is of spinal cord from E-160 rats. We can see small and very dark stained neurons in both photographs. Irregular arrangement of cells can be appreciated in both photographs. Cytoplasm and nucleus cannot be identified differently. Neurons are also exhibiting clumping of nuclear material without distinct nucleolus. Fig. 9 is of spinal cord from E-160 rats showing the ventral horn in dorsoventral view.

Neurons in ventral horn (N) are showing morphological changes. Cells are darkly stained and nucleus is heterochromatic. Neurons exhibit nuclear swelling, extensive clumping of nuclear material and nucleolus is not prominent.

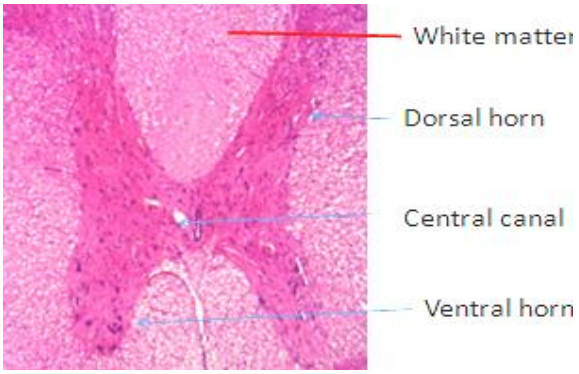

Fig-1: Representative Photomicrograph showing dorsoventral view of the spinal cord of in transverse section from the control group at low magnification reveals central grey and peripheral white matter. The dorsal and ventral horns can be easily identified. Paraffin section, H&E, X100

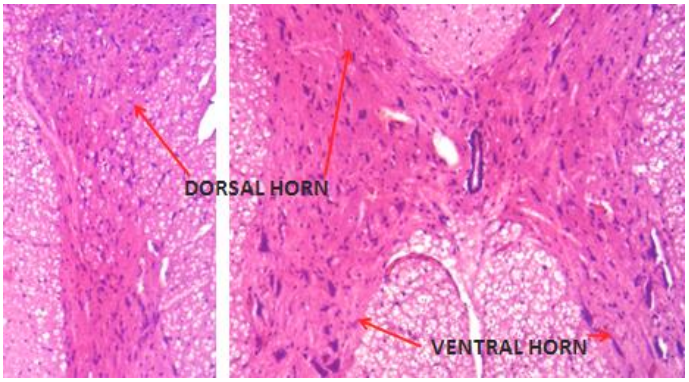

Fig-2: Representative Photomicrographs showing a dorsolateral view of the spinal cord of control group reveal dorsal and ventral horn of the grey matter of the control group at low magnification. The picture is showing normal distribution and normal cell morphology within ventral and dorsal horns. Paraffin sections, H&E, X200

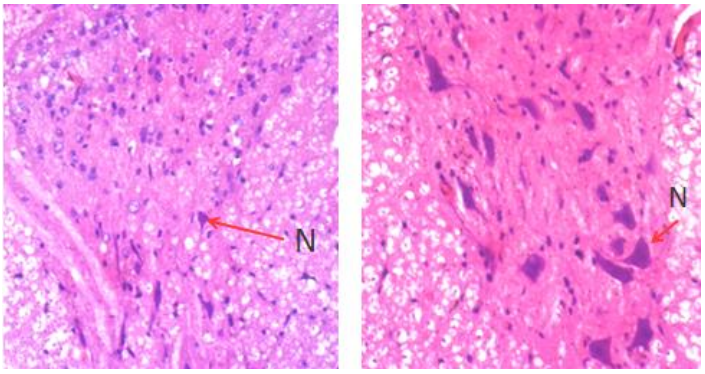

Fig-3: Representative Photomicrograph of spinal cord from control rats is showing dorsal and ventral horn. In the dorsal horn we can see that the most prevalent neurons are of small size and belong to the category of interneurons. However, some neurons have medium size somata. In ventral horn we can see large multipolar motor neurons of normal shape and size. H&E, X400

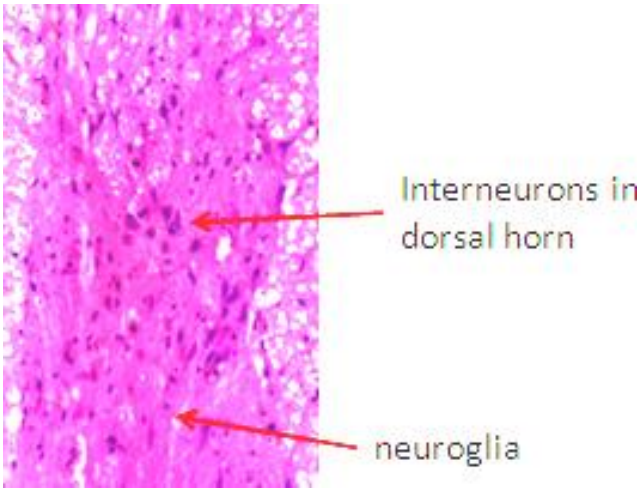

Fig-4: Representative Photomicrograph of the spinal cord from the control group at high magnification is showing middle part of dorsal horn. Neuronal cell bodies and neuroglial cells can be well identified. This picture is revealing normal histology of this part. Paraffin section, H&E, X400

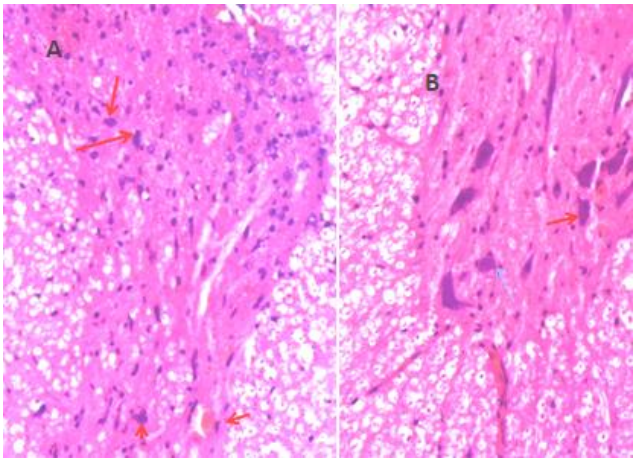

Fig-5: Representative Photomicrographs of spinal cord from E-80 is showing dorsal (A) and ventral horn (B). Neurons in the dorsal horn are very small size and their nucleus is heterochromatic. The same thing can be observed in ventral horn interneurons as well. In picture B, few neurons are very darkly stained and nucleus cannot be recognized. (H&E, X400)

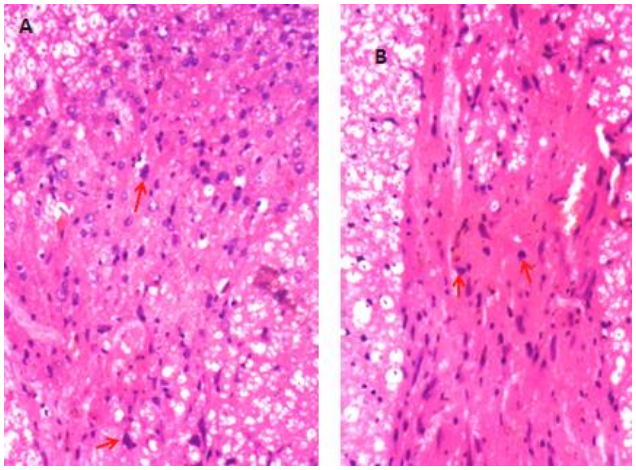

Fig-6: Representative photomicrograph is from dorsal (A) and middle (B) part of the dorsal horn of spinal cord from E-120 rats. Both pictures are show the irregular and loosely arrangement of neurons. They are darkly stained and size is reduced. Nucleus and nucleoli cannot be differentiated. H&E, X400

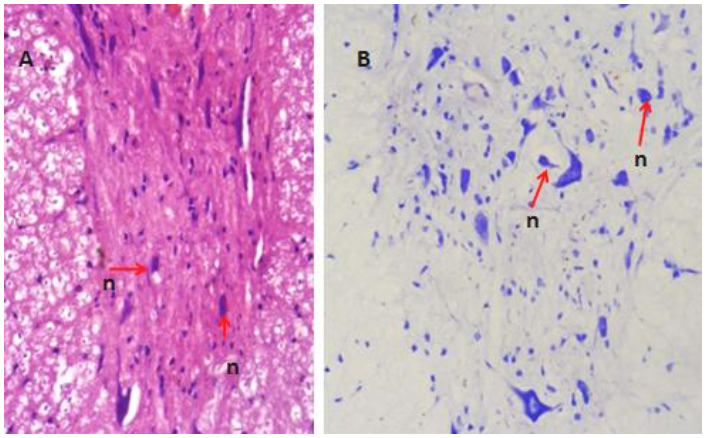

Fig-7: Representative Photomicrographs from E-120 rats are showing the ventral horn of the spinal cord. Few neurons in some parts are very small and very darkly stained (red arrow). Cytoplasm is totally absent. The nucleus is highly heterochromatic with invisible nucleoli

In fig B, in lower field and in the centre typical normal looking multipolar neurons can been seen

B-cresyl violet staining, A- H&E, X400

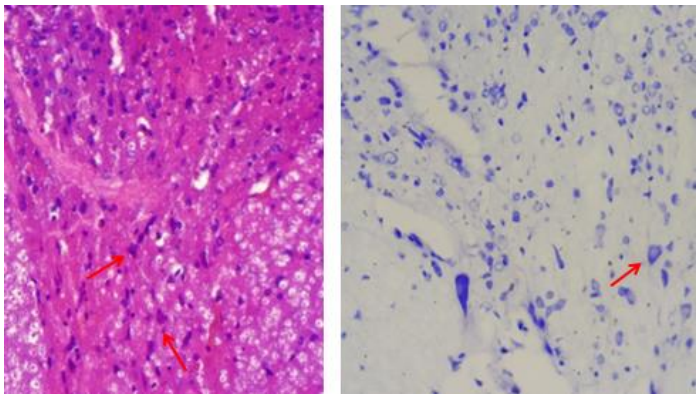

Fig-8: Representative Photomicrograph is of spinal cord from E-160 rats. We can see small and very darkly stained neurons in both photographs (Red arrow). In picture B, there is a medium size normal looking neuron with prominent nucleus. Irregular arrangement of cells can be appreciated in both photographs. Cytoplasm and nucleus cannot be identified differently. Neurons are also exhibiting clumping of nuclear material without distinct nucleolus. A-H&E, B-Cresyl violet X400

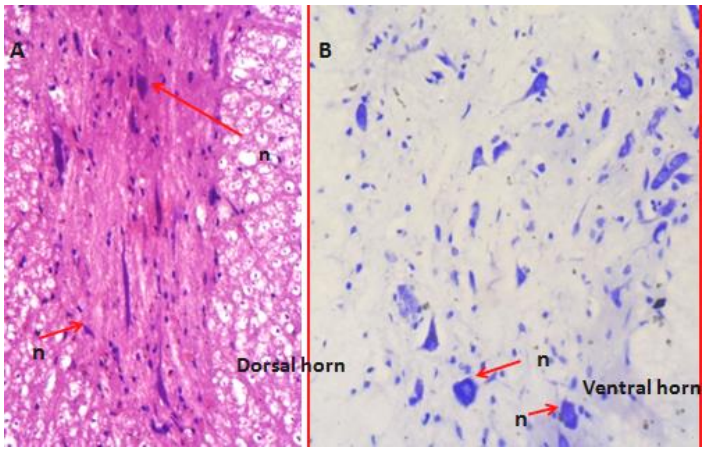

Fig-9: Representative Photomicrograph is of spinal cord from E-160 rats showing the ventral horn in dorsoventral view. Neurons in ventral horn (n) are showing morphological changes. Cells are darkly stained and many of them have elongated appearance. Some neurons are highly heterochromatic. A- H&E, B-cresyl violet, X400

DISCUSSION

The interaction of radio-frequency electromagnetic radiation (RF-EMR) with the brain is a serious concern of our society. A large proportion of users consist of children and teenagers, due to the wide and growing use of mobile communication, there is increasing concern about the interactions of electromagnetic radiation with the human organs in general and the brain in particular.

Brain a very important part of the human body which in addition to playing a crucial role in controlling human behavior is also affected by a plethora of factors and has been identified as an important site for neurogenesis in adults and therefore, has now become an area of intense research.

In this study, GSM (0.9 GHz/1.8 GHz) mobile phone in silent mode (no ring tone & no vibration) was placed in the cage and the rats were exposed to RF-EMR by giving complete missed calls (of 45 seconds duration each) one after the other, every day for 4 weeks, then these rats were sacrificed, dissected, processed, following standard protocol and observed under light microscopy for comparison of the selected areas of control with identical areas of different experimental groups. In humans, the motor systems project fibers from the cerebrum to the brainstem. Complex behaviors such as social interactions, behavior, thought, judgment, learning, working memory, speech and language were facilitated by the neural networks in the prefrontal lobe of the cerebrum. Spinal cord receives the motor fibers from the motor cortex which constitutes the upper motor neuron synapses with lower motor neuron which innervates the skeletal muscles. Damage to motor areas of the cortex can lead to certain types of motor neuron disease resulting in loss of muscular power and precision rather than total paralysis. It has also been associated with neurological diseases like amyotrophic lateral sclerosis, senile dementia, Parkinson's disease and Alzheimer's disease [5].

Spinal cord receives primary afferent fibers from peripheral receptors located in widespread somatic and visceral structures, and sends motor axons to skeletal muscle. It also contains the cell bodies of all the preganglionic neurons responsible for the sympathetic innervations of cardiac and smooth muscle and secretory glands, and for the parasympathetic innervations of smooth muscle in the erectile tissues of external genitalia, pelvic viscera and distal part of the hindgut. The action potential in the peripheral receptors initiates propagation of electrical impulse, bypassing the cell body and continue to propagate reaching the synaptic terminal in the spinal cord.

Advancements in the Electromagnetic Field (EMF) technologies and communications have greatly increased the human population exposure to EMFs. Many studies have demonstrated that EMF does affect the nervous tissue in one or the other way. Since the brain and spinal cord is a very important part of the human body which is concerned with monitoring the whole human body. The present study was designed to evaluate the effects of EMF. The study was designed and executed carefully so that the brain is not unduly disturbed by other factors (sound or vibration) during the course of study. Therefore, in this study whatever morphological changes have been observed, they are to be considered to be purely due to EMF (Keeping a GSM (0.9 GHz/1.8 GHz; mobile phone in silent mode - no ring tone & no vibration). In the present study, it is observed that there is a decrease in a number of ganglionic cells of dorsal root ganglion in the experimental group as well as decrease in size of ganglionic cells due to the harmful effects of EMF on scheduling death of cells and their apoptosis. These morphological changes can be because of decreased activity of the nucleus and its result is decreased activity of the cell. These findings are in agreement with studies of [6, 7]. Rat embryos were exposed to different doses of radiation, sacrificed and subjected to histological processing.

Observation of the light microscopic study revealed the developing neurons suffered a damage which was dose dependent and persisted in spite of giving exposure free period between two the exposures. The present study also showed the effects of RF-EMR cells of the ventral and dorsal horn of the spinal cord which was in agreement with study of [8]. Rats were irradiated with doses of 1000–3000 rads to the cervical spinal cord and subsequently given a paralytic dose of p-Bromophenylacetylurea. The nuclear populations in the degenerating dorsal columns were determined and it was found that a significant suppression of cell proliferation occurred after all three dose levels. The cell populations in the shielded parts of the tracts rostral of the irradiated zone were not affected.

Free radicals also play an important role in aging processes which have been ascribed to be a consequence of accumulated oxidative damage to body tissues [9], and involvement of free radicals in neurodegenerative diseases, such as Alzheimer's, Huntington, and Parkinson, has also been suggested [10]. The nutritional status of an individual can be a factor which relies on the free radicals. E.g. availability of dietary antioxidants, consumption of alcohol and the amount of food consumed. Various life conditions, such as psychological stress [11] and strenuous physical exercise [12], have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some

individuals may be more susceptible to the effects of RFR exposure because of increase production of free radical. These findings indicate that exposure to EMF has a detrimental effect on the neurons of the cerebral cortex and spinal cord. At the molecular level EMF produces biological stress and **free radical**, which can make the susceptible animal population prone to increase permeability of BBB, congenital malformation, tissue and cell damage or death [13] and free radicals can cause oxidative stress at the cellular level, interfering with protein synthesis. These components also play an important role in acute inflammation, endothelial destruction, resulting in tissue edema. It has been postulated that EMF-exposure produces high levels of oxidative stress as a result of its effect on the immune response [14] and oxidative stress leading to cellular dysfunction may be the end result of long term exposure to EMF.

Many molecules such as hormones, amino acids, potassium undergo frequent fluctuations in blood brain barrier (BBB) especially after stressful conditions resulting changes in the neuronal excitability. The BBB in adults is a highly selective semipermeable border of a highly specialized basal membrane, a large number of pericytes embedded in the basal membrane and astrocytic end feet. The endothelial cells form the barrier proper, the brain endothelial cells vary from endothelial cells of organs in two important ways. First, In between Brain and endothelial cells have continuous tight junctions. These tight junctions prevent para cellular movement of molecules. Second, there are no intracellular vesicles. The endothelial cells of the brain create a barrier between the blood and the brain. The effects of continuous-wave, sinusoidally amplitude-modulated and pulsed 591-MHz RFR were compared after five minutes of exposure at power densities of 10 and 20 mW/cm2 (SARs at the cerebral cortex were 1.8 and 3.6 W/kg).

Modulation frequencies (4-32 Hz) of varying intensities were used in the amplitude-modulation mode. The effect of NADH level across the modulation frequencies was insignificant. Furthermore, pulsed radiations of 250 and 500 pps were compared with power densities ranging from 0.5-13.8 mW/cm2. There was a significantly more effective NADH concentration in the cerebral cortex with 500pps radiation than 250pps radiation. These experimental changes observed when the cerebral cortex issue temperature was normal; the authors anticipated that it is due to direct inhibition of the electron transport functions in the mitochondria by RFR-induced dipole molecular oscillation in divalent metal containing enzymes or electron transport sites [15]. A synapse is a neuro neuronal junction, where an electrical or chemical signal is transmitted. At the synapse presynaptic neuron comes in close opposition with the post synaptic neuron, a link between the two membranes carrying out the signaling process. Astrocytes regulating synaptic transmission. There are two fundamentally different types of synapses- (i) in chemical synapse, the presynaptic neuron releases a chemical called a neurotransmitter that binds to receptors located in the postsynaptic cell, generally implanted in the plasma membrane. The neurotransmitters released at the synaptic cleft may either excite or inhibit the post synaptic neuron. (ii) In an electrical synapse, the presynaptic and postsynaptic cell membranes are connected by special channels called gap junctions that are capable of passing electric current, causing voltage changes in the presynaptic cell propagated to the postsynaptic cell. Electrical synapse helps in the rapid transfer of signals from one cell to the next.

Thus looking the complexity of synaptic structure, functions and the input pattern it may be concluded that even a minor morphological change in the synaptic structure is likely to have major functional consequences unless repaired very fast or else compensated intrinsically by the system. Thus the findings of such study can assume more significance if conducted in conjunction with the behavioral study also.

From the findings of the present study it appears pertinent that in order to protect the population living around base stations and users of mobile handsets, governments and administrative authorities should implement safety standards, which decipher to limits on exposure levels below a certain value. International Commission for Non-Ionizing Radiation Protection (ICNIRP) is the most has recommended standards, and has been adopted so far by more than 80 countries. ICNIRP proposes two safety levels for radio stations: one for occupational exposure, another one for the general population. Currently there are efforts underway to harmonize the different standards in existence.

Radio based licensing procedures have been established in the majority of urban spaces regulated either at municipal/county, provincial/state or national level. Mobile telephone service providers are, in many regions, required to obtain construction licenses, provide certification of antenna emission levels and assure compliance with ICNIRP standards and/or to other environmental legislation. Many governmental bodies encourage competition between the telecommunication companies trying to achieve sharing of towers so as to decrease the environmental and cosmetic impact. Installation of new antennas and towers have been a rejection by the communities. The Federal Communications Commission (FCC) of the U.S has based its standards primarily on the standards established by the Institute of Electrical and Electronics Engineers (IEEE), specifically Subcommittee 4 of the "International Committee on Electromagnetic Safety".

WHO established the International Electromagnetic Fields (EMF) Project in 1996 in response to public and governmental concern to assess the scientific evidence of possible adverse health effects from electromagnetic field. WHO conducted a formal risk assessment of all studied health outcomes from radiofrequency field exposure by 2016, and as noted above. The International Agency for Research on Cancer (IARC), a WHO specialized agency, conducted a review, and documented the carcinogenic potential of radiofrequency fields, as from mobile phones in May 2011.

CONCLUSION

WHO identifies and promotes research priorities for radiofrequency fields and health to fill gaps in knowledge through its research agendas.

WHO develops public information materials and promotes dialogue among scientists, governments, industry and the public to raise the level of understanding about potential adverse health risks of mobile phones. Therefore, human beings can be prevented from the ill effects of electromagnetic radiation.

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