Effect of Chronic Exposure to GSM 900 and 1800 MHz Radio-Frequency Electromagnetic Field Induces Cognitive Impairment, Neurotransmitters and Histopathological Changes in Rat Brain

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Abstract - Introduction: Today there is an upsurge in the public and media concern about the potential health risk of radio frequency field exposure. Interaction of radio frequency electromagnetic radiation with the brain is a precarious apprehension in our society. Objective: The current study was aimed to evaluate the possible implications on cognitive function on exposure to radio frequency-electromagnetic radiation (RF-EMR) in male rats at 900 MHz and 1800 MHz frequencies for 2 h per day for 3 months. Materials and methods: Wistar rats were categorized into 3 groups (n = 12) viz., sham exposure, exposure to 1800 MHz and exposure to 900 MHz. At the end of the study effect on cognitive function and behavioral pattern viz., elevated plus maze, T-maze, step down passive avoidance and locomotor activity were performed. Alterations in neurotransmitters level and histopathology of brain were analyzed. Results: Effect on elevated plus maze and T-maze showed significant increase in transfer latency as compared with control and step down passive avoidance and locomotor activity was found to be significantly decrease as compared with control. The brain level of dopamine, nor-adrenaline, GABA and acetylcholinesterase enzyme activity was found to be significantly lowered. Histopathological findings of brain showed a marked congestion of the cerebral blood vessels and few of the pyramidal cells and neuroglial cells show degenerative changes with mild inflammatory infiltration. Conclusion: Thus, our results suggest that exposure to RF-EMR certainly causes alteration in cognition function due to alteration in brain transmitter levels.

Keywords: RF-EMR, elevated plus maze, T-maze, step down passive avoidance, locomotor activity, dopamine, nor-adrenaline, GABA, acetylcholinesterase enzyme.

I. INTRODUCTION

In everyday life, we are constantly surrounded by electromagnetic fields (EMF). There are two means of sources: natural sources of EMF, such as the earth's magnetic field and man-made sources, such as power cables, domestic appliances, radio stations, mobile phones, wireless networks and radars. Among them mobile phones use modulated radio frequency electromagnetic fields (RF-EMF) to transfer information. Mobile phones have been a part of everyday life and been an unprecedented growth in the global communication industry that has resulted in a dramatic increase in the number of wireless devices. Mobile services are one of the fastest growing mobile telephonic industries in the world. According to the Telecom Regulatory Authority of India, the composition of telephone subscribers using wireless form of communication in urban area is 63.27% and rural area is 33.20% [1]. As on May 2014, the number of mobile phone users in India was 910 million (second highest in the world) and that of mobile towers was more than 6 lac. This has led to the mushrooming of supporting infrastructure in the form of cell towers, which provide the link to and from the mobile phone [2].

Mobile phones emit RF-EMF using specified frequency bands during the transmission phase. While speaking during a phone call the rate at which radio frequency (RF) energy is actually absorbed in a body is called the "Specific Absorption Rate (SAR)". It is usually expressed in Watts per kilogram (W/kg) or milliwatts per gram (mW/g). SAR is set at 1.6 W/kg averaged over 1 g of body tissue in the US and Canada and 2 W/kg averaged over 10 g of body tissue in countries that adopted the ICNIRP guidelines. There is an increasing concern about the interactions of electromagnetic radiation with the human organs, particularly with the brain. The brain and nervous system have long been considered as sensitive targets for the effects of RF fields. Calcium ions play an essential role in brain function, because a small amount of calcium must enter the cytosol of the neuron before it can release its neurotransmitters. Electromagnetically-induced membrane leakage would increase the calcium levels in the neurons so that they quickly release their neurotransmitters. This improves the reaction time towards simple stimuli, but it can also trigger the spontaneous release of neurotransmitters to transmit spurious signals, which makes the brain hyperactive and less able to concentrate [2,3].

Studies by Finnie et al. have detected no consistent evidence of any field-induced changes in gene and protein expression following low-level exposures. There was no evidence that short- or long-term exposure to 900 MHz field produced microglial activation in the cortex or

hippocampus of mice [4]. There was no evidence of apoptosis and/or increase in degeneration of (dark) neurons in the brain of juvenile rats after acute, low-level exposure to pulsed 900 MHz fields [5]. Previously, it had reported that an equivalent exposure had caused widespread damage to neurons [6]. Tarantino et al. [7] had investigated the delayed effects of long-term, continuous, whole-body exposure of rabbits to 650 MHz, typical of some broadcasting signals. No effects were observed on body weight and health of the animals, but progressive changes were observed in brain morphology with a gradual increase in apoptosis. Mausset-Bonnefont et al. had investigated the effects of acute exposure of the head of rats to high power (but not hyperthermic) 900 MHz field on the levels and binding properties of *N*-methyl-D aspartate (NMDA), GABA-A receptors and dopamine transporters in the cortex, striatum and hippocampus. Significant changes were reported, particularly for the decreased levels of GABA-A receptors in the hippocampus, increased dopamine transporters in the striatum and decreased NMDA receptors in the cortex [8].It has long been recognized that the exposure of animals to RF field at thermal levels may affect their behavior and disrupt performance of learned tasks, but this does not exclude the possibility that low-level exposures may endanger subtle behavioral or cognitive changes under certain circumstances [9]. Narayanan et al. had placed a mobile phone in silent but vibratory mode beneath the cage that containing young adult rats. Each day for 4 weeks, these animals were exposed to the fields associated with 50 missed calls and then their spatial learning capabilities were tested using a water maze. Significant differences in behavior were observed [10].

The current project envisages to probe the effect on long-term exposure of RF-EMR (900 MHz and 1800 MHz) on neurotransmitter level and its disparity on behavioral and cognitive function in male Wistar rats.

II. MATERIALS AND METHODS

Experimental animals

Inbred healthy male albino Wistar rats (aged 6–8 weeks old) were used in this experiment. The rats were housed in polypropylene cages inside a temperature- and humidity-controlled environment with free access to food and water *ad libitum*, with a 12 h light and 12 h dark cycle. All the experiments were carried out with prior approval from the institutional animal ethics committee. Care was taken to handle the rats in a human manner, and all precautions were taken to use the minimum number of animals required to generate significant data.

Experimental design

Animals were categorized into three groups: group I (n = 12), normal control; group II (n = 12) exposed to RF-EMR 900 MHz and group III (n=12), exposed to RF-EMR 1800 MHz (out of n=12, n=6 were used to investigate on neurotransmitter levels and n=6 were used to investigate behavioral changes to avoid the effect on neurotransmitters due to the behavioral studies and chaos between results). The radio frequency electromagnetic radiation generator (manufactured by AND Solutions, India) was used to emit 900MHz and 1800MHz frequencies, SAR set at 1.6 W/kg and power density was 0.9 watt/m^2(which was fixed statically by manufacturer as per IEEE guidelines) via monopole antenna that directed the signal to the cages. The antenna generated uniform RF radiations as a genuine GSM mobile phone. The length of the monopole was set so that the antenna is resonant at the operating frequency. The antenna had an Omni-directional pattern in the azimuth plane through which the rats were to be uniformly distributed. Male Wistar rats were placed individually in a hexagonal acrylic plastic cage (40 cm in diameter) which was divided into six compartments and exposed to RF-EMR daily 2 h for 3 months. The cage was designed in such a way that the animals were not restrained and ventilated by drilling holes. The animals were left for 3 days for acclimatization. Control animals underwent the same transportation, habituation and handling procedure without switching on the signal generator i.e. no RF-EMR exposure. The antenna was placed 30 cm above the ground. (Fig. 1)

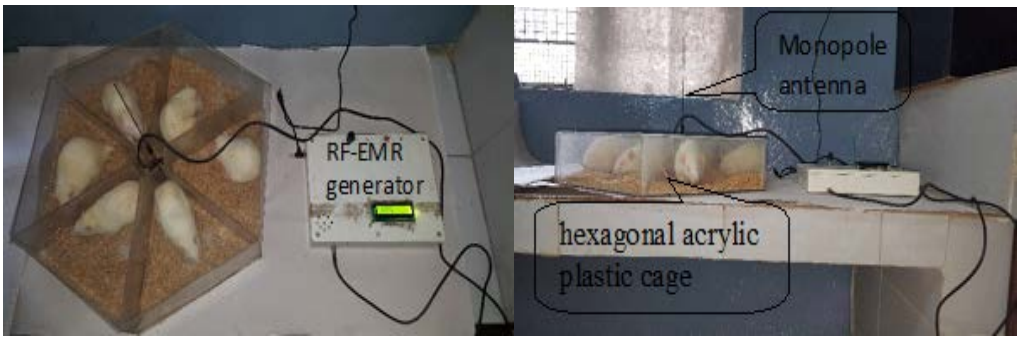

Fig.1: Male Wistar rats under RF-EMR exposure placed in hexagonal acrylic cage by placing the monopole antenna in the center of the cage.

At the end of the study elevated plus maze, T-maze, step down passive avoidance and locomotor activity were carried out. Rats were sacrificed by cervical dislocation to estimate neurotransmitter levels, acetylcholinesterase enzyme activity and histopathology of the brain.

Evaluation of learning and memory in rats using elevated plus maze

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two closed arms (16 cm × 5 cm × 12 cm). The arms extended from a central platform (5 cm × 5 cm), and maze was elevated to a height of 25 cm from the floor. On the first day, each rat was placed at the end of an open arm, facing away from the central platform and the time taken to enter the one of the closed arms with all the four paws in the closed arm was taken as the transfer latency (TL). If the rat did not enter into one of the closed arms within 90 sec, it was gently pushed into one of the two covered arms and the TL was assigned as 90 sec. The rat was allowed to explore the maze for 20 sec and then returned to its home cage. Twenty-four hours later, the rats were again placed on the elevated plus maze individually as before and transfer latency was recorded. Transfer latency measured on 1st and 2nd day was served as a parameter for acquisition and retrieval respectively. Memory retention was examined [11].

Evaluation of spatial working memory in rats using T maze

T-maze was used to evaluate spatial working memory in rats. T Maze was made up of transparent Perspex material which consisted of a stem, a starting box of dimensions 86.5× 8 cm and two arms, left and right arm dimensions 62×18 cm were the goal areas, used to keep food pellets at the end of either arms. The instrument was at the height of 87.5 cm from the ground. Animals were brought into the experiment room at least one day before starting the experiment to condition the animals to the lab conditions. The day before the experiment, food was given to the animals around 15:00 hr. and the food was withdrawn after an hour of feeding. The same feeding procedure was followed for three remaining experimentation days.

The experiment protocol involves three phases. The three phases were carried for three days. Each day the experiment was started at the 10:00 AM, between each trail the animals were transferred to their home cages and allowed to stay for some time before subjecting to the next trail. On the first day, each rat placed on the maze completed 15 trials to become familiarized with the maze and access food. Second day is the acquisition where rats are again subjected to the trails as the same time of the first day. The rats were trained until they attained a criterion of nine correct arm choices of 10 consecutive trails. The rats were transferred to their home cage between the trails. Third day is the retrieval where three trails for each animal were taken. Number of correct responses and time taken to reach the food (TRF) in each trail were noted [12]

Evaluation of learning and memory in rats using step down passive avoidance

Passive avoidance behavior which is based on negative reinforcement was recorded to examine long-term memory. The test involves training of rats to avoid punishment (normally an electric shock) by curbing a normal behavior (such as an exploratory behavior). At specified intervals after training, the animals were tested again for retention of such learning. The apparatus consists of The apparatus consisted of a box (27 × 27 × 27 cm) having three walls of wood and one wall of Plexiglas, equipped with the floor of electrifiable grid with a wooden platform of dimensions 8 x 8 x 5 cm was used as the shock free-zone (SFZ), in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period.

The experiment protocol involves three phases. The three phases were carried for three days. First was the familiarization where, the test rat was placed on the SFZ located in the center of the electrifiable grid floor of the chamber and the latency to descend was measured. After 30 sec of exploration, it was returned to the home cage. On the second day test rat was placed on the SFZ and allowed to explore for 60 sec. When the animal stepped off this SFZ, it received a shock of 1 mA. The shock procedure was repeated whenever the animal stepped down. After 3-5 trials (within 5 min) the animals acquired a passive avoidance, i.e., refrained from stepping down the grid floor. Criterion was reached when the animals remained on the platform for full of 60 sec. Animals not meeting these criteria of learning in 5 trials were rejected. Third day was the retention test where retention parameters such as step down latency (SDL), time spent in shock zone (TSZ) and step down errors (SDE) were noted 24 hours after training[13].

Evaluation of locomotor activity in rats using actophotometer

The locomotor activity was studied using the actophotometer. The movement of the animal interrupts the beam of light falling on a photocell at which the count was recorded and displayed digitally. Each rat was placed individually in the actophotometer for 10 min and the basal activity was obtained. The rats were observed in a square open field arena (68×68 ×45 cm) equipped with 2 rows of 8 photocells, sensitive to infrared light, placed 40 and 125 mm above the floor, respectively. The photocells were

spaced 90 mm apart and the last photocell in a row was spaced 25 mm from the wall. Measurements were made in the dark in a ventilated, sound-attenuating box [14].

Estimation of dopamine and nor-adrenaline in rat brain homogenate

Estimation of dopamine and nor-adrenaline was followed according to Schlumpf et al. Dopamine and nor-adrenaline levels were measured at 330 and 395 nm as excitation and 375 and 485 nm as emission wavelength, respectively, using spectrofluorimeter.

On the day of experiment, rats were sacrificed by cervical dislocation, whole brain was dissected out and the sub-cortical region (including the striatum) was separated. Briefly, 0.5 g of tissue was weighed and homogenized in 25 ml of HCl–butanol for 1 min. The sample was then centrifuged for 10 min at 2000 rpm. An aliquot supernatant phase (5 ml) was removed and added to centrifuge tube containing 12.5 ml of heptane and 8 ml 0.1 M HCl. After 10 min of vigorous shaking, the tube was centrifuged under the aforementioned conditions to separate the two phases, and the overlaying organic phase was discarded. The aqueous phase (1 ml) was then extracted for dopamine and nor-adrenaline assay. The extraction procedure was carried out at 0°C [15,16].

Estimation of GABA content in brain homogenate

The brain gamma amino butyric acid (GABA) content was estimated as specified by Lowe et al. Rats were sacrificed by decapitation, the brains were rapidly removed and hippocampus was isolated, blotted, weighed and transferred in ice cold 5 mL trichloroacetic acid (10% w/v), homogenized and centrifuged at 10,000 g for 10 min at 0°C. A sample (0.1 mL) of tissue extract with 0.2 mL of 0.14 M ninhydrin solution in 0.5 M carbonate-bicarbonate buffer (pH 9.9), kept in a water bath at 60°C for 30 min then cooled and treated with 5 mL of copper tartrate reagent (0.16% disodium carbonate, 0.03% copper sulphate and 0.0329% tartaric acid). After 10 min, the fluorescence reading was measured at 377 and 451 nm as excitation and emission wavelength, respectively.

GABA was determined by the measurement of the formed fluorescent product resulting from the reaction of GABA with 0.14 M ninhydrin in an alkaline medium, in the presence of glutamate. The GABA content in brain was expressed in µg per mg of wet brain tissue [17].

Estimation of acetylcholinesterase in rat brain homogenate

Acetylcholinesterase (AChE) enzyme activity was estimated by Elman method. The rats were decapitated by cervical dislocation, brains are removed quickly and placed in ice-cold saline. Thalamus, hypothalamus and hippocampus are quickly dissected out on a petri dish chilled on crushed ice. The tissues were weighed (250 mg) and homogenized in 0.1M Phosphate buffer (pH 8). Further 0.4ml aliquot of the homogenate was added to a cuvette containing 2.6 ml phosphate buffer (0.1M, pH 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm using a spectrophotometer. When absorbance reaches a stable value, it is recorded as the basal reading. 20µl of substrate i.e., acetylthiocholine was added and change in absorbance was recorded for 10 minutes at an interval of 2 min. Change in the absorbance per minute is thus determined[18].

Histology

The brain was carefully dissected and fixed in 10% buffered formalin (with pH 7.4) for 24 h. It was then dehydrated in ethanol, defatted in xylene and embedded in paraffin. Care was taken to ensure that the brains of all rats were oriented in the same direction during embedding to minimize the differences in the angles at which the brains were sectioned. A single investigator processed all brains to maintain consistency in histological analysis and stained with haematoxylin and eosin (H&E) according to the standard procedure and observed under the magnification 400X. The brain parenchyma from cerebral region was studied and identified for pyramidal cells, neuroglial cells and blood vessels.

Statistical analysis

All data were expressed as mean ± SEM and analyzed with one way analysis of variance (ANOVA) between the groups and followed by Tukeys Multiple Comparison Test to assess differences between the groups using graph pad prism (Ver. 5). Probability values $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered significant.

III. RESULTS

Our investigational studies revealed that RF-EMR exposure affected the behavior, neurotransmitter level and acetylcholinesterase activity in the brain.

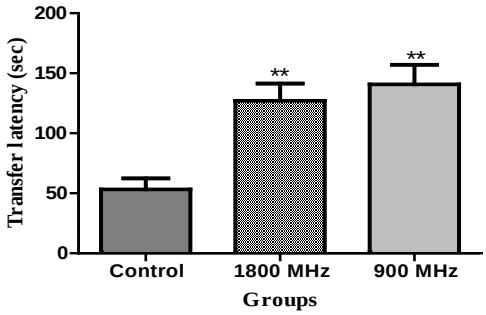

Fig. 2: Effect of RF-EMR on transfer latency in elevated plus maze on retrieval day. $P < 0.05$, ** denotes significant difference $p < 0.01$.

Effect on cognitive function

Our result of the elevated plus maze test demonstrated a remarkable change in behavior. More anxiety and poor learning was observed in test animals as compared with control. On the retrieval day, the transfer latency of rats exposed to RF-EMR of 1800MHz and 900MHz was found to be significantly higher as compared with sham-exposed rats (Fig. 2).

While the result of T maze test was found significant alteration on transfer latency in comparison with control group. On the retrieval day, the transfer latency of rats exposed to RF-EMR of 1800 MHz and 900 MHz was found to be significantly higher as compared with sham-exposed rats (Fig. 3).

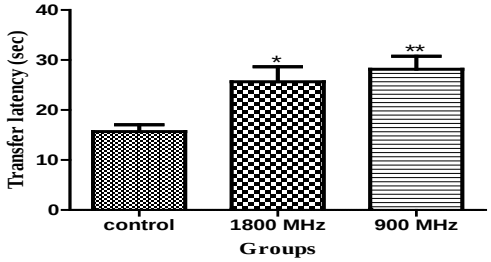

Fig. 3: Effect of RF-EMR on transfer latency in learning and memory in T-Maze on retrieval day. * denotes significant difference $p < 0.05$, ** denotes significant difference $p < 0.01$.

In step down passive avoidance test, it was observed that on the retrieval day, step down latency (SDL) and time spent in shock-free zone of rats exposed to RF-EMR 900 MHz was significantly lower and that of 1800 MHz was non-significant as compared with sham-exposed rats (Fig. 4).

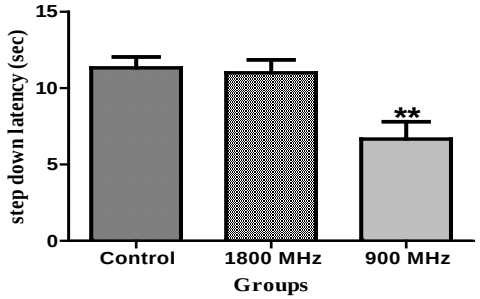

Fig. 4: Effect of RF-EMR on memory and learning by measuring step-down latency in step-down passive avoidance apparatus on retrieval day**.** ** denotes significant difference $p < 0.01$.

Locomotor activity was performed to evaluate the CNS depressant effect. Locomotor activity in rat exposed to 1800 MHz and 900 MHz was significantly decreased as compared with sham-exposed rats (Fig. 5).

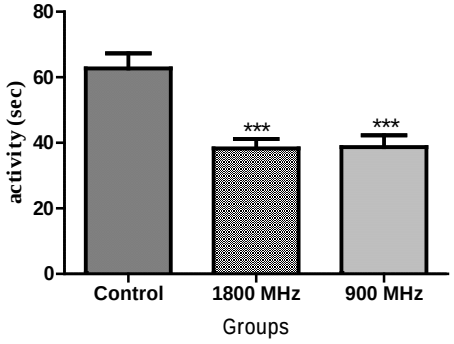

Fig. 5: Effect of RF-EMR on locomotors activity using actophotometer in rats. *** denotes significant difference $p < 0.001$.

Effect on brain neurotransmitter level

Significant changes were observed in brain neurotransmitter levels. Rats exposed to RF-EMR at 1800 MHz and 900 MHz frequencies had brain dopamine levels of 4.743 ± 3.64 ng/mg and 38.49 ± 2.50ng/mg, respectively, of which 1800 MHz was found to be significantly lower than sham-exposed rats (47.69 ± 5.61 ng/mg) (Fig. 6).

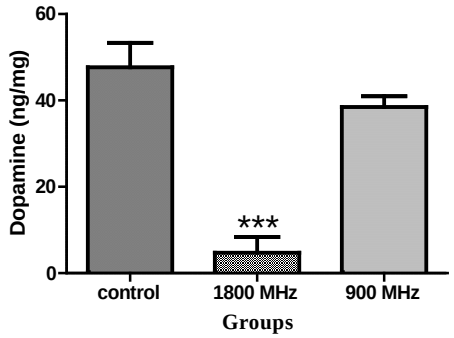

Fig. 6: Effect of RF-EMR on brain dopamine levels in rats. *** denotes significant difference $p < 0.001$.

Moreover, rats exposed to RF-EMR 1800 MHz and 900 MHz frequency had brain noradrenaline levels of 6.660 ± 1.083 ng/mg and 3.855 ± 0.521 ng/mg, respectively, which was found to be significantly lower as than sham-exposed rats (12.04 ± 0.625 ng/mg) (Fig. 7).

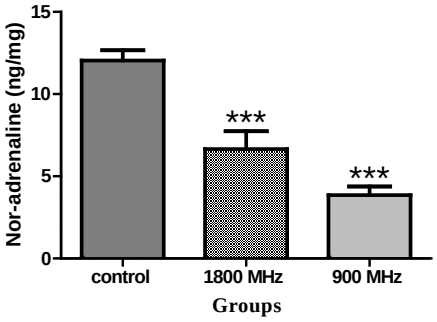

Fig. 7: Effect of RF-EMR on brain nor-adrenaline levels in male rats. *** denotes significant difference $p < 0.001$.

Furthermore, rats exposed to RF-EMR at 1800 MHz and 900 MHz frequency had brain GABA levels of 1.103 ±

0.06518 µg/mg and 0.9860 ± 0.09917 µg/mg, respectively, which was significantly lower than sham-exposed rats (1.690 ± 0.07864 µg/mg) (Fig. 8).

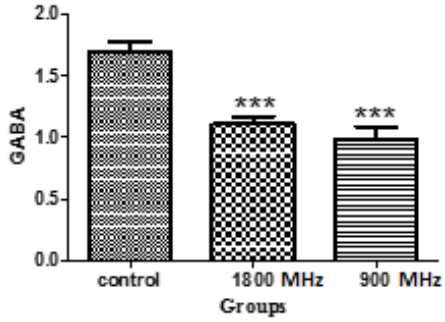

Fig. 8: Effect of RF-EMR on brain GABA levels in rats. *** denotes significant difference $p < 0.001$.

Acetylcholinesterase activity also showed a significant difference in the brain. Rats exposed to RF-EMR at 1800 MHz and 900 MHz frequencies showed 1.735 ± 0.01867 µ moles/min/mg tissue and 1.687 ± 0.2013 µ moles/min/mg tissue, respectively, which was significantly lower than sham-exposed rats (2.398 ± 0.2284 µ moles/min/mg tissue). (Fig. 9).

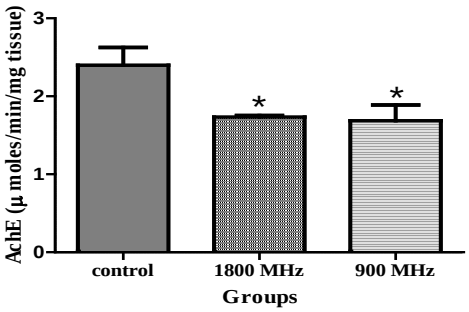

Fig. 9: Effect of RF-EMR on brain acetylcholinesterase in rats. * denotes significant difference $p < 0.05$.

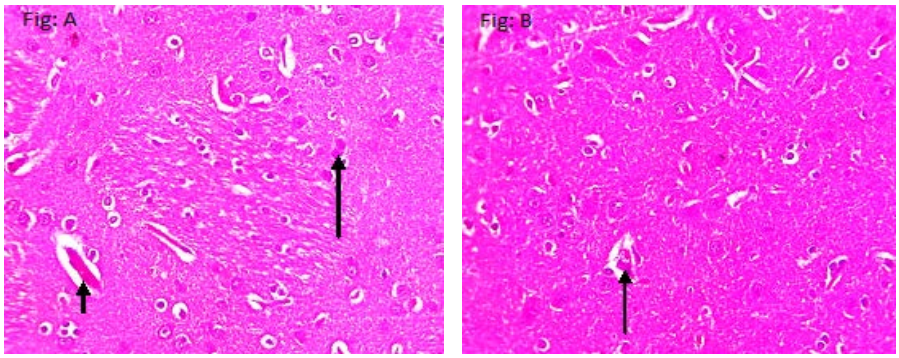

Fig. 10: Section of H & E stained brain exposed to 1800 MHz rat under 400X showing brain parenchyma. Pyramidal cells and neuroglial cells show degenerative changes [A, long arrow], mild inflammatory infiltration [B, arrow], congested blood vessels [A, short arrow].

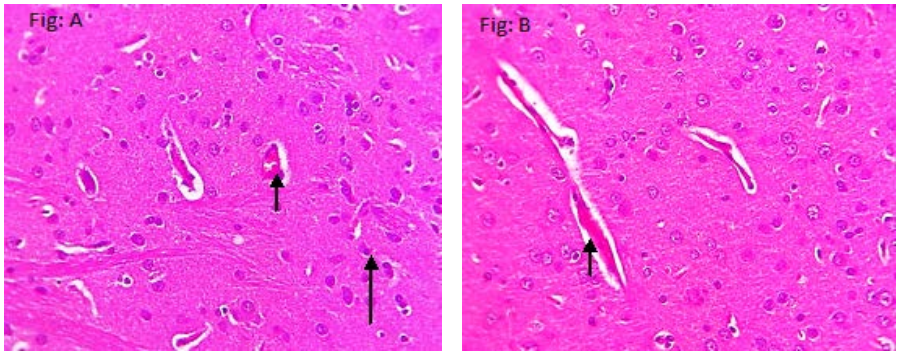

Fig. 11: Section of H & E stained brain exposed to 900 MHz rat under 400X showing brain parenchyma. Pyramidal cells and neuroglial cells show degenerative changes [A, long arrow], mild inflammatory infiltration [B, arrow], congested blood vessels [A, short arrow].

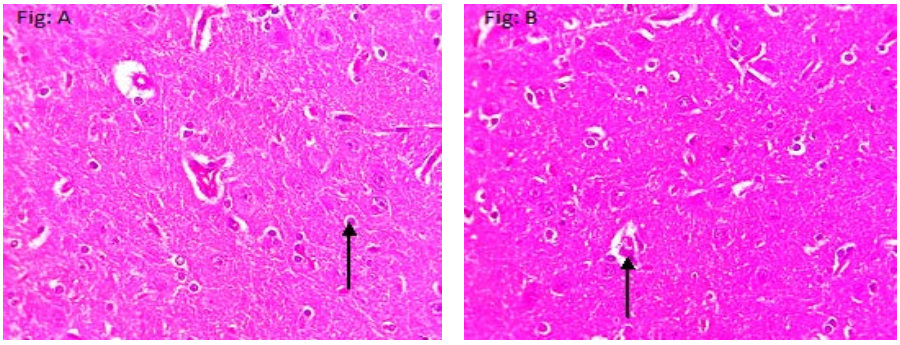

Fig. 12: Section of H & E stained brain of normal rat under 400X showing brain parenchyma. Pyramidal cells and neuroglial cells appear intact [A, arrow]. Blood vessels appear unremarkable [B, arrow].

Histological study

There were observable histological changes in the rats exposed to radiation when compared to the sham-exposed rats. The photomicrograph of brain parenchyma of rats exposed to 1800 MHz and 900 MHz showed (Figs. 10 and 11) a marked congestion of the cerebral blood vessels and few of the pyramidal cells and neuroglial cells showed degenerative changes (10A and 11A, long arrow) with mild inflammatory infiltration (10B and 11B, arrow). Few of the blood vessels appeared congested (10A and 11A, short arrow). The photomicrograph of a rat brain of the control (Fig. 12) showed intact architecture of brain parenchyma. Most of the pyramidal cells and neuroglial cells appeared intact (Fig. 12A, arrow). The blood vessels appear unremarkable (Fig. 12B, arrow).

IV. DISCUSSION

The brain and nervous system have long been considered sensitive targets for the effects of low RF fields. Depending on the amount of movement of the animal, the energy absorption pattern in its body could become either more complex and unpredictable or more uniform. The pattern of energy absorption inside an irradiated body is non-uniform, and biological responses are dependent on distribution of energy and the body part that is affected. RF-EMR emitted from mobile phone could influence anxiety like behavior in rats [9]. Critical analysis of the current study revealed that chronic exposure to RF-EMR from mobile phone could affect the well-being of rats.

Rats exposed to both 1800 MHz and 900 MHz showed decrease in memory and learning, and increase in anxiety was observed. Imbalance between neurotransmitter levels could also cause anxiety-like behavior, which can be correlated with the present study. Alteration in dopamine, nor-adrenaline, GABA and acetylcholinesterase activity were observed due to increased stress and stress-coping mechanisms. Effect of RF-EMR spatial memory performance in rats showed significant decrease of memory and learning capabilities in rats exposed to radiation. Effect of RF-EMR on learning and memory was studied using a step-down passive avoidance method. The results stated that there was a significant decrease in memory and learning in rats exposed to radiation. This showed that the animals after being exposed to aversive stimulation (foot shock) in the passive avoidance task did not remember this task to some extent on the following day, and this clearly indicates the impairment of the memory.

A number of clinical and experimental studies have shown the role of hippocampal formation and related structures in the medial temporal lobe in learning and memory [19] [20]. In rats, bilateral lesion of the specific areas of the hippocampus (CA_1 and CA_3) produced greater impairments in the performance of passive avoidance task [21]. These studies suggest the involvement of the hippocampal system in associative learning processes; in memory, it also could be due to either loss of neurons (cell death) or altered morphology of principle neurons in the basolateral nucleus. The radiation from cell phones, even at one-hundredth of the permitted SAR value, can open the blood brain barrier in rats so that the protein molecules as large as albumin could enter their brains [22]. Later experiments by Salford showed that this was associated with the death of neurons [23]. An immediate effect may not be expected because the brain has spare capacity. However, prolonged or repeated exposure to cell phone or similar radiation would be expected to cause a progressive loss of functional neurons and result in early dementia and Alzheimer's disease in humans.

In the current study, we have investigated that significant change was observed in brain neurotransmitter levels. Level of dopamine was significantly decreased in rats exposed to 1800 MHz. The chemical is involved in controlling emotions, pleasure, movement and incoming information. Low dopamine levels in the motor areas of the brain may result in abnormal nerve-firing patterns within the brain that causes impaired movement leading to consequences, such as like Parkinson's disease, restless leg syndrome, anxiety, attention deficits, cognitive impairment, confusion, depression, blunted effect, fatigue, sleepiness and memory impairment [24]. Significant decrease in nor-adrenaline levels in rats was observed and may linked to lower arousals, lower alertness and depression. It helps promote vigilant concentration and is different from dopamine in the fact that dopamine has a greater influence on cognition. In a stressful condition, norepinephrine is secreted by the sympathetic nervous system that leads to an increased amount of heart contractions. Decrease in the locomotor activity of rat's shows the animal was prone to stress and depression condition, which can be correlated with the decrease level of noradrenaline [24].

Brain GABA levels were also found to be significantly lowered in rats exposed to 1800 MHz and 900 MHz. GABA is primarily known for its ability to keep in a relaxed state; it actually plays a crucial role in regulating many aspects of mood, attention, cognition, and sleep. It control nervous signals in the retina and the central nervous system; therefore, insufficient GABA usually leads to insomnia, depression, anxiety, mood disorder, excessive stress, hypertension, motion sickness, low level of digestive enzymes, epileptic seizures, panic disorders and low growth hormone levels. Dutta et al. had showed that these disorders are brought by the modifications in the activity of the acetylcholinesterase (AChE); there is, therefore, well deterioration of the cell functions [25]. Kunjilwar and Behari

found modifications of the AChE by long-term application of these modulated RF-EMR [26].

Histopathology of brain showed structural changes in the brain cells of the rats exposed to mobile phone radiation as compared with sham-exposed rats. These changes in the histology is in consonance with the report given by other researchers, such as Laila K. Hanafy et al., where they observed histological changes in the different visceral organs after exposing 15 rats to mobile phone radiation daily for 4 weeks (1 h/day) [27]. Hence, the possible mechanism could be due to increased vulnerability to free radicals generation. Boris et al., have also shown that the first changes occurred at the neuronal structure, followed by glial and endothelial cell damages [28].

Thus, alteration in neurotransmitter levels could affect the cognitive function and behavior of the exposed rats, which could be detrimental to overall health. But the study is not asserted by our research and it has to be endorsed by doing further research and more findings.

V. CONCLUSION

Analyses showed various changes in the neurotransmitter levels which resulted a profound effect on behavioral and cognitive function in rats exposed to RF-EMR of 1800 MHz and 900 MHz. The most significant observation was the alteration of brain neurotransmitter levels of dopamine, nor-adrenaline, GABA and brain acetylcholinesterase activity levels with marked congestion of cerebral blood vessels and mild inflammatory infiltration in pyramidal and neuroglial cells of brain parenchyma.

VI. DECLARATION OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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