

Mobile phone induced cognitive and neurochemical consequences

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ARTICLE INFO

Keywords:

Microwave
Brain
Cognitive
Hippocampus
Oxidative stress

ABSTRACT

With the rapid advances in technology, extensive use of mobile phones has increased the risk of health problems. This study was performed to find out the effect of mobile phone frequency on male *Wistar* rats. Animals were divided into two groups ($n = 6$ in each group). Group one was considered as control and group two (experimental group) was exposed to microwave radiation (2100 MHz) for 4 hours/day (5 days/week) for 3 months. Exposure of microwave radiation frequency showed significant alterations in cholinesterase activity, muscular strength, learning ability and anxiety. MWR exposure was also associated with significant alteration in the oxidative defense system and hippocampus degeneration. Histopathological observations clearly depicted the neural degeneration. Thus, it can be concluded that MWR significantly affects the central nervous system and may lead to many severe illnesses. This study may reveal a platform to understand its toxic effect and can further be used for amendment in current guidelines of mobile radiation.

1. Introduction

Non-ionizing radiation (NIR) is widespread in human environment. The most frequent sources of NIR are mobile phones and cell towers, which emits potential microwave radiation (MWR) in human environment. Recently, the level of electromagnetic radiation was increased by a thousand fold from artificial sources. There are 7.4 billion telecommunication subscribers in the world. India is the second largest user of telecommunication and third largest user for internet. This data shows that we are the key player in the mobile market. Mobile has become an indispensable part of our life. The increasing mobile phone mediated microwave exposure is an alarm for human health. Mobile phone radiation generally affects the brain, especially central nervous system. However, central nervous system is largely affected area, as 80% of the radiation emitted by mobile phone is absorbed by the brain (Kesari et al., 2013). Extensive use of mobile phone shows some physiological effects like, insomnia, dizziness and lethargic behavior. It also caused neurological damage followed by headaches, changes in sleep patterns, modifications in neuronal electrical activity, increases in the permeability of the blood–brain barrier (BBB) and disturbances in neurotransmitter release. Concomitantly, growing evidences suggested that MWR also induces oxidative stress in the brain and in other tissues which plays a critical role in DNA damage, abnormal cell proliferation, inflammation and mitochondrial dysfunction (Kivrak et al., 2017; Sahin

et al., 2016). Oxidative stress is an imbalance between the production of reactive species which may alters the learning behaviour, memory and increases anxiety like behavior (Patki et al., 2013). However, there are various studies which suggest that MWR depicted the changes in cognitive functions, but the mechanisms behind cognitive changes have not been clearly defined yet. Hippocampus is more sensitive to oxidative stress and responsible for memory, therefore, we have assessed the different region of hippocampus to link the toxicity with cognitive and behavioural alterations. This study suggests that microwave possess toxic effect on the brain, which may lead to generation of reactive oxygen species (ROS) which further caused neural and genomic damages. Therefore, this study was designed to underlying mechanisms for such radiation-related cognitive deficits. The principal objective of this study was to find out the effect of continuous electromagnetic exposure for 3 months.

2. Materials and methods

2.1. Chemicals

All chemicals were of analytical grade and procured from Sigma Aldrich Company (USA), E Merck (Germany). The other reagents used in the study will be procured from Ranbaxy, Sigma and Rankem etc.

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<https://doi.org/10.1016/j.jchemneu.2019.101684>

Received 23 June 2019; Received in revised form 20 September 2019; Accepted 20 September 2019

Available online 22 September 2019

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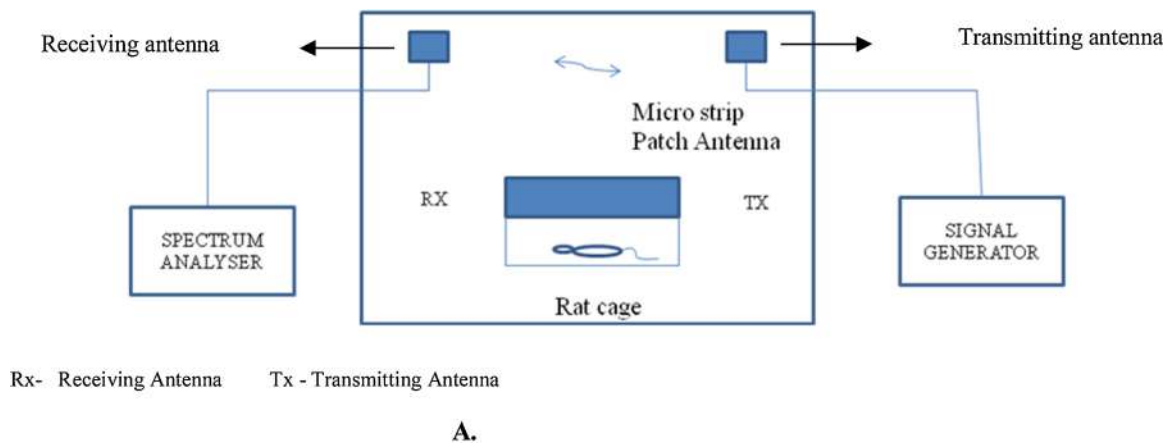


Fig. 1. A. Layout of exposure setup. B. Schematic design of exposure setup for microwave exposure of rats with all apparatus used. The transmitter and receiver antenna (patch) is at distance R from the rat cage.

2.2. Animals care and maintenance

Male *Wistar* rats (160 ± 10 g, b.wt.) were procured from DRDE, Gwalior. Animals were housed under standard conditions ($25 \pm 2^\circ\text{C}$ temperature, 60%–70% relative humidity and 14 h light and 10 h dark). The rats were fed on a standard pellet diet and water *ad libitum*. Animals used in this study were treated and cared in the animal facility Jiwaji University, according to the guidelines recommended by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The reference number is 1854/GO/Re/S/16/CPCSEA/IAEC/JU/24.

2.3. Microwave exposure setup designing

The exposure system was designed at frequency of 2100 MHz to expose the animals. The system is designed by Electronics Department of Madhav Institute of Technology and Science (MITS), Gwalior, India. Computer simulation technology (CST) supported the designing of antenna which efficiently radiate and receive the electromagnetic waves. Two antennas were used for the exposure setup. One for transmitting purpose (Tx) and another for receiving purposes (Rx). The antennas were mounted on a wooden box by facing each other with dimensions length (L) 100 cm $\times$ width (W) 70 cm $\times$ height (H) 40 cm. The transmitting antenna was connected to the signal generator (SMC 100,

Rohde and Schwarz GmbH and Co. Germany) to transmit signals at the frequency 2100 MHz and power 0.00 dB (1 mW). These transmitted signals were received by receiving antenna which was further measured with spectrum analyzer (ROHDE & SCHWARZ FS315) to track the receiving signals. Rat cages were placed near the transmitting antennas and the distance between both antennas was 1 m. Fig. 1A and B are depicting the layout and schematic design of exposure setup. Dosimetry of the microwave was assessed at a specific absorption rate (SAR) and power density. The calculation of the whole body specific absorption rate (SAR) was measured. The localized SAR of the brain is calculated on the basis of electric field and the tissue density as mentioned by different authors (Sun et al., 2017; Panagopoulos et al., 2013). The calculation formula applied for measurement is-

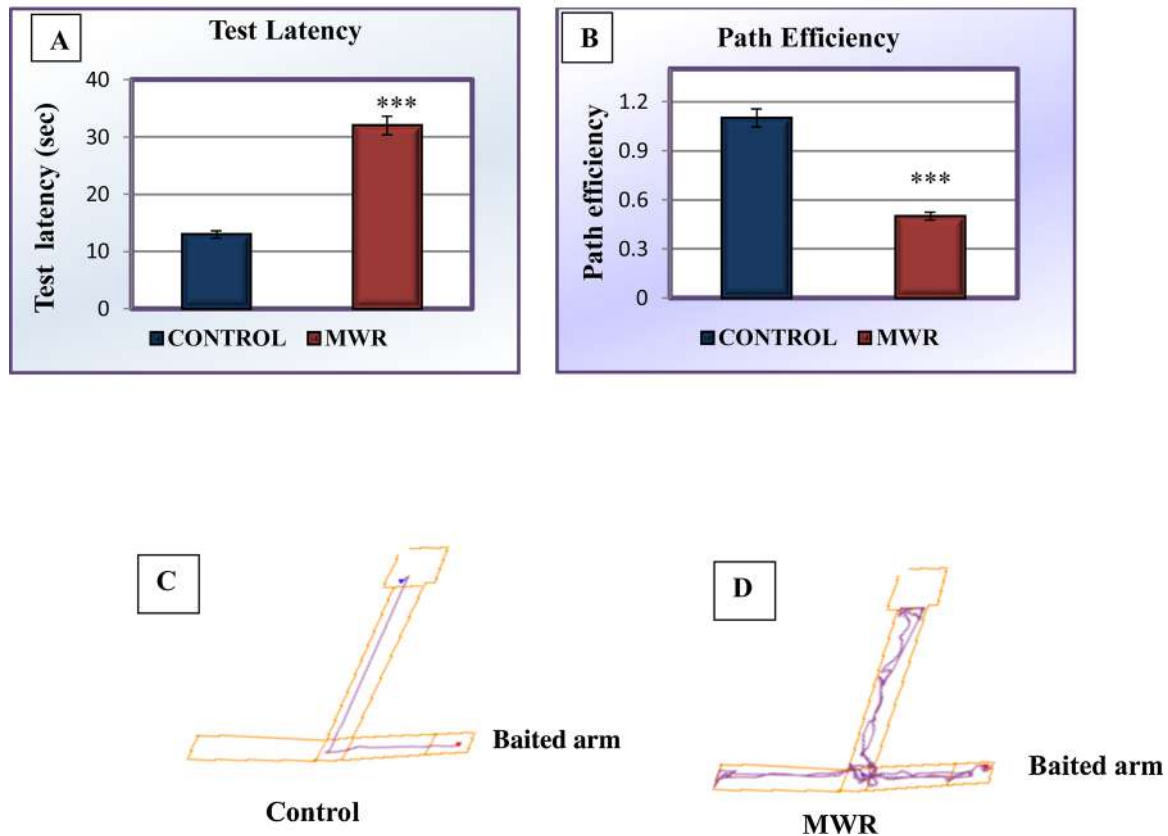
$$\text{SAR} = \sigma E^2 / 2\rho \text{ (W/Kg)}$$

Where,

- σ - electrical conductivity
- ρ - sample density (1000 kg m^3)
- E - root mean square of induced electric field strength (V/m) in tissue

The average localized SAR for brain region is 0.453 W/kg at a maximum power density ($8.237 \mu\text{W/m}^2$). In India the limit for localized

T Maze test



Graph 1. Effect of MWR on working memory in T Maze test after 90 days of MWR exposure, effect of MWR on test latency (A) and path efficiency (B), where (C) is the track report of control group (D) is the track reports of exposed group. Values are mean $\pm$ S.E; N = 6, significant at student t-test Microwave radiation (MWR) vs Control at $p \leq 0.001$.

SAR value of the microwave exposure is 1.6 W/kg. In the present study, SAR is much lower than the restricted limit.

2.4. Experimental protocol

Male Wistar rats were exposed with 2100 MHz frequency at a specific absorption rate (SAR) of approximately 0.453 (W/kg), 4 h/day and (5days/week) for a total time span of 90 days. A whole set of experiment was divided into two groups control and microwave exposed group. At the end of exposure tenure, these animals were sacrificed by decapitation and brain areas were dissected out, weighed and subjected to various assessments.

Group I: These rats ($n = 6$) were maintained in standard conditions similar to the experimental group (without near source of MWR).

Group II: Rats ($n = 6$) constantly exposed to MWR 4 h/day (Frequency 2100 MHz and average SAR 0.453 W/kg) for 90 days.

The conditions of exposure were determined in accordance of other expertise (Bedir et al., 2018; Nisbet et al., 2016). The exposure procedure was carried out in a temperature maintained room under controlled lighting conditions. Control groups were placed in the same condition without any exposure.

2.5. Behavioral tests

All animals were tested after 3 months of exposure. Because of high inter-individual variability, the initial testing before the exposure served as a control for correct random assignment into the groups, i.e. Behavioral differences between groups at the beginning. Behavioural tests were recorded and analyzed by automated tracking software Ethovision XT 10.1 (Noldus, Wageningen, Netherlands).

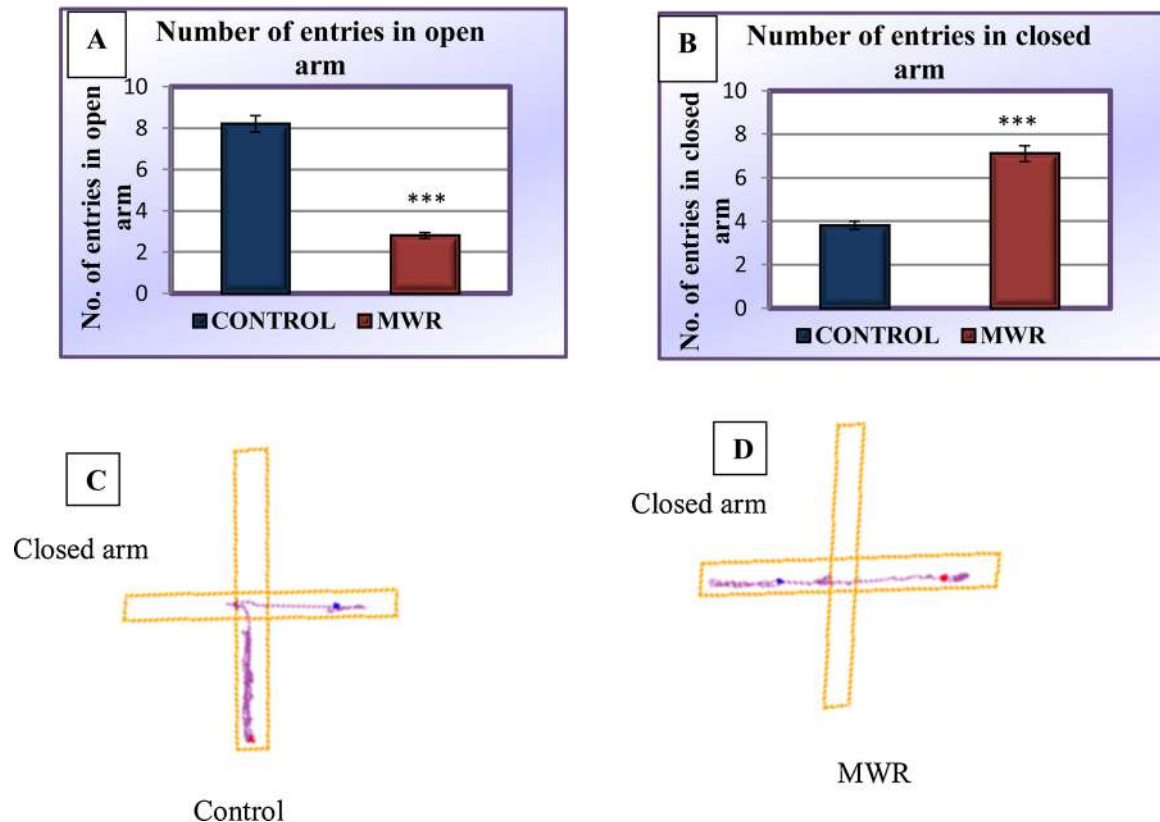
2.5.1. Elevated plus maze

Elevated plus maze (EPM) is a test to assess anxiety, fear like behaviors in rodents (Pellow et al., 1985) EPM is being performed in understanding the basis of emotionality related to the anxiety, stress and fear (Carobrez and Bertoglio, 2005). The behavior of the animals was recorded with the camera placed just above to the setup which is further connected to computer with "ANY" maze software (Columbus instruments, USA). The number of entries in open and closed arm was the measure of anxiety and fear like behavior.

% frequency and % time were calculated with the following formula-

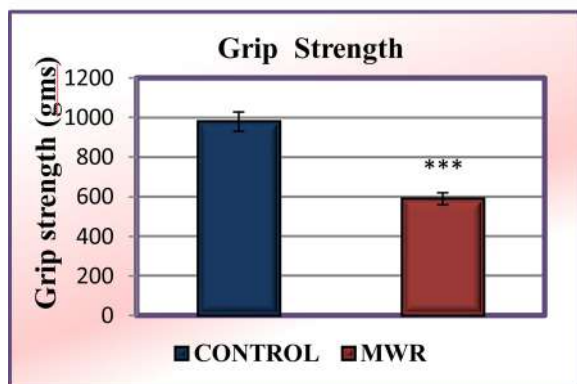
- % Open arm frequency = $[\text{frequency in open arms} / (\text{frequency in open arms} + \text{frequency in closed arms})] \times 100$
- % Open arm time = $[\text{time spent in open arms} / (\text{time spent in open} + \text{time spent in closed})] \times 100$

Elevated plus maze test



Graph 2. Elevated plus maze test was performed to detect the level of anxiety. Effects of exposure to EMF on the number of entries in open arm (A), entries in close arm (B). (C–D) showed track report where (C) control rats (D) exposed rats. Data are given in % (mean $\pm$ S.E.) N = 6, significant at student *t*-test Microwave radiation (MWR) vs Control at $p \leq 0.001$.

Muscular strength test



Graph 3. Depicted muscular strength test of animals. Data are given in gms (mean $\pm$ S.E.). Values are mean $\pm$ S.E.; N = 6, significant at student *t*-test Microwave radiation (MWR) vs Control at $p \leq 0.001$.

arms + frequency in closed arms)] $\times 100$

2.5.2. Spatial working memory

T-maze was used for testing the spatial working memory. In this task, the animals were trained to locate the baited arm (arm having eatable reward) based on their memory of previously visited arm (Cinzia et al., 2013). Preinstalled “ANY” Maze software version 4.82 (Stoelting, USA) was used to track the detail with the help of a camera.

2.5.3. Forelimb grip strength test

To assess neuromuscular function, grip strength test was performed. Grip strength meter (Columbus, USA) was used for this experiment.

2.6. Tissue biochemical assays

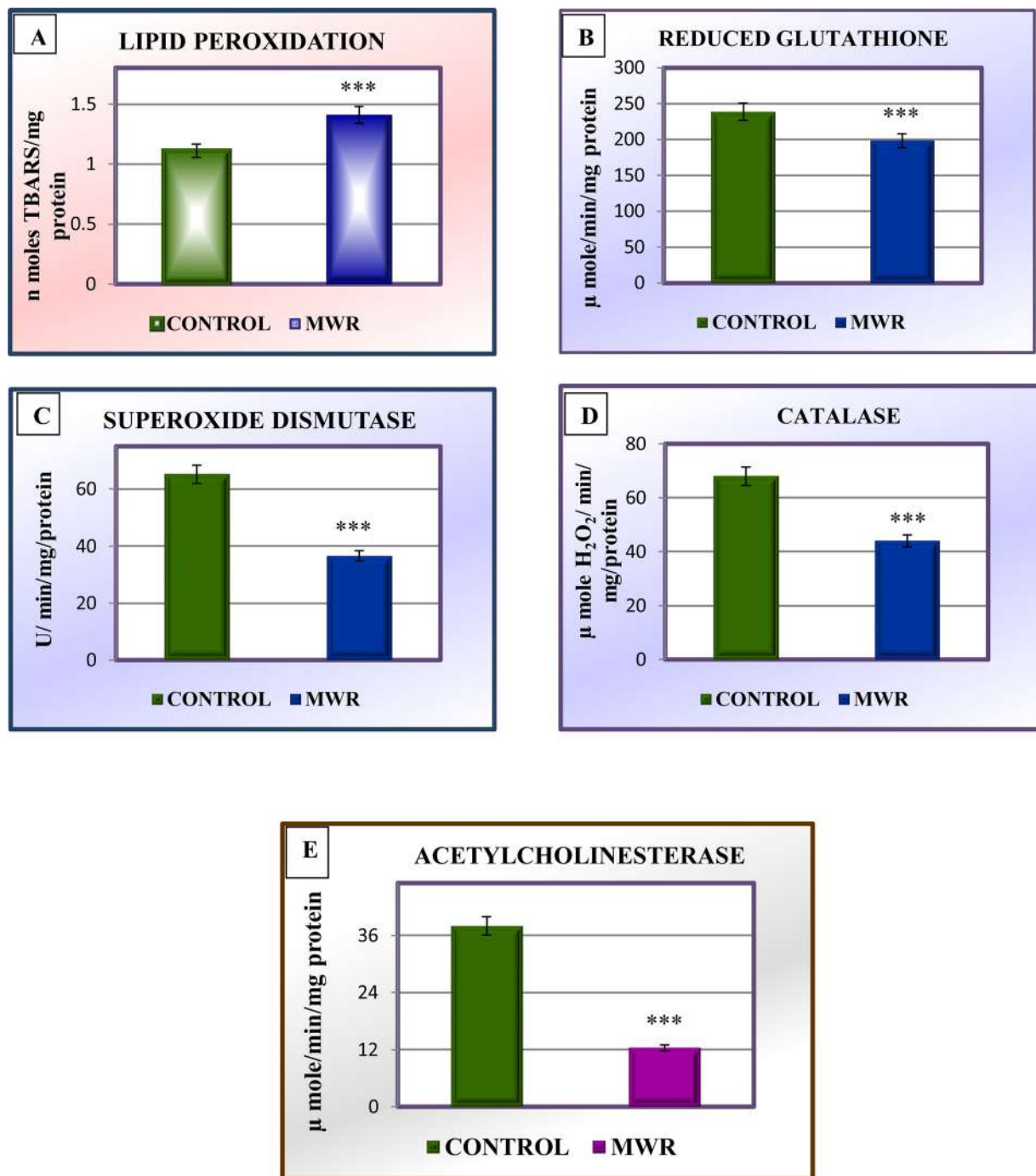
Immediately after the necropsy, brain tissues were excised, rinsed in ice cold normal saline and blotted dry for tissue biochemical estimations. Tissues were homogenized with a Remi Motor Homogenizer (RQ-122) using glass tube and teflon pestle and were immediately processed to determine lipid peroxidation (LPO) (Sharma and Murti, 1968), reduced glutathione (GSH) (Biochemistry, 1976), superoxide dismutase (SOD) (Misra and Fridovich, 1972), catalase (CAT) (Packer and Glazer, 1990) acetyl cholinesterase (AChE) (Courtney and Francisco, 1961).

2.7. Statistical analysis

Data were subjected to statistical analysis through student's *t*-test considering $P \leq 0.001$. Results are presented as mean $\pm$ S.E., of six animals used in each group.

2.8. Histopathological observations

Histopathological observations were observed by fixing the tissue in 10% formalin and processed for paraffin sectioning as per previously described method (Longa et al., 1989). Briefly, after fixation, tissues were carefully washed with water and dehydrated with a graded series of ethyl alcohol. Further, the tissues were cleared in toluene and infiltrated in the wax (Sigma, m.p.56–58 °C) for proper impregnation. Tissue blocks were made in the paraffin and serial coronal sections of



Graph 4. Effect of MWR on antioxidant defence system. Lipid peroxidation due to microwave radiation(A), depletion in reduced glutathione level (B), effect on superoxide dismutase activity (C), effect on catalase (D) and acetylcholinesterase (E) Values are mean $\pm$ S.E; N = 6, significant at student *t*-test Microwave radiation (MWR) vs Control at $p \leq 0.001$.

the hippocampus were cut at a thickness of the 10 μ m using WESWOX-MT0A microtome. The sections were stained by haematoxylin and eosin staining.

3. Results

3.1. Behavior alteration

The T maze test was performed to assess spatial learning and memory by assessing the test duration and path efficiency. In this task, the animals learn to find the baited arm (arm having eatable reward)

based on their memory of previously visited arms. Chronic exposure of microwave showed significant increase in test latency (Graph 1 A) and decrease in path efficiency (Graph 1 B) as compare to control group ($P \leq 0.001$). Modifications in path efficiency and test latency indicated the alteration in spatial working memory which is further confirmed by the track reports. The result describes that the control group easily found the baited arm (Graph 1 C) while the exposed group took longer time to find the baited arm (Graph 1 D). The results emphasized on the memory loss.

Number of entries in close arms and open arm of elevated plus maze is a measure to represent the anxiety level in rats. Data analysis

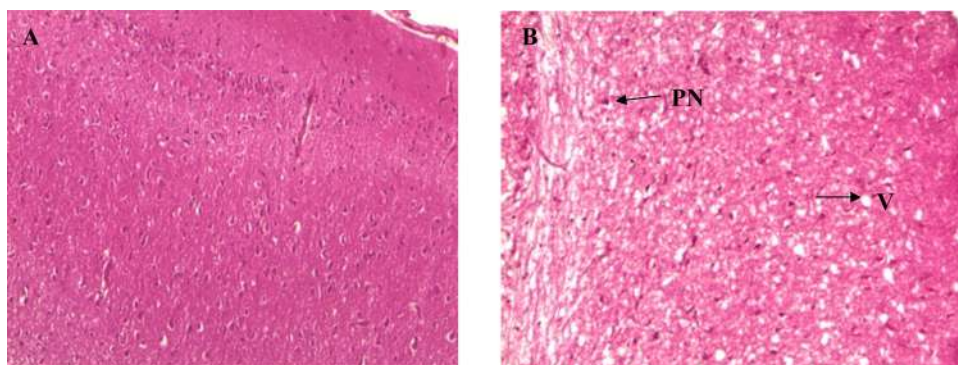


Fig. 2. Cerebral cortex of control rats (Scale bar = 50 μ m) showed normal healthy well arranged neuron with central nuclei and granular cytoplasm of rat brain (A). Exposure of MWR causes irregular arrangement of neurons, increase in perivascular spaces, vacuoles and pyknosis (B) (X 100). Abbreviations: PN, Pyknotic nuclei; V, Vacuoles.

revealed that exposed animals showed a significant decrease in open arm entries (Graph 2 A) and increase in closed arm entries (Graph 2 B) as compare to control group. It indicates the anxiety and fear like behavior in the exposed group ($P \leq 0.001$). Track report (Graph 2 C) shows that the control group animal spent equal time in both open and closed arms, whereas exposed group spent more time in closed arm (Graph 2 D).

Muscular strength was measured by the grip strength meter to assess the effect of MWR on the neuromuscular strength of the animals. Grip strength test (Graph 3) showed that grip strength was decreased in the MWR exposed rats when compared to control rats ($P \leq 0.001$). The values at all time points are significant as compared to the control group. Each animal was tested again at 2 min interval. Six readings per animal in total were recorded.

3.2. Tissue biochemical observations

Exposure of MWR to the experimental animals (Graph 4 A, B) caused a significant increase in membrane lipid peroxidation (LPO) with a potential fall in reduced glutathione (GSH) as compared to control group. After chronic exposure of MWR, the enzymatic defense system was affected, a significant reduction was observed in superoxide dismutase (SOD) and catalase (CAT) activities as compared to the control group showed in Graph 4 C, D ($P \leq 0.001$).

Acetylcholine (ACh) is a chemical that activates the neurons by working at neuromuscular junctions for muscular contraction. In the current study, we have assessed the effect of exposure on the acetylcholinesterase enzyme (AChE) in the synaptosomes of brain tissue. Graph 4 E depicts AChE activity, which indicated a significant inhibition of AChE activity in the brain ($P \leq 0.001$) as compared to control group. These alterations were confirmed by statistical analysis. Aggregation of reactive oxygen species (ROS) may be a possible cause for inhibition of AChE release from synaptosomes and blocking acetylcholine receptor.

3.3. Histopathological examination and analysis of rat brain

The cell population was estimated in cerebral cortex and CA1, CA2, CA3, DG regions of the hippocampus. Cerebral cortex of control rats showing normal neurons with central nuclei and granular cytoplasm (Fig. 2A), while MWR frequency exposed rat showed vacuolization, increase in perivascular spaces, demyelination and reduced nerve fibers in the brain (Fig. 2B). Serial sections passing through the brain were studied for cytological changes in neuron cell bodies (Fig. 3). Hippocampus of control rats showed a normal, healthy, well arranged pyramidal neuron with central nuclei and granular cytoplasm in CA1, CA2, CA3 and DG region of rat brain as shown in Fig. 3A, C, E, G respectively. H & E stain clearly showed that the exposure of MWR cause irregular arrangement, pyknosis, increase in perivascular spaces and degenerated neuron in different region of hippocampus as CA1, CA2, CA3 and DG shown in Fig. 3B, D, F, H respectively.

Fig. 4 explains that the cerebellum region of the brain. Control rat showed well formed regularly arranged layer of Purkinje cells (Fig. 4A, C). MWR exposure caused loss in the number of Purkinje cells with degenerated nuclei and tiny dendrites in cerebellum of the brain (Fig. 4B, D).

4. Discussion

Mobile technology for the digital India has grown and flourished nationally and internationally. International Agency for Research on Cancer (IARC) has identified that electromagnetic radiation from mobile phones and other wireless devices constitute a possible human carcinogen “2B” (Miller et al., 2018; IARC, 2011). Effect of mobile phone radiation exposure depends on its frequency and duration of use. 2100 MHz microwave frequency has been selected for exposure of rats because telecommunication industries are mostly using this frequency in India.

Exposure system was designed in such manner so that radiation similar to mobile signals was emitted by a standard dipole antenna instead of mobile phone. Animals were exposed in an open environment (without any shielding material) to simulate the real exposure condition, as people are generally used to talk in an open environment. This study demonstrated a noticeable link between the reduced cognitive ability in MWR exposed animals coupled with various neurological alterations.

The anxiety level of animals was assessed through elevated plus maze test. Number of entries in open and closed arm is an indicative of anxiety (Ehlers and Todd, 2017). The relationship between anxiety, close and open arm entry is still not well established. In some studies, decreased entries in close arm showed the anxiolytic status. Whether in our study MWR exposure showed decreased number of entries in the open arm because anxiolytic animals avoided the entry in open arm and tried to remain in close or safe place (Liu et al., 2008; Cui et al., 2007; Rodgers et al., 1997; Wąsik et al., 2019). Learning and memory paradigms were confirmed by T Maze, which showed loss in the learning behavior in the exposed rats. Various scientists reported the similar observations after chronic MWR exposure due to its lower penetration power (Mor et al., 2006).

Lipids play an important role in the membrane integrity, intracellular signal transmission and act as a barrier to mark the boundaries of the cells. Excessive amount of free radical production alters the maintenance of antioxidant defence mechanism and promotes membrane damage by lipid peroxidation (Mironczuk-Chodakowska et al., 2018). Chronic exposure of MWR disturbed the antioxidant defense mechanism and affected the membrane integrity. It grounds the leakage of various intracellular components into extracellular compartments (Yakymenko et al., 2018). In the present study manifestation of augmented free radicals in exposed rats were measured in term of TBARS level, an indicator of lipid per oxidation which instigates the pathophysiological modifications in biological system. MWR mediated free radicals caused an oxidative degradation of lipid molecules by stealing

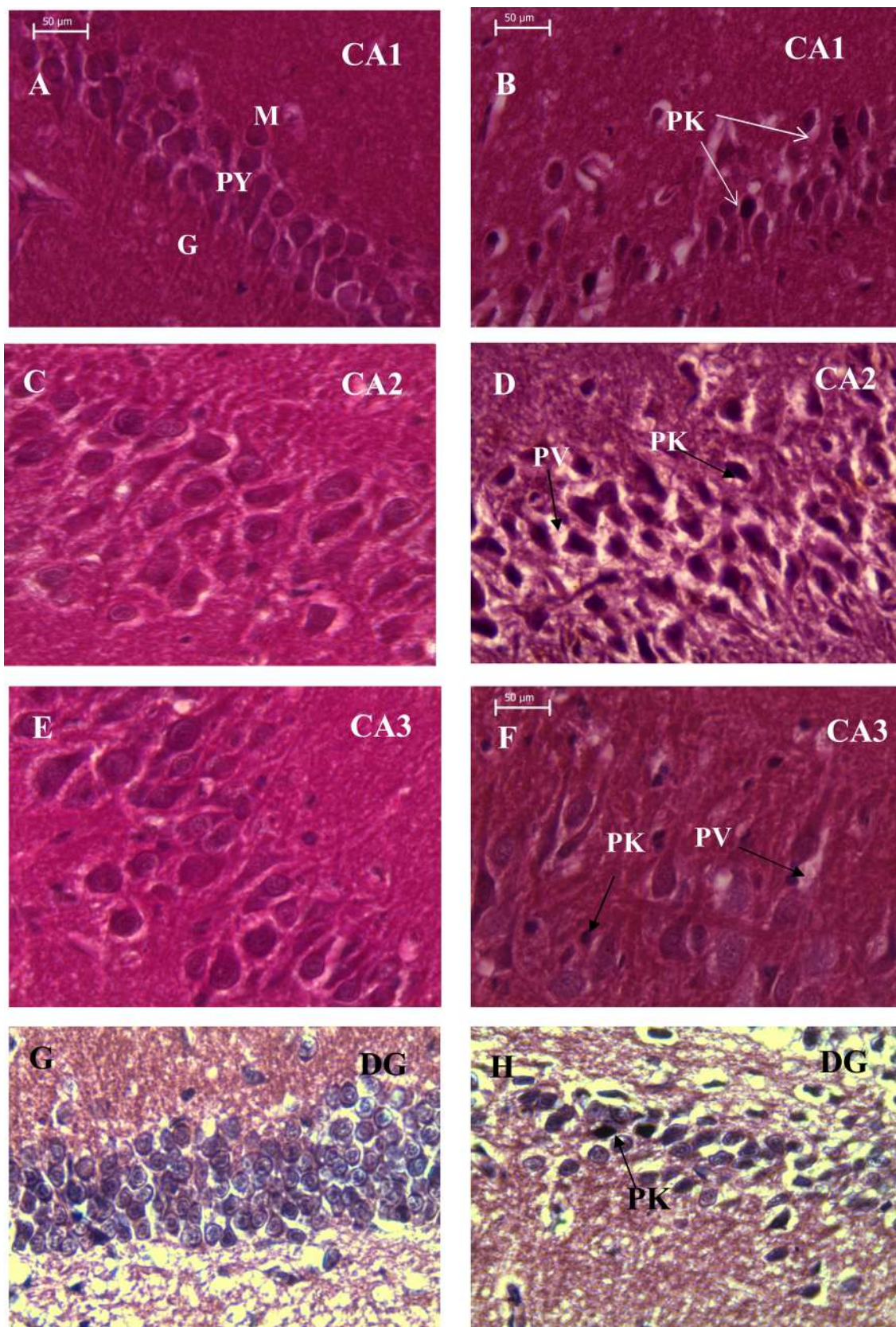


Fig. 3. Hippocampus of control rats (Scale bar = 50 μm) showed normal healthy well arranged neuron with central nuclei and granular cytoplasm in CA1, CA2, CA3 and DG region of rat brain Fig. 3A, C, E, G. It is clearly showed that exposure of MWR cause irregular arrangement of neurons, increase in perivascular spaces and pyknosis, there was also a significant decrease nerve fibers in the different region of hippocampus Fig. 3B, D, F, H. Abbreviations: M, Molecular layer; G, Granular layer; PY, Pyramidal layer; PK, Pyknotic nuclei; PV, Perivascular spaces.

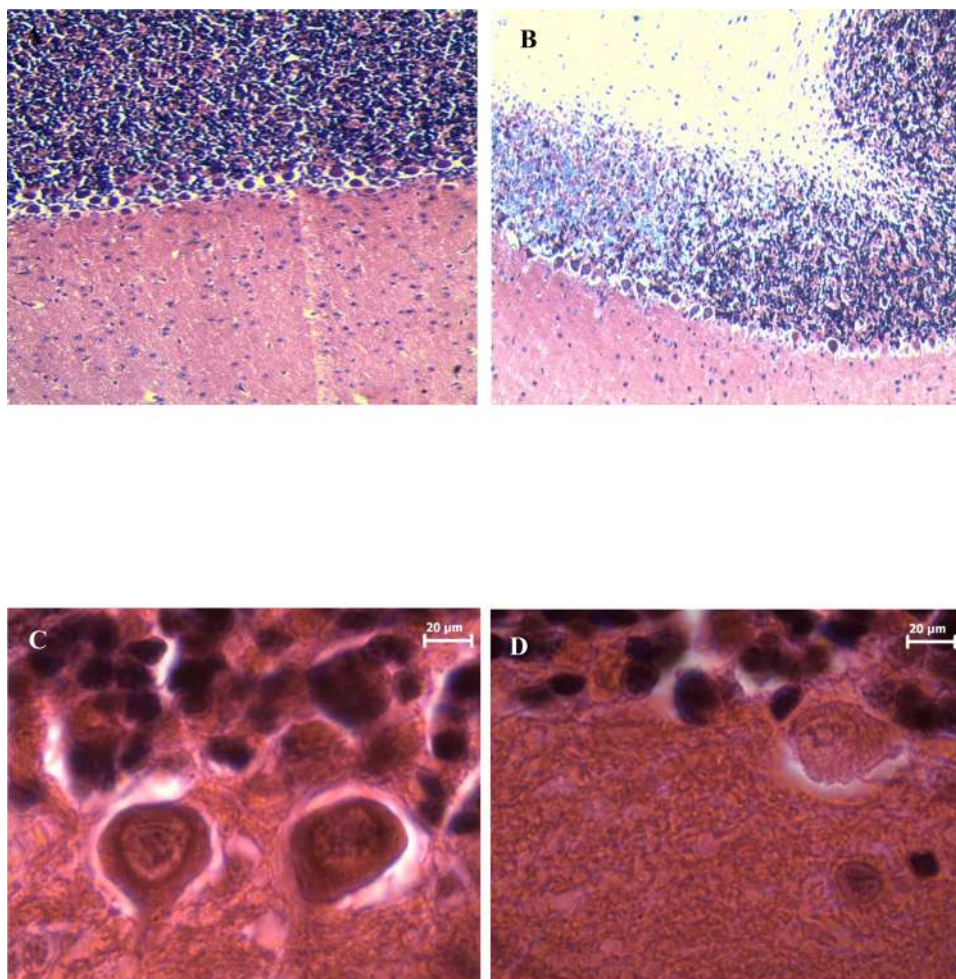


Fig. 4. Coronal sections were studied for cytological changes in neuron cell bodies. Cerebellum of control rats (X 100) showed normal healthy well arranged Purkinje cells with central nuclei and granular cytoplasm (A), H & E staining clearly showed that exposure of MWR cause loss in the number and also alter the morphology of purkinje cells (B), it can also be seen at higher magnification (Scale bar = 20 μ m). Control group shows well form Purkinje cells (C) while MWR cause loss in number as well as alterations in the morphology of Purkinje cells (D).

electrons from the lipids in cell membranes which results in cellular damage in the brain. It was noted that the increased rate of ROS in an organism releases calcium in its free form which disturbs membrane structure and enzyme activities increased free radical formation, resulting in cell damage directly or indirectly (Görlach et al., 2015).

GSH is an important cellular antioxidant and an indicator of cellular health. It is clinically related to a potential pathomechanism to localize structural alterations in specific brain regions responsible for cognition and behavioral alterations (Langbein et al., 2018; Fernandez-Fernandez et al., 2018; Kumar et al., 2010). The present study also depicted that MWR significantly decreased the GSH content in the brain tissue which is necessary for neutralization of ROS by its own oxidation into GSSG (Kivrak et al., 2017; Deshmukh et al., 2013). Lowered GSH/GSSG ratio in the brain is an indicative of redox imbalance in exposed rats.

Superoxide dismutase (SOD) is an antioxidant enzyme that dismutates superoxide radicals such as $O_2^{\cdot -}$. MWR caused significant decrease in the amount of SOD which was used to convert free radicals into H_2O_2 generated by MWR exposure. This excessive amount of H_2O_2 was consumed through CAT extensively and resulted in diminished catalase enzyme activity (Shehu et al., 2016). Neurons, cells probably have a narrow redox range for determining the steady state condition. Therefore, negligible change in redox level could alter neuron functions drastically and can lead to morphological or cellular modifications which results alteration in the oxidative stress induced neuronal transmission, neuronal function and overall brain activity (Wang and

Michaelis, 2010; Xie et al., 2010).

Brain resilience is associated with high oxidative stress and low antioxidant status (Freret et al., 2015; Salim et al., 2018). The findings suggest that MWR mediated oxidative stress altered the brain resilience and is also associated with anxiety (Hjemdal et al., 2011).

The research findings established a significant decline in the Acetylcholinesterase (AChE) activity. Depletion in AChE activity has a strong association with oxidative stress (Singh et al., 2013). Oxidation and cholinergic function are thought to be responsible for the memory disorders. Reduction of cholinergic markers is critical components for memory deficits (Terry and Buccafusco, 2003). Alteration in neurotransmitter was also reported as an impact on the functional status (neurobehavioral). In this study, AChE is determined as a net output of sensory, motor and cognitive functions in the nervous system which in turns makes it a potentially sensitive endpoint of EMR-induced neurotoxicity (File et al., 2000). We found that EMR exposed animals were poorly performing on T maze task revealing a reduced working memory. It may be due to the direct inhibitory effect of EMR radiations on cholinesterase activity. The hippocampus is associated with learning and memory (Deng et al., 2010) and alterations to the hippocampus lead to learning deficits and other disorders, like autism, Alzheimer (Shilyansky et al., 2010).

The present collective evidence grounds the consideration that EMR induces oxidative stress, reduced redox metabolism and depleted cholinesterase activity in the hippocampus. Later, the triangular associative

interplay between three factors in hippocampus results in memory deficit, poor motor coordination and anxiety behavior following mobile emitted radiations. The previous literature sounds for similar findings where high radiofrequency electromagnetic radiation exposure induces cognitive impairment and stress-related behaviors in rats (Wang et al., 2017). These findings were linked to the selective permeability and ionization properties of radio waves which allows them to cross the blood brain barrier (Sirav and Seyhan, 2009). Similarly the excessive levels of ROS produced during RF-radiation exposure were closely related to neural cell apoptosis (Kesari et al., 2011). Behavior and oxidative effects were further verified through histological aspects. Histological expression of different brain regions responded in variable manner (Sandhir et al., 1994). It was observed in various regions of rat brain, like CA1, CA2, CA3 and DG region of the hippocampus of exposed rat showed intense degeneration (Wang et al., 2017). The hippocampus is responsible for the control of some behavioral and cognitive functions, which includes storing and retaining information in the learning process (Altun et al., 2017). Chronic exposure of MWR showed the irregular arrangement of neurons. Neuronal damage was also confirmed by an increase in distorted and darkly stained nuclei. Significantly decreased number of pyramidal neurons and granule cells were found in the hippocampus of MWR exposed group. Scientific fraternities of MWR research have also reported almost similar findings with strong support of these observations (Wang et al., 2017; Kakkar and Kaur, 2011). As DG is accountable for neurogenesis, an exposure of 2100 MHz alters neurogenesis by decreasing in the number of granular neurons in DG region. This state of affairs may have increased neurological or concurrent behavioral defects (Bas et al., 2009). This study can be distinguished from the previous literature since we evaluated the oxidative damage induced by RF-radiation in brain related to cognitive and cholinergic activity. The originality of the present study is based on our investigation and confirmation of both the damaging effects of EMF on histological as well as neurochemical aspects. More efforts are required to better elucidate the pathophysiological changes that underpin the progression and severity of brain disorders in rats as well as human tissues following mobile-phone exposure.

5. Conclusion

Evidence of this research work supports the hypothesis that MWR may hamper the body defence mechanism, due to generation of free radicals. These free radicals then conjugate with GSH to be eliminated from the body and lower the GSH content in the body. Excessive generation of free radicals ruptures the cell membrane and shows increase in lipid peroxidation. These results further demonstrate the toxic effect by SOD and CAT deprivation the brain tissue of animals at the dose of 0.453 (W/kg).

The study further concludes that the toxic effects of MWR exposure elevated the intracellular oxidative stress, and neuron degeneration which directed the cognitive and behavioural abnormalities and further increases the risk of neurological disorders. In this context, mobile phones radiation exposure causes health issues. Thus, more advanced technologies with few biological effects should be developed.

Declaration of Competing Interest

The authors declare no conflict of interest in the present work.

Acknowledgments

The author would like to thank Jiwaji University, Gwalior for lab facilities and Madhav Institute of Technology and Science, Gwalior for support in calculations of the measurement uncertainty of exposure set up. This work was financially supported by UGC, India, meritorious student research fellowship in Biosciences. The fellowship reference number is 25-1/2014-15/(BSR)/7-97/2007/(BSR)-SSZ-1015/2015.

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