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Exposure of Radiofrequency Electromagnetic Radiation on Biochemical and Pathological Alterations

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Abstract:

Introduction: In the era of globalization, too much dependency on mobile phones is a cause of concern.

Objective: The present study was designed to evaluate the risk assessment of microwave radiation (MWR) at 1800 MHz frequency and specific absorption rate 0.433 (W/kg) on male Wistar rats.

Methodology: Animals were divided into two groups: the first group is the control group, and the second group was exposed to 1800 MHz radiation for 90 days at 4 h/5 days/week in a month.

Results: Chronic exposure of MWR may alter GSH homeostasis due to alteration in various GSH cycle regulating enzymes such as GR, GPx, GST, and G6PDH which showed an imbalance in GSH content and causes an increase in the oxidative stress and release of inflammatory cytokines. A remarkable increase in the DNA damage was seen due to disorganization and pyknosis of neurons in exposed animal's brain when compared with the control group ($P \leq 0.05$). There was also a significant decline in AChE level.

Conclusion: The study concludes that MWR may cause neurochemical and pathophysiological damage by initiating the inflammatory process in various brain regions, especially in hippocampus and cerebral cortex. These effects are further associated with a remarkable elevation in the genotoxicity of neurons with reference to the control group.

Key Words:

Inflammation, microwave, pathophysiology, Pyknosis, rats

Key Message:

Excessive use of mobile phones is a cause of concern, it causes inflammatory, neurochemical and pathophysiological damage to the brain.

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Microwave radiation (MWR) is widespread in the environment and connected to everyone through mobile phones and their towers. About 95% of the total global population reside in the area covered by a cellular network.[1] India has the second-highest number of smartphone users (approx 1.3 billion) next to China; thus, a large population is under risk due to excess use of mobile phones without knowing their health consequences. Therefore, it is a matter of serious concern.[2] The present study was designed to investigate the effect of MWR (1800 MHz frequency) on oxidative stress and inflammation through genotoxicity and histopathological observations in rat brain.

Materials and Methods

Animals and chemicals

In the present investigation, male *Wistar* rats (160 ± 10 g, body weight) were taken as an experimental model. Animals were housed under standard conditions (25 ± 2°C temperature 60%–70% relative humidity, and 14 h light and 10 h dark) and fed on a standard pellet diet and water *ad libitum.* Animals were cared according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments (CPCSEA) on Animals, with the reference number 1854/GO/Re/S/16/CPCSEA/IAEC/JU/24.

All chemicals used in the study were of analytical grade and procured from Sigma Aldrich

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Company (USA), Hi-Media, E Merck (Germany) Fisher Scientific and Rankem.

Microwave exposure

Animals were exposed as described in sharma *et al*., 2019.[3] The setup was established by the electronics department, Madhav Institute of Technology and Science, Gwalior, India. The aim was to establish an exposure system, possibly to radiate microwave radiation homogeneously in the surrounding for freely moving rats. The microstrip patch antenna at 1800 MHz frequency was designed realized and characterized to make an exposure array. The signal was generated by transmitting antenna and received by receiving antenna which was further connected to a spectrum analyzer (ROHDE & SCHWARZ FS315) to track the receiving signal. Animal cages were placed nearer to transmitting antenna with the power 0.00 dbm (1 mW).[3] Localized specific absorption rate (SAR) of the brain was calculated from electric field and the tissue density.[4] The calculation of SAR was done from the formula given in the equation.

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

- E is the local electric field modulus (V/m)
- σ is the electrical conductivity
- ρ is the sample density (1000 kg m^3).

The calculated SAR for the brain was 0.433 Watt/kg at a maximum power density which was lower than the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines (1.6 Watt/kg).

The calculated power density of antenna was 11.638 $\mu W/m^2$. All the exposure procedure was carried out in a temperature control room under constant light and dark condition.

Experimental design

Male *Wistar* rats were exposed to 1800 MHz frequency at a specific absorption rate of approximately 0.433 (W/kg), for 90 days and the animals were exposed for 4 h/day for 5 days in a week. The experiment was divided into two groups, consisting of six rats in each group.

Group I served as a control group; these animals did not expose to microwave but kept in similar condition as exposed group.

Group II: Animals exposed to MWR at 1800 MHz for 4 h per day for 90 days.

All the exposure procedures were carried out in a temperature control room under constant light and dark condition.

At the end of the experiment, these animals were euthanized and blood was collected according to Riley (1960).[5]

Tissue biochemical assays

After decapitation, brain tissue was collected, then rinsed in ice-cold normal saline and kept in deep freezer till further biochemical estimations. Tissues were homogenized through Remi motor homogenizer (RQ-122) using glass tube, teflon pestle and immediately processed to determine reduced glutathione (GSH),[6] oxidized glutathione (GSSG),[6] glutathione peroxidase (GPx),[7] glutathione reductase (GR),[8] glutathione-S-transferase (GST),[9] glutathione-6-phosphate dehydrogenase (G-6-PDH),[10] and acetylcholinesterase (AChE).[11] Levels of inflammatory cytokines (Interleukin-6 and TNf-α) were estimated by ELISA at a wavelength of 450 nm according to the manufacturer's instructions (R&D Systems) from Rat ELISA Kit of RayBio.

Acetylcholinesterase (AChE) activity

Acetylcholinesterase activity was assessed in the synaptosomes. For the synaptosomes preparation, 1 ml of 10% homogenate (w/v) was prepared from brain tissue in 0.25M sucrose solution and centrifuged the same as earlier; the supernatant was taken and again centrifuged at 2000 rpm for 10 min; the supernatant was taken in the falcon and centrifuged for 14,000 g for 60 min; the supernatant was discarded, and pellet was taken and dissolved in 2 ml of sucrose solution (0.32M). These synaptosomes were assessed for AChE activity.

For AChE estimation, the reaction mixture was prepared with 20 µl aliquot in 2.6 ml PO^-_4 buffer (0.1M), and further 0.1 ml DTNB (0.01M, dissolved in PO^-_4 buffer) was added and incubated for 5 min at 37°C. The reaction was initiated in the addition of 20 µl of acetylthiocholine iodide (0.075M dissolved in DH_2O) and observation was taken for 5 min at 412 nm.

Reduced/oxidized glutathione

Approximately 250 mg of tissue was homogenized in the solution of 3.75 ml phosphate EDTA buffer (0.1 M sodium phosphate buffer containing 5.0 mM EDTA, pH 8.0) and 1.0 ml of 25% of HPO_3 which were used as protein precipitant. GSH or GSSG was assayed in the supernatant obtained after centrifugation at 10,000 g for 30 min. 0.5 ml of supernatant was taken and 4.5 ml of phosphate EDTA buffer (pH 8.0) was added for the estimation of GSH. The final mixture contained 0.1 ml tissue supernatant, 1.8 ml PO_4 EDTA buffer, and 10 µl of O-phthalaldehyde. Fluorescence was determined after 15 min of incubation (room temperature) at 412 nm with activation at 350 nm.

For GSSG, 0.5 ml of supernatant was added to 0.2 ml of N-ethyl amide (0.04 M) and incubated at room temperature for 30 min. 4.3 ml of 0.1N NaOH was added, out of that 0.1 ml was used for GSSG assay in the manner identical to GSH. 0.1N NaOH was employed as diluents in place of phosphate EDTA buffer. Finally, the observation was taken at 420 nm with activation at 350 nm.

Glutathione reductase

GR was estimated by measuring the conversion rate of NADPH to NADP. 10% homogenate was prepared in 1.15% KCl and centrifuged for 10 min at 10,000 rpm. After centrifuging, acquired supernatant was taken for the test. Reaction mixture consists of 0.7 ml phosphate buffer, (GSSG), 0.1 ml homogenate, and 0.1 ml oxidized glutathione. Then, it was incubated at room temperature for 10 min. NADPH (0.1 ml) was added to initiate the reaction. Absorbance change/min was recorded at 340 nm for 5 min. Specific activity was expressed in the term of µM NADPH oxidized/min/mg protein.

Glutathione peroxidase

In 1.15% KCl, tissues were homogenized to prepare 10% homogenate with 10,000 rpm. Supernatant for the test has been

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processed. The reaction mixture was produced by adding 0.05 ml homogenate with 0.05 ml sodium azide, 0.3 ml sodium phosphate buffer, 0.01 ml NADPH, 0.1 ml reduced glutathione, and 0.05 ml glutathione reductase mixed well and incubated for 10 min at room temperature. The reaction was initiated after adding 0.05 ml H_2O_2 and change in optical density/min was documented for 5 min at 340 nm. The activity of glutathione peroxidase was assessed in μM NADPH oxidized/min/mg protein, expressed as a specific activity of NADPH as 6300/m/cm.

Glutathione-S-transferase

10% homogenate was prepared in 1.15% KCl and centrifuged at 10,000 rpm for 10 min and the supernatant was obtained for the test. The reaction mixture is made up with 0.5 ml phosphate buffer and 50 μl CDNB. 50 μl homogenate was added to the reaction mixture and then incubated at room temperature for 10 min. 25 μl of GSH was added to start the reaction, and optical density change per minute was documented for 5 min at 340 nm.

Glucose-6-phosphate dehydrogenase

The 10% homogenate was produced in 1.15% KCl; the supernatant was obtained for the assay after centrifugation at 10,000 rpm for 10 min. The reaction mixture containing 0.1 ml $MgCl_2$, 0.1 ml glucose-6-phosphate, 1.5 ml tris HCl buffer, 1.0 ml distilled water, and 0.1 ml diluted sample was incubated for 10 min at room temperature. 0.2 ml NADP+ was added to initiate the reaction of the mixture and change in absorbance/min was recorded for 5 min continuously at 340 nm. Specific activity was expressed in term of μM NADPH formed min^{-1} mg^{-1} protein by the motor extinction coefficient of NADPH as 6300 M^{-1} cm^{-1}.

Assessment of DNA damage

Single-cell gel electrophoresis was used for the measurement of DNA damage by Alkali method termed as comet assay.[12] The analysis proceeds upon the concept that strand breakage of the supercoiled duplex DNA undergoes to the shrinkage of the size of the big molecules as these strands can be stretched out by alkaline buffer. Besides, under extremely alkaline environment, there is denaturation, unfolding of the duplex DNA and expression of alkali labile spots as breakage of single strand and negatively charged DNA molecule turns out to migrate freely in the electric field and approaching to the anode. Comet formation occurs by two principles, viz., DNA migration and tail length. DNA migration is a property of both the size and the number of damaged ends of the DNA and tail length develops bearing initial damages and then extends to maximum dependent on the electrophoretic condition. Fluorochrome (ethidium bromide) is applied for staining the DNA appropriately. The eluted DNA fragments appear like a comet tail under fluorescence microscope.

Observations were documented by software Leica Q Win V3. As many cells were measured (25–30 cells per slide), the statistical distribution of the individual cell measurements represents the amount of DNA damage of the cell sample.

Interleukin-6 and TNf-α

Levels of pro-inflammatory cytokines were estimated by ELISA according to the manufacturer's instructions (R&D Systems) from Rat ELISA Kit of RayBio with the lot number (Interleukin-6 # 0826160724), (TNf-α # Lot # 0812160709). Rat ELISA Kit from RayBio was used for the assay. This assay works on the quantitative sandwich enzyme immunoassay technique. 100 μl of samples and standard were added in triplicate to the precoated well plate; then, 100 μl of biotinylated antibody was added to each well as a conjugated antibody for 1 h. Washed for four times, after washing, 100 μl of streptavidin horseradish peroxidase was added to each well. Incubated for 45 min followed by 100 μl of tetramethylbenzidine substrate solution for 30 min. Finally, 50 μl of stop solution was added to each well and absorbance was detected from an Elisa plate reader at 450 nm. The whole experiment was performed at room temperature. The sample values were analyzed according to the standard curve.

Histopathological observations

Serial sections passed through the brain were studied for cytological changes by light microscopy. Tissues were fixed in 10% formalin briefly; after fixation, tissues were carefully washed with water and then dehydrated with a graded series of ethyl alcohol. Then, the tissues were cleared in toluene. After clearing, tissues were infiltrated with paraffin (Sigma, m. p. 56–58°C) and the blocks were made.[13] Blocks of the hippocampus were sectioned at a thickness of the 10 μm using WESWOX-MT1090A microtome. After sectioning, slides were processed for haematoxylin and eosin stain. The observation was taken from the light microscope (Nikon Eclipse Ni).

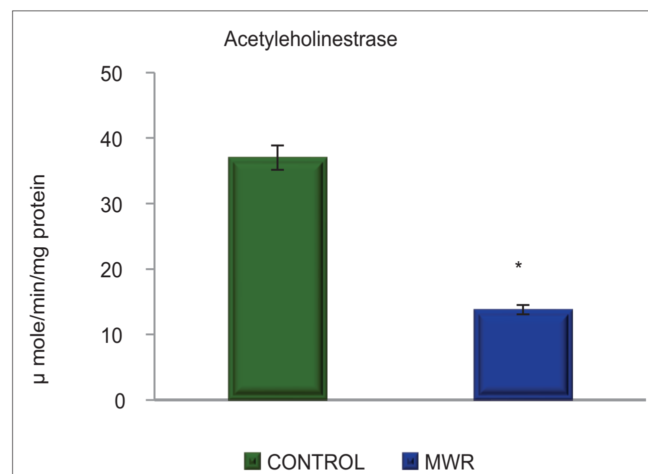
Statistical analysis

Data were subjected to statistical analysis with student's t-test considering significant $P \leq 0.05$.[14] Results are presented as mean ± S.E. of six animals in each group.

Results

Tissue biochemical observations

AChE activity in the synaptosomes of brain tissue of exposed and control groups [Graph 1] was reported. The toxic effect of radiation was clearly observed by significant inhibition of AChE activity in the brain ($P \leq 0.05$) after 3 months of exposure. Asterisk (*) indicates a significant difference between treatment groups with reference to the control group.



Graph 1: Effect of MWR on acetylcholinesterase activity, Values are mean ± S.E; N = 6, significant at student t-test, microwave radiation vs Control at $P \leq 0.05$

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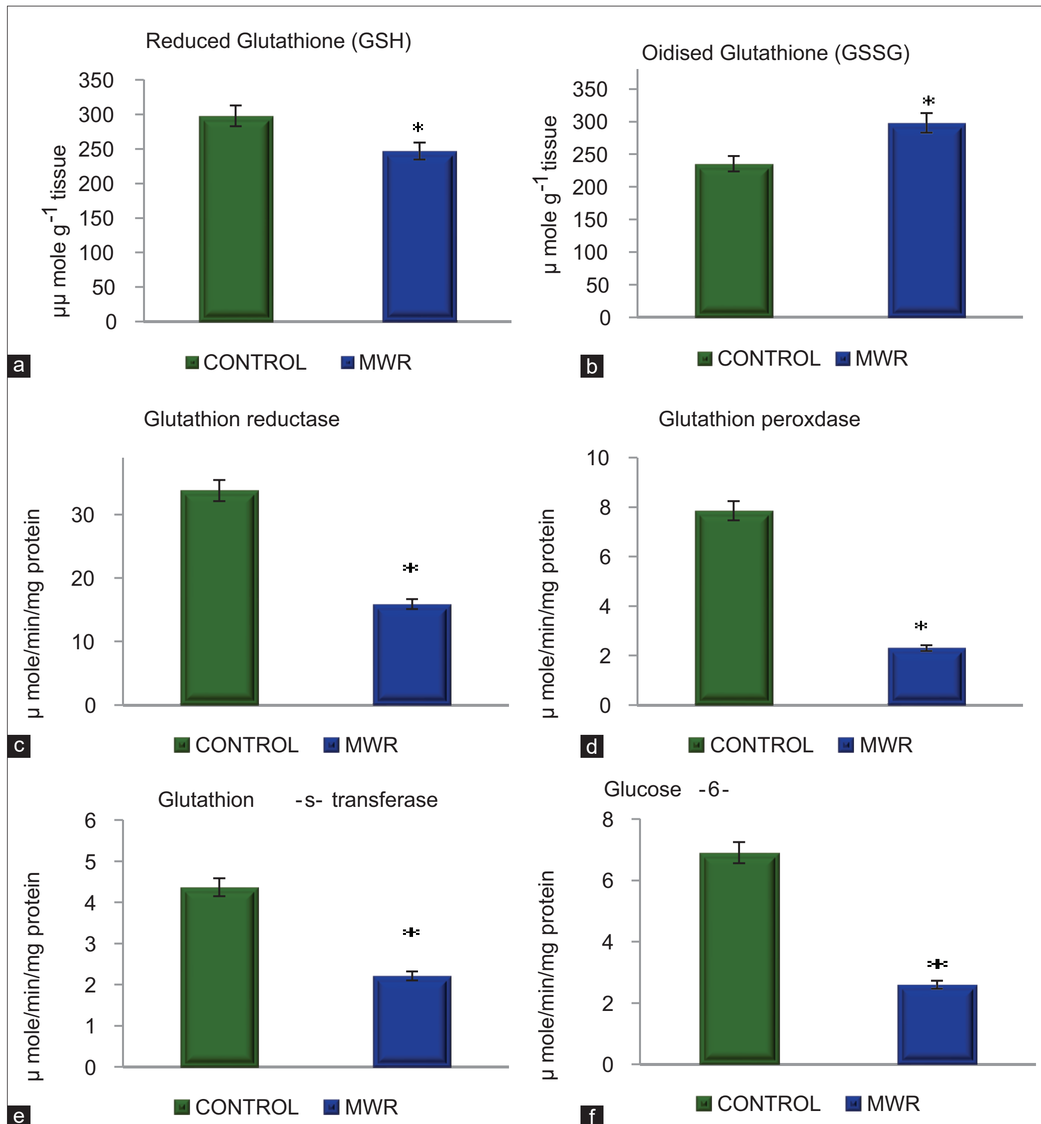
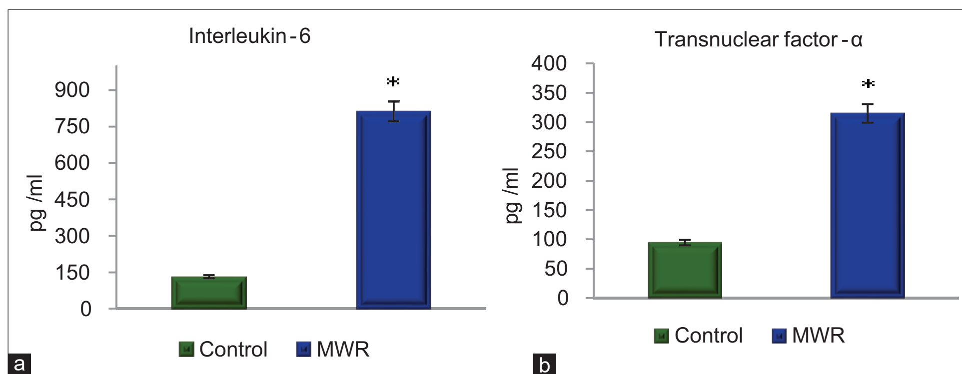


Graph 2: Effect of MWR on glutathione homeostasis. Reduced glutathione GSH (a), Oxidized glutathione GSSG (b), effect on Glutathione reductase (c), effect on glutathione peroxidase (d), glutathione s transferase (e) and glutathione 6 phosphate dehydrogenase activity (f). Values are mean ± S.E; N = 6, significant at student t-test, microwave radiation vs Control at $P \leq 0.05$



Graph 3: Effect of 1800 MHz microwave frequency on proinflammatory cytokines. Interleukin-6 (a), transnuclear factor- α (b). Values are mean ± S.E; N = 6, significant at student t-test, microwave radiation vs Control at $P \leq 0.05$.

Aggregation of reactive oxygen species (ROS) may be a possible cause for inhibition of AChE release from synaptosomes and blocking ACh receptor.

Glutathione redox cycle is a mechanism which scavenges lipid peroxides and hydrogen peroxide. The toxic effect of MWR on GSH cycle enzymes with reference to the control

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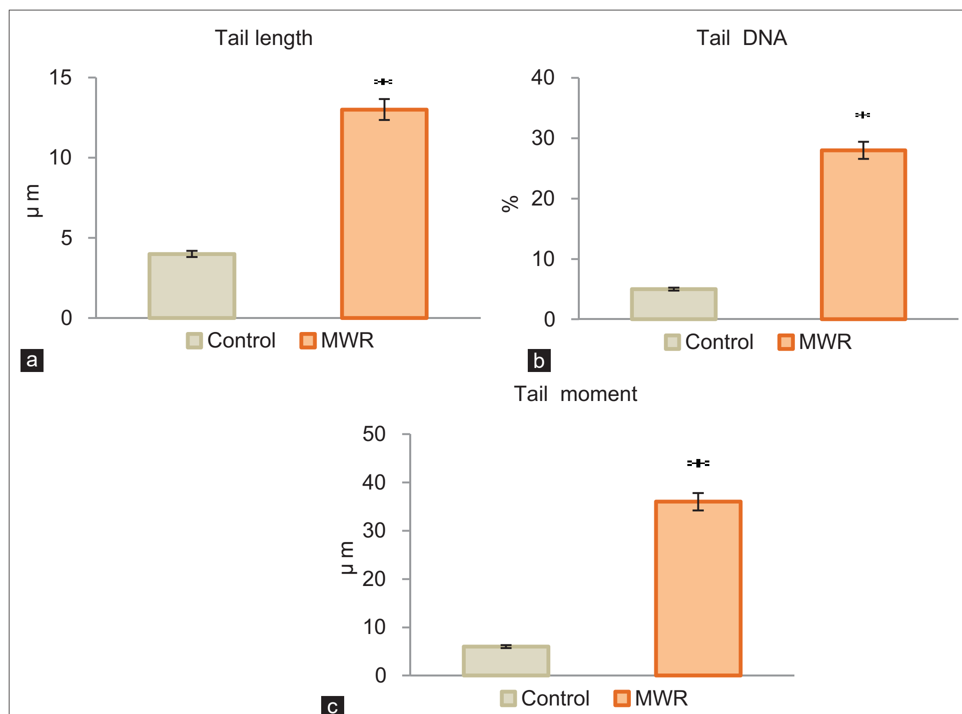

Graph 4: Effect of 1800 MHz microwave frequency on DNA damage in brain tissue. Increase in tail length (a), tail DNA (b), tail moment (c). Values are mean ± S.E; N = 6, significant at student t-test microwave radiation vs Control at $P \leq 0.05$

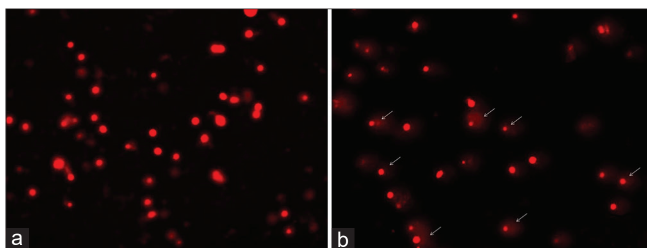

Figure 1: The extent of DNA damage showed in the microscopic observation. Control group showed intact spherical cellular structure (a). Exposed group shows a significant comet-like tail (arrow) depicted damaged DNA (b)

group was expressed in Graph 2. MWR exposure causes a significant decrease in GSH content [Graph 2a]. The maintenance of the cellular level of GSH was disturbed by a redox reaction to detoxify ROS, where GSH itself converted into GSSG. Thus, there was a significant elevation in GSSG content in the exposed group [Graph 2b]. GSH cycle mediates the level of reduced glutathione; this process involves many other enzymes as GR, GPx, GST, and G-6-PDH. Microwave absorption caused overproduction of free radicals that oxidises the GSH into GSSG and alters the level of GSH cycle regulating enzymes. Graph 2c and d showed that administration of MWR exposure caused a significant decrease in the activity of GR and GPx ($P \leq 0.05$). Due to exposure of MWR, the GSH need to achieve their reduced state by consuming GR.

MWR is responsible for significant inhibition ($P \leq 0.05$) in the enzymatic activity of Glutathione-S-Transferase (GST) showing in Graph 2e, due to the low level of GST, the amount of free radicals can not be neutralize thus the level of free radicals increases.

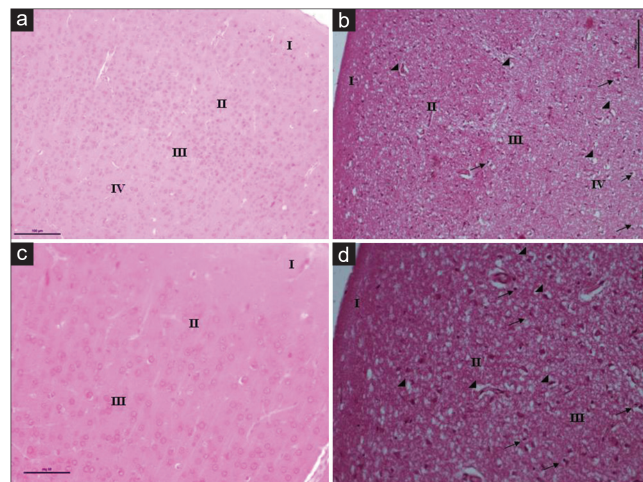

Figure 2: Control group a and b shows healthy equally distributed cortical neurons (Scale bar = 50 µm). Exposed group c and d shows increase in peri-cellular spaces (arrow head) and pyknosis in the nuclei (arrow) of all these layers of cerebral cortex

G-6-PDH plays a key role in the pentose phosphate pathway by maintaining the level of the co-enzyme nicotinamide adenine dinucleotide phosphate (NADPH). The NADPH in turn maintains the level of glutathione in the cells and protects them against oxidative damage. Microwave exposure at 2100 MHz frequency showed significant decrease in G-6-PDH activity Graph 2 Fat $P \leq 0.05$ indicating oxidative damage.

Estimation of Neuro-inflammatory markers

Oxidative stress initiated the inflammatory process in the brain of exposed animals. Chronic MWR exposure at 1800 MHz showed a significant effect on inflammation. Increase in the

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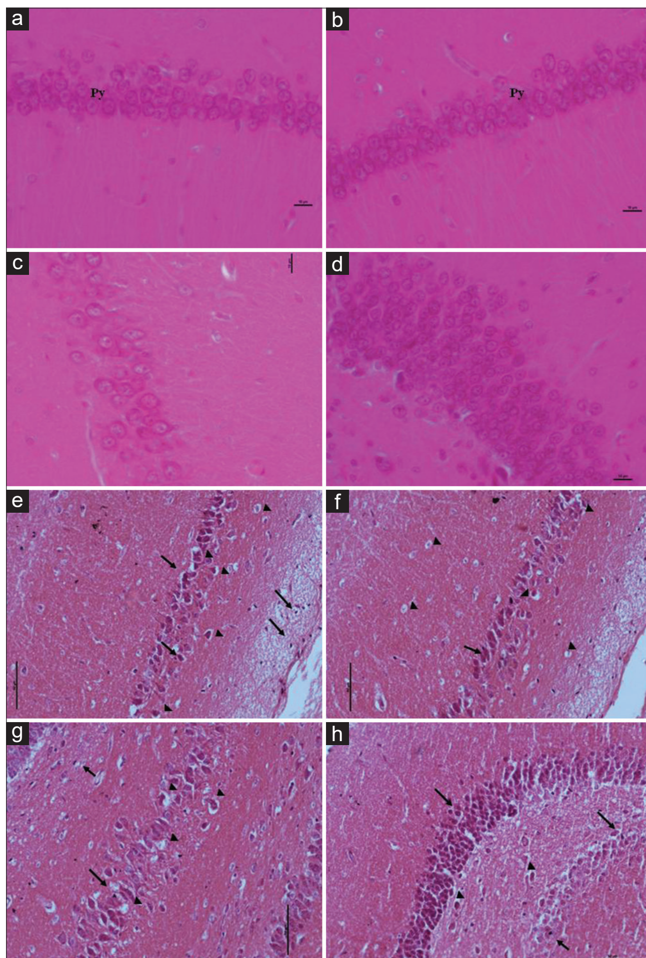

Figure 3: Depicts hippocampus of control rats. Regularly arranged and healthy neurons with central nuclei were seen in CA1 (a), CA2 (b), CA3 (c), and DG of rat brain (d) (Scale bar = 50 μm). Hippocampus of MWR exposure showed irregular arrangement, increase in perivascular spaces (arrow head) and pyknosis (arrow), in CA1 (e), CA2 (f), CA3 (g), and DG (h) at the scale bar = 50 μm

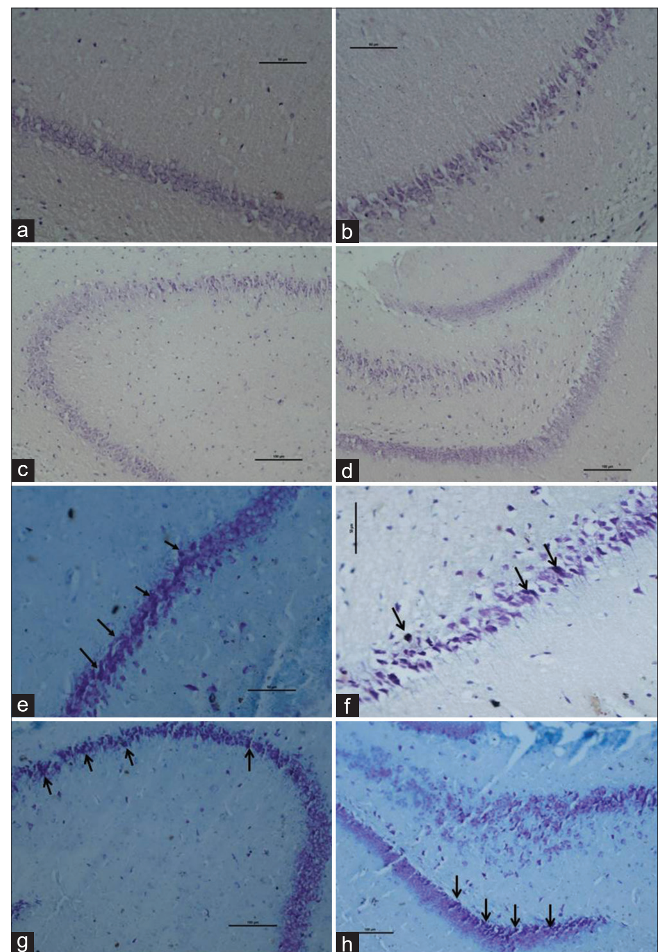

Figure 4: Hippocampus control group showed regularly arranged healthy pyramidal neuron layer in CA1 (a), CA2 (b), CA3 (c), and DG (d) at the scale bar = 50 μm. Hippocampus of MWR exposure showed darkly stained pyknotic neurons in (arrow) CA1 (e), CA2 (f), CA3 (g), and DG (h) region

level of inflammatory cytokines, specially proinflammatory cytokines IL-6 [Graph 3a] and TNF-α, [Graph 3b] ($P \leq 0.05$) was observed at the lower level from their normal values due to cellular stress in the body. Data significance was checked by the student's t-test.

Assessment of DNA damage

Comet assay

The alkaline comet assay is a sensitive method for measuring DNA strand breaks. A statistically significant qualitative and quantitative increase in the oxidative DNA damage was observed in the brain of exposed rats. The damage was confirmed in terms of increase in tail length [Graph 4a], tail DNA [Graph 4b], and tail moment [Graph 4c] with reference to the control group. The genotoxic extent was observed by software Leica (Q Win V_3) depicted in Figure 1a and b at $P \leq 0.05$ of significance.

Light microscopic studies

As different regions of the brain are related to different functionalities, we have assessed cerebral cortex and hippocampus region. The cell population was estimated in the cerebral cortex and hippocampus (CA1, CA2, CA3, and DG) region of the brain.

Histopathological observation of the cerebral cortex of rats showed different neuronal layers in the rat brain as molecular layer (I), external granular layer (II), pyramidal layer (III), and inner granular layer (IV). Control group animals showed well-formed regularly arranged neuronal layers with central nuclei of cerebral cortex in rat brain [Figure 2a and 2b]. Histopathology of microwave treated rats showed an irregular arrangement of neuronal layer along with increased number of vacuoles. Toxicity was further confirmed by darkly stained clumped pyknotic neurons. The number of neuronal cells was also decreased showing in Figure 2c and d.

Hippocampus of control rats [Figure 3] showed well-formed regularly arranged healthy neurons with central nuclei and granular cytoplasm. Neurons were regularly arranged in molecular, granular, and pyramidal neuron layers in CA1 [Figure 3a], CA2 [Figure 3b], CA3 [Figure 3c], and DG [Figure 3d] regions. H and E stain clearly depicts alteration in histo-architecture in the hippocampus of the exposed group.

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Hippocampus of microwave exposed group [Figure 3], at the scale bar of 50 μm, showed the degenerated irregularly arranged neurons with a significant increase in vacuolation, perivascular spaces, and reduced nerve fibers in the brain of exposed rats as evident in the CA1 [Figure 3e], CA2 [Figure 3f], CA3 [Figure 3g], and DG [Figure 3h] regions. Cell damage was clearly showed in the brain cells with darkly stained nuclei of the neurons. The results confirmed pronounced degenerating in the hippocampus region of the exposed brain. In the cresyl violet staining, it was observed that exposure of MWR shows pyknosis in terms of prominent dark stained pyramidal neuron in hippocampus [Figure 4 in CA1 (4A), CA2 (4B), CA3 (4C), and DG (4D)]. Animals of the control group showed healthy neurons [Figure 4 in CA1 (4E), CA2 (4F), CA3 (4G), and DG (4H)].

Discussion

In this study, we have observed the effect of 1800 MHz microwave exposure in rats. Brain uses a large amount of energy because of the high metabolic rate which makes it more susceptible to oxidative stress as compared to other organs. Increase in ROS causes necrosis and some neurological disorders such as multiple sclerosis, Alzheimer's disease, spinal cord injury, and epilepsy.[15,16]

GSH redox imbalance plays a key role in the onset and progression of many neurological disorders like Alzheimer's disease, by the alteration in the GSH/GSSG ratio.[17] There is a correlation between the GSH/GSSG ratio and cognitive performance in Alzheimer's patients; therefore, brain GSH is considered as an important biomarker for neurological alterations.[18,19] Exposure of microwave poses a significant fall in the level of GSH and elevation in oxidized glutathione. GR is a cytosolic enzyme involved in the production of glutathione by reduction of oxidized glutathione. This process needs NADPH, produced in the pentose phosphate pathway. A significant decrease was observed in neural GR activity after MWR exposure, which might be due to either free radical-dependent inactivation of enzyme or depletion of its co-substrate (GSH and NADPH). These findings are supported by several investigators.[20-22] GST is a phase-2 enzyme that plays a vital role in the detoxification and transports of many DNA alkylating agents, carcinogens, and environmentally hazardous chemicals. It also catalyzes the conjugation of GSH with various and endogenous toxic compounds. GST can reduce peroxides with the help of GSH and act as a producer for the scavenger of cytotoxic and genotoxic compounds. In our study, GST activity was decreased in MWR treated animals. The overproduction of free radicals as indicated by increased levels of GSSG by MWR might be associated with depletion of GST activity. Reduction of GST markedly promotes the formation of free radicals by MWR. The enzymes of the GSH cycle, which linked to maintaining a normal GSH level in the body, are also related to several psychiatric diseases.[23]

MWR causes a significant elevation in the DNA fragmentation in rat brain.[24,25] Microwave exposure did not show any detectable genotoxic effect directly, however, causes damage to the DNA by interfering the repair mechanism of the body and by increasing oxidative stress. In the case of oxidative damage, free radical interacts to DNA with the addition of bases or abstraction of hydrogen atoms from sugar moieties in the DNA strands.[26,27] The toxic effects have been linked to the selective permeability and ionizing properties of microwaves which helps them to cross blood-brain barriers.[28]

Synaptosomes are generally used to study synaptic transmission between the neurons because they hold the molecular machinery for the uptake, storage, and release of various neurotransmitters. They regulate membrane potential by presynaptic receptors, translocate metabolites, and ions and when depolarized release various neurotransmitters including amino acids, acetylcholine, peptides, and catecholamines in Ca_2^+ dependent manner.[29,30] Since in the early nineties, findings have clearly shown that some neurotransmitter systems are selectively altered in brain diseases.[31]

Depletion in cholinesterase activity in the present study might be associated with redox changes, induced by oxidative stress. The cholinergic function is believed to be responsible for memory disorders.[32]

The relationship between oxidative stress and AChE activity in the different brain regions has been reported earlier.[33] Reduction of cholinergic markers is a critical component for memory deficits.[34,35] The hippocampus is associated with learning and memory. Alteration to the hippocampus leads to learning deficits and other disorders like autism and Alzheimer's.[36] Similarly, it has been stated that GSH alteration is associated with cognitive impairment which, in turn, related to neurophysiologic alterations.

The free radical generated due to microwave exposure may stimulate immune response specially the T helper cells, which causes the release of certain pro-inflammatory cytokines as Il-6 and TNF-α that may lead to an inflammatory inequity. In the present study it is suggested that microwave exposure caused alteration in the level of proinflammatory cytokines.[19] Inflammatory cytokines release is related to the genesis of brain white matter lesions[37] which refers to the areas of the central nervous system and mainly made up of myelinated axons; hence, directly or indirectly increase in the inflammatory cytokines may modulate the cell to cell communication in the brain and mediates neurodegeneration.[38]

The hippocampus is responsible for cognition and behavior. Different brain regions had different reactions to the oxidative stress rather than the whole brain.[39] The brain of exposed rat showed degeneration of neuron cells in various regions.[40] Exposure of MWR showed that irregularly arranged neuron cells.[21] Neural toxicity is also confirmed by an increase in the number of this distorted and darkly stained nucleus and a decrease in the total number of pyramidal neuron in the CA region of the hippocampus.[41] As the hippocampus involves storing and retaining information in the learning process, MWR exposure negatively affects the memory and learning process.[42] As DG is associated with neurogenesis, an exposure of 1800 MHz frequency alters neurogenesis by decreasing in the number of granular neurons and this state of affairs may have increased neurological or concurrent behavioral defects[43]

In conclusion, the study showed the mechanism of MWR toxicity at the frequency of 1800 MHz and the SAR value 0.433 W/kg.

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MWR exposure contributes to the poor defense capacity of the brain against ROS due to compromised GSH homeostasis and causes a condition of oxidative stress. These changes modulate the immune responses by producing inflammatory cytokine. Oxidative stress leads to DNA fragmentation and initiated neuronal cell death in the hippocampus region verified by histopathological observations. Thus, strengthening the statement that MWR could be a huge risk of induction of brain damage, though further investigations are needed to unblemished the mechanism in detail.[44,45]

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Conflicts of interest

There is no conflicts of interest.

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