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Oxidative damage in the liver and brain of the rats exposed to frequency-dependent radiofrequency electromagnetic exposure: biochemical and histopathological evidence

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ABSTRACT

The study aimed to discover a link between the liver and brain's functional status due to frequency dependent-radiofrequency electromagnetic radiation (RF-EMR). Forty *Wistar* rats were randomly classified as control (sham-exposed) and EMR exposed groups. Animals were exposed to 900, 1800, and 2100 MHz with the specific absorption rate (SAR) 0.434 (W/Kg), 0.433 (W/Kg), and 0.453 (W/Kg) respectively. Animal exposure was limited at 1 h/day, 5 days/week for 1 month with a restricted power density (900 MHz- 11.638 $\mu W/m^2$, 1800- 11.438 $\mu W/m^2$ and 2100 MHz frequency- 8.237 $\mu W/m^2$). Exposure at various frequencies showed a frequency-dependent change in the body weight and hematologic parameters (RBCs, WBCs, platelets, hemoglobin, and hematocrit) as compared with the control group ($p \leq 0.01$) ($p \leq 0.001$). A significant elevation in serum transaminases and bilirubin, urea, uric acid, and creatinine was noted, whereas albumin significantly decreased after EMR exposure ($p \leq 0.01$) ($p \leq 0.001$). The blood glucose, lipid peroxidation, triglycerides, and cholesterol were elevated while adenosine triphosphatases, acetylcholinesterase, and tissue antioxidants such as glutathione, superoxide dismutase, catalase, *glutathione reductase*, glutathione Peroxidase, *glutathione-S-transferase*, and glucose-6-phosphate dehydrogenases were decreased significantly ($p \leq 0.001$). Histopathological observations of the liver showed centrilobular mononuclear cell infiltration and swelling in sinusoidal spaces, while in the brain degenerated pyramidal and Purkinje neurons were seen. Furthermore, Substantial evidence was found that the brain is more susceptible to oxidative mutilation compare to the liver of exposed animals. In conclusion, RF-EMR exposure showed oxidative damage to the liver, increasing the incidence of brain damage in a frequency-dependent manner.

HIGHLIGHTS

- EMR exposure showed frequency-dependent toxicity.
- Alterations in blood profile and modifications in the serological markers.
- Increasing lipid peroxidation indicating membrane damage.
- Inhibition of acetylcholinesterase activity affecting cholinergic neurotransmission.
- EMR exposure resulted in the loss of cellular energy and production of excess amounts of ROS thereby altering several antioxidant enzymes.
- Histopathological evidence of severe degenerative changes in the liver and brain.

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Radiofrequency exposure; free radicals; liver; neurodegeneration; oxidative stress

Introduction

Radiofrequency is omnipresent in the environment so the level of electromagnetic radiation is exceeding beyond the present guidelines of the national safety standards [1]. The major sources of electromagnetic radiation to human exposure are telecommunication devices. Telecommunication technology is growing day by day as 2 G, 3 G, 4 G and now 5 G is about to introduce. Globally, it is estimated that there are over 5.2 billion users of mobile phones and this number will keep on growing [2,3]. Every technical advancement raises certain health concerns, and mobile phones are no exception. The use of a mobile phone is increasing to a large extent without knowing the health consequences; thus, the medical evaluation of exposed and unexposed individuals is needed.

Mobile phone transmitted electromagnetic radiation (EMR) has been listed as a potential carcinogen to humans by the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [4]. Previous literature already have been established the harmful

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effects of EMR on the body. EMR penetrates the animal body and causing damage by changing the cell membrane potential, dipoles, and ion concentration. EMR has the potential to cause oxidative stress in different organs by free peroxidation and modifying antioxidant activity [5]. An increase in oxidative stress leads to lipid peroxidation and oxidative damage, ultimately affecting many systemic disturbances causing cell death [6,7]. The harmful effect of EMR was reported on various organs, including the testes, lungs, kidneys, liver, and brain [8,9]. Electromagnetic waves can be absorbed by a variety of body tissues, including the liver and brain, depending on where the handset is carried [10]. The conductivity and dielectric properties of different tissues influence the rate of EMR absorption. The conductivity depends on the tissue density, which is different in various organs [11]. Because of the cell phone's proximity to the brain, it has been suggested that EMR may affect the nervous system [12]. Electrical conductivity increases in the brain due to high water content, which makes it hypersensitive to EMR [13]. On the other hand, the brain uses around 20% of the oxygen in the body and contains polyunsaturated fatty acids, which are one of the main targets of reactive oxygen species making the brain more vulnerable to oxidative damage [14].

Electromagnetic exposure also effects the liver, like other toxicants, EMR-induced ROS are also primarily detoxified in the liver and cause hepatotoxicity [15]. It is already documented that the whole body's electromagnetic exposure is related to harmful effects on various organs [15–17]. However, it remains uncertain if the brain's oxidative stress caused by EMR is linked to liver damage. As neurodegeneration has a strong association with liver injury, brain activity declines when the liver is not able to expel toxins from the bloodstream [13]. A damaged liver can negatively effect the bloodstream that may further affect the nervous system [18]. The effect of EMR on the liver should be noticed in association with brain damage; thus, this research was founded on the need to resolve this problem. Therefore, the present study is an approach to elaborate on the possible association of liver injury in conjunction with the degenerative changes of the brain following mobile radiation exposure.

Materials and methods

Chemicals

All the required chemicals, diagnostic kits, and solvents were of analytical grade acquired from Sigma Aldrich (USA), Ranbaxy Pvt. Ltd. (India), E- Merck (Germany), Himedia Laboratories (Mumbai MH, India), Erba Pvt. Ltd. (India).

Animals care and maintenance

All India Institute of Medical Sciences (AIIMS), New Delhi, provided male *Wistar* rats (150 ± 10 g, *b.wt.*). The animals were kept under normal conditions (25 ± 2 °C temperature, 12-h light, and 12-hour dark condition and relative humidity of 60%–70%) to the animal house of Jiwaji University. The animals were given a normal pellet diet and free access to water. This work was ethically approved by the Jiwaji University according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines. All the animals were maintained with recommended guidelines (1854/GO/Re/S/16/CPCSEA/IAEC/JU/24).

Exposure set up for animal study

Animals were exposed to the exposure set up at three different mobile phone frequencies to find out their ill effects as described in our previous study [5,19]. This exposure array depicts the experimental exposure system for freely moving rats, possible to radiate homogeneously in the environment. For this purpose rectangular microstrip patch antennas were designed at 900, 1800, and 2100 MHz frequencies.

Dosimetry characterization

Dosimetry is the amount of dose of EMR frequency absorbed into the body. It was expressed as power density and specific absorption rate.

Specific Absorption Rate (SAR) was quantified from the electric field followed by tissue density. The formula below was used to calculate the result. The value of SAR was calculated from the electric field and tissue density [20]. The electric fields of 900, 1800, and 2100 MHz are 15.613, 18.192, and 17.660 V/m, respectively.

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

where E = local electric field modulus (V/m)σ= electrical conductivityρ= sample density (1000 kg m^3)

In India, the recommended localized SAR value of mobile phone exposure is 1.6 W/kg. The calculated SAR of the proposed antenna was used to radiate below the SAR limit of the International Commission on Non-ionizing Radiation Protection (ICNIRP) standard. SAR value of 900, 1800, and 2100 MHz are 0.434 (W/kg), 0.433 (W/kg), and 0.453 (W/kg), respectively.

The power density of the antenna was measured by power meter and calculated by the following formula-

$$PD = Pt/4\pi r^2$$

where PD = power density, Pt = transmitted power, r = range from antenna, that is, radius of the sphere. The calculated power densities of different antennas were lesser than the decoded limit of the ICNIRP. Power density for 900 MHz was 11.638 μW/m^2, 1800 was 11.438 μW/m^{2}and 2100 MHz frequency was 8.237 μW/m^2.

The schematic design of an exposure system is depicted in Figure 1(A). As wood is a poor conductor so it was used to isolate the outer radiation from the exposure array by covering it with a wooden box with the dimensions (length: 100 cm × width: 70 cm × height: 40 cm) (Figure 1(B,C)). Two antennas were designed for each frequency and mounted by facing each other on a wooden box. Out of these two, one was for transmitting purposes and the other was for receiving purposes.

The transmitting and receiving antennas were connected to a signal generator (ROHDE & SCHWARZ) and a spectrum analyzer (ROHDE & SCHWARZ FS315 - 9kHZ to 3 GHz) to monitor the signals. Animals were exposed in the plexiglass cages with the dimension height – 16, length – 40, and width – 24 cm. In the exposure system cages were kept horizontally, nearer to the transmitting antenna exposed at 25 ± 2 °C and 60%–70% relative humidity. Emitted EMR radiations were measured by a spectrum analyzer and power meter connected through an SWR bridge.

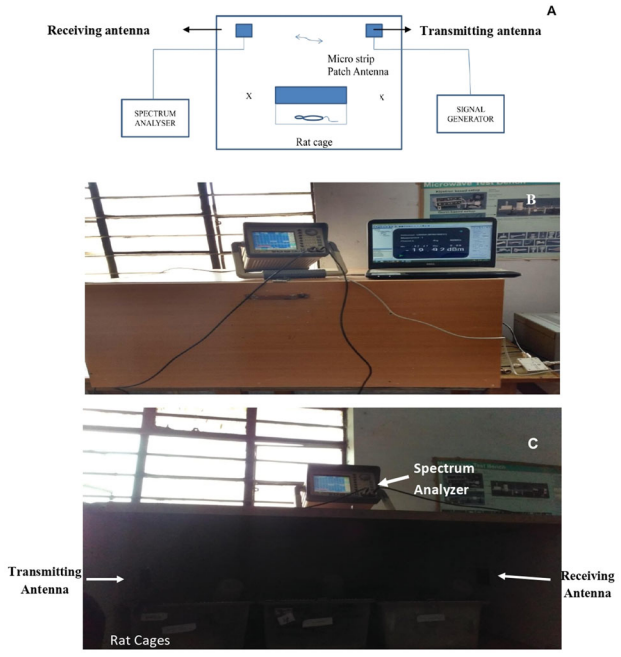


Figure 1. (A). Schematic design of exposure setup. (B–C). Layout of exposure setup for EMR exposure of rats with all apparatus used.

Experimental protocol

Male *Wistar* rats were exposed for 1 month at 900, 1800, and 2100 MHz frequency for 60 min per day (5 days/week). Power density for 900 MHz was 11.638 μW/m^2, 1800 was 11.438 μW/m^2 and 2100 MHz frequency was 8.237 μW/m^2. Forty *Wistar* rats were divided and categorized into the following groups-

Group Ist: These rats ($n = 10$) were kept in standard conditions and served as the control group (Sham exposed).
Group IInd: Animals ($n = 10$) exposed at 900 MHz frequency.
Group IIIrd: Animals ($n = 10$) exposed at 1800 MHz frequency.
Group IVth: Animals ($n = 10$) exposed at 2100 MHz frequency.

Collection of blood and preparation of serum

Blood was collected from each animal immediately before the sacrifice by puncturing the retro-orbital plexus [21].

Hematological parameters

Various hematological parameters as red blood corpuscles, hematocrit, and hemoglobin quantification were performed with an automatic counter ABX MICROS 60 from HORIBA. The measurement was done with 10 microliters of blood obtained from control as well as treated animals.

Serological parameters

The blood samples were kept for 30 min at 37 °C and the clot was smoothly detached from the wall of the test tubes. These tubes were centrifuged for 20 min at 2000 rpm to collect hemolysis-free clear serum, which was later stored at −20 °C. The serological parameters were also observed in terms of blood sugar [22], Albumin (BCG Dye method), bilirubin (Diazo method), Aspartate transaminase (AST), and Alanine transaminase (ALT) [23], urea (GLDH-Urease method), uric acid (Modified Trinder method), and creatinine (Jaffer's method). These parameters were estimated by the kits (Erba, India Pvt Ltd.) using a serum autoanalyzer (Micro Lab 200, Merck).

Tissue biochemical assays

Liver and brain tissues were isolated shortly after the necropsy and kept (−20 °C) for biochemical assessments. Tissues were homogenized with a Remi Homogenizer (RQ-122) using a teflon pestle and then processed to assess reduced glutathione (GSH) [24], lipid peroxidation (LPO) [25], catalase (CAT) [26], superoxide dismutase (SOD) [27], adenosine triphosphatase (ATPase) [28], acetylcholinesterase (AChE) [29], glutathione reductase [30], glutathione peroxidase [31], glutathione-S-transferase [32], and glucose-6-phosphate dehydrogenase [33].

Histopathological observations

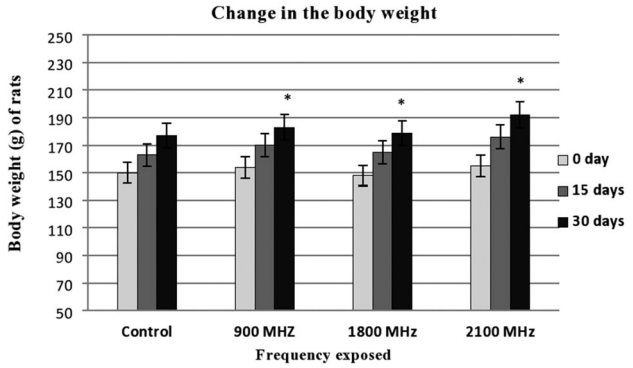
Rats were anesthetized using diethyl ether and then transcardially perfused with PBS (0.01 M; pH 7.4) along with fixation in 4% paraformaldehyde in 0.01 M PB (pH 7.4). Tissues were sliced and fixed in the same fixative for another 24 h. After fixation, a graded sequence of ethyl alcohol was used to carefully wash and dehydrate tissues. Then, the tissues were cleared in toluene and infiltrated with paraffin (Sigma, m. p.56–58 °C) [34]. Further, the embedding of the tissues was done in the paraffin for block preparation. Liver and brain tissue blocks were sectioned using the WESWOX-MT1090A microtome at 5 and 10 μm of thickness respectively.

Statistical analysis

Data were statistically analyzed via one-way analysis of variance (ANOVA) considering statistical significance at 5% level, $p \leq 0.01$, and $p \leq 0.001$ [35]. Results are written as mean ± S.E. of the 10 animals taken in every group. Statistical analysis was performed using GraphPadIn Stat 3.10 software package.

Result

The study was performed to examine, whether the exposure of telecommunication electromagnetic frequencies causes oxidative stress in the liver and brain tissue followed by hematological and histopathological variations. The change in body weights is shown in Graph 1 exhibits the significant difference in average body weight of exposed and control animals. The bodyweight of control group animals was gradually increased over time. While the exposed animals exhibited an increase in body weight more prominent than that of the control. The change in body weight was observed in a frequency-dependent manner ($p \leq 0.01$).



Graph 1. Body weight of *Wistar* rats exposed to electromagnetic exposure at the frequencies tested. Data are mean ± S.E.; $N = 10$; @ =Significant at 5% for ANOVA; * EMR vs Control, at $p \leq 0.01$.

Besides, we have performed hematological parameters, results showed significant variations in the hematological markers of the experimental animals. A substantial reduction in RBC count was noted in the exposed group concerning the control group (Table 1) ($p \leq 0.001$). The PCV is also known as hematocrit and represents the percentage of RBC in blood. There is a direct relationship between RBC, hematocrit, and hemoglobin concentration (Table 1). Hence, a decrease in hemoglobin leads to a decrease in RBCs and hematocrit values due to EMR exposure. Platelet count was also affected due to EMR exposure Table 1. Hematological variations progressively increased in a linear relationship with the increase in frequency (900, 1800, 2100 MHz). The most prominent effect was seen on 2100 MHz frequency at the 5% level of ANOVA.

ALT is a transferase enzyme mainly associated with the liver that converts proteins into energy for the liver cells. AST is a significant enzyme of amino acid metabolism that belongs to the class of transferases. It catalyzes the transition of the amino group from aspartate to glutamate in a reversible manner. The release of intracellular serum transaminases (AST and ALT) in the circulation is an evident indicator of liver injury. The amount of AST and ALT were raised (Table 2) after the exposure of EMR frequencies for 30 days. Statistically, a significant variance was perceived at 900, 1800, and 2100 MHz frequency exposure, confirming frequency-dependent EMR toxicity at 5% of ANOVA ($p \leq 0.001$).

Table 2 showed a significant surge in blood glucose in EMR-exposed group, whereas the control group showed a normal level of blood glucose. A maximum increase in blood glucose level was noticed in the 2100 MHz frequency-exposed group when compared with other frequencies ($p \leq 0.01$).

The level of albumin and bilirubin was also assessed in case of EMR exposure as shown in Table 2.

Table 1. Effect of different electromagnetic frequencies on the blood profile.

	RBCs (10^6/μL)	Hematocrit (%)	Hemoglobin (g/dL)	Platelets (10^3/μL)
Control	8.02 ± 0.44	47.2 ± 0.77	16.6 ± 0.91	859 ± 0.47
EMR 900 MHz	6.82 ± 0.37*	36.5 ± 0.89*	13.7 ± 0.75*	574 ± 0.21*
EMR 1800 MHz	5.67 ± 0.31*	34.8 ± 0.78*	11.8 ± 0.65*	510 ± 0.38*
EMR 2100 MHz	3.65 ± 0.20*	31.4 ± 0.76*	9.81 ± 0.54*	434 ± 0.23*
F Value (at 5% level)	11.37[@]	12.69[@]	18.78[@]	35.67[@]

Data are mean ± S.E.; $N = 10$; [@] = Significant at 5% for ANOVA; * EMR vs Control, at $p \leq 0.001$.
EMR: Electromagnetic radiation; MHz: Megahertz; RBCs: Red blood corpuscles.

Table 2. Effect of EMR on liver biomarkers and blood glucose.

	AST (IU/L)	ALT (IU/L)	Blood Glucose (mg/100ml)	Albumin (g/dl)	Bilirubin (g/dl)
Control	52.1 ± 0.49	41.5 ± 1.4	97.5 ± 1.9	4.20 ± 0.51	0.30 ± 0.03
EMR 900 MHz	99.1 ± 4.91*	97.0 ± 1.3*	112 ± 3.5*	3.60 ± 0.44*	0.71 ± 0.08*
EMR 1800 MHz	113 ± 16.7*	108 ± 0.99*	128 ± 2.7*	2.85 ± 0.35*	1.10 ± 0.13*
EMR 2100 MHz	130 ± 9.23*	121 ± 1.50*	131 ± 1.70*	1.90 ± 0.23*	1.41 ± 0.17*
F Value (at 5% level)	41.63[@]	51.35[@]	44.22[@]	36.57[@]	95.15[@]

Data are mean±S.E.; $N = 10$; [@]= Significant at 5% for ANOVA; *EMR vs Control, at $p \leq 0.001$, blood glucose is significant at $p \leq 0.01$.
EMR: Electromagnetic radiation; MHz: Megahertz; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase.

Continuous exposure to EMR for one month causes a notable decrease in the albumin content, while the level of bilirubin was increased. Alteration in the albumin and bilirubin showed a frequency-dependent effect on the exposed animals ($p \leq 0.001$). As the frequency increases, the level of albumin and bilirubin changes accordingly.

Table 3 demonstrated the effectiveness of the EMR frequencies on renal function tests. EMR exposure instigated a notable increase in urea, uric acid, and creatinine indicating renal damage ($p \leq 0.001$). The changes were seen in a frequency-dependent manner, as effects were more prominent with an increase in the frequency. 2100 MHz showed maximum toxicity, in comparison to 900 and 1800 MHz.

The EMR toxicity was also associated with various biochemical deviations, and these could usually accredit to the peroxidation of membrane lipids and depletion in the antioxidant defense system. LPO was assessed in terms of the Thiobarbituric acid reactive substances (TBARS). Table 4 indicates a statistically significant escalation in the lipid peroxidation level with 30 days of EMR exposure. 2100 MHz showed a maximum increase in the TBARS level as compared to 900 and 1800 MHz exposure ($p \leq 0.001$). Brain tissue showed prominent damage in comparison to liver tissue.

Glutathione, a tripeptide is recognized for the removal of potentially toxic electrophilic compounds. EMR absorption modified the GSH level in the liver and brain tissues. Table 4 showed that the GSH content was significantly decreased after EMR exposure, prominently in brain tissue. The frequency-dependent decline was observed when compared with different experimental

Table 3. Toxic profile of electromagnetic radiation frequencies on serological biomarkers.

	Urea (mg/dl)	Uric acid (mg/dl)	Creatinine (mg/dl)
Control	26.9 ± 1.48	0.87 ± 0.03	0.16 ± 0.62
EMR 900 MHz	48.0 ± 1.75*	1.82 ± 0.10*	0.62 ± 0.34*
EMR 1800 MHz	61.8 ± 1.74*	2.76 ± 0.15*	0.71 ± 0.39*
EMR 2100 MHz	64.4 ± 2.00*	2.95 ± 0.16*	0.78 ± 0.43*
F Value (at 5% level)	42.09[@]	70.59[@]	80.56[@]

Data are mean ± S.E.; $N = 10$; [@]= Significant at 5% for ANOVA; *EMR vs Control, at $p \leq 0.001$.
EMR: Electromagnetic radiation; MHz: Megahertz.

groups. This confirmed that 2100 MHz frequency showed the most toxic effect when compared to 900 MHz and 1800 MHz frequencies ($p \leq 0.001$).

Exposure at different mobile phones frequencies also inhibited the activities of GSH cycle enzymes in the liver and brain tissues. These are the enzyme involved in the recycling of reduced glutathione pool. Table 5 depicted the activity of GSH cycle enzymes such as GR, GPx, GST, G-6-PDH. The activities of these enzymes decreased significantly at different mobile phone frequencies, that is, 900, 1800, and 2100 MHz exposed rats, compared with the control animals. At the highest frequency, the maximum inhibition was recorded as compared to 900 MHz and 1800 MHz frequency ($p \leq 0.001$).

SOD and catalases are the enzymes involved in the intracellular degradation of $^{\circ}O_2$ and H_2O_2. Table 6 represents the activity of SOD in the liver and brain tissues. The activity of SOD decreased in EMR exposed groups concerning the control group at a 5% level of ANOVA. 2100 MHz group showed maximum toxicity ($p \leq 0.001$).

A considerable increase of catalase activity in liver and brain tissues was observed after 1 month of

Table 4. EMR-induced toxicity in tissue biochemical parameters.

	Lipid peroxidation (n moles TBARS/mg protein)		Reduced glutathione (μ mole/g)	
	Liver	Brain	Liver	Brain
Control	0.33 ± 0.01	0.39 ± 0.02	432 ± 37.3	291 ± 39.6
EMR 900 MHz	0.68 ± 0.03*	1.24 ± 0.68*	329 ± 28.3*	252 ± 41.2*
EMR 1800 MHz	0.93 ± 0.05*	1.44 ± 0.07*	253 ± 31.2*	241 ± 45.5*
EMR 2100 MHz	1.21 ± 0.06*	1.98 ± 0.10*	241 ± 29.7*	201 ± 39.5*
F Value (at 5% level)	78.87[@]	89.13[@]	29.13[@]	26.21[@]

Data are mean ± S.E.; $N = 10$; [@] = Significant at 5% for ANOVA; * EMR vs Control, at $p \leq 0.001$.
EMR: Electromagnetic radiation; MHz: Megahertz; TBARS: Thio barbituric acid reactive substances.

Table 5. Effect of electromagnetic radiation exposure on GSH cycle enzymes

	GR (μmole/min/mg protein)		GPx (μmole/min/mg protein)		GST (unit/min/mg protein)		G-6-PDH (μ mole/min/mg protein)	
	Liver	Brain	Liver	Brain	Liver	Brain	Liver	Brain
Control	6.33 ± 0.34	34.4 ± 1.90	5.52 ± 0.30	8.51 ± 0.47	3.61 ± 0.19	4.93 ± 0.27	14.1 ± 0.77	7.94 ± 39.6
EMR 900 MHz	3.98 ± 0.22*	23.7 ± 1.31*	3.25 ± 0.17*	5.14 ± 0.28*	2.45 ± 0.13*	3.16 ± 0.16*	9.52 ± 0.52*	4.93 ± 41.2*
EMR 1800 MHz	3.13 ± 0.17*	18.4 ± 0.97*	2.14 ± 0.11*	4.31 ± 0.25*	2.24 ± 0.12*	2.94 ± 0.12*	8.21 ± 0.45*	3.41 ± 45.5*
EMR 2100 MHz	2.44 ± 0.13*	16.8 ± 1.01*	2.03 ± 0.11*	4.01 ± 0.21*	2.11 ± 0.09*	2.34 ± 0.10*	6.43 ± 0.35*	3.04 ± 39.59*
F Value (at 5% level)	62.95[@]	41.96[@]	82.87[@]	50.30[@]	25.94[@]	40.23[@]	42.38[@]	72.09[@]

Data are mean ± S.E.; $N = 10$; [@] = Significant at 5% for ANOVA; *EMR vs Control, at $p \leq 0.001$.
EMR: Electromagnetic radiation; MHz: Megahertz; GR: Glutathione reductase; GPx: Glutathione peroxidase; GST: Glutathione-S-transferase; G-6-PDH: Glucose-6-phosphate dehydrogenases.

Table 6. Noxious effect of EMR on enzymatic oxidative stress markers.

	Superoxide dismutase (U/min/mg protein)		Catalase (μ mole H_2O_2/min/ mg protein)	
	Liver	Brain	Liver	Brain
Control	61.9 ± 3.42	62.9 ± 2.94	67.4 ± 8.33	76.7 ± 2.76
EMR 900 MHz	49.4 ± 2.73*	48.4 ± 2.18*	54.2 ± 6.69*	55.2 ± 1.98*
EMR 1800 MHz	45.2 ± 2.49*	46.2 ± 2.08*	47.1 ± 5.80*	47.8 ± 2.64*
EMR 2100 MHz	44.6 ± 2.46*	41.3 ± 1.41*	41.3 ± 5.06*	42.8 ± 1.98*
F Value (at 5% level)	9.85[@]	13.36[@]	17.42[@]	26.88[@]

Data are mean ± S.E.; $N = 10$; [@] = Significant at 5% for ANOVA; * EMR vs Control, at $p \leq 0.001$.
EMR: Electromagnetic radiation; MHz: Megahertz.

Table 7. Electromagnetic radiation mediated alterations in ATPase and AChE activity.

	ATPase (mg Pi/100g/min)		AChE (μ mole/min/mg protein)
	Liver	Brain	Brain
Control	1582 ± 19.92	1641 ± 13.92	41.0 ± 1.96
EMR 900 MHz	1345 ± 20.41*	1278 ± 11.23*	33.2 ± 1.90*
EMR 1800 MHz	1298 ± 25.31*	1107 ± 11.99*	30.4 ± 1.73*
EMR 2100 MHz	1141 ± 15.47*	988.4 ± 10.46*	23.1 ± 1.34*
F Value (at 5% level)	17.17[@]	18.97[@]	20.66[@]

Data are mean ± S.E.; $N = 10$; [@] = Significant at 5% for ANOVA; *EMR vs Control, at $p \leq 0.001$.
EMR: Electromagnetic radiation; MHz: Megahertz; ATPase: Adenosine triphosphatase; AChE: Acetylcholinestrase.

exposure to telecommunication frequencies (Table 6) ($p \leq 0.001$). The treated group of all three frequencies showed significant arrest in the enzymatic activity in comparison to the control group, but the maximum enzyme arrest was seen on the highest frequency (2100 MHz). Alterations were more pronounced in the brain tissue than the liver.

ATPase activities in the liver and brain are mentioned in Table 7. The toxic symptom of EMR exposure was depicted by the statistically significant diminution of ATPase activity. The inhibition was more prominent in the brain tissue than the liver. The telecommunication frequencies at 900, 1800, and 2100 MHz showed an ill effect concerning control, whereas 2100 MHz was found to be the most toxic and showed maximum depletion in ATPase activity ($p \leq 0.001$).

AChE is a molecule related to neurotransmission. Its function is to terminate the neurotransmission by quick hydrolysis of acetylcholine released from the motor neurons. EMR exposure showed clear inhibition of acetylcholinesterase activity (Table 7) of the brain in a frequency-dependent manner ($p \leq 0.001$).

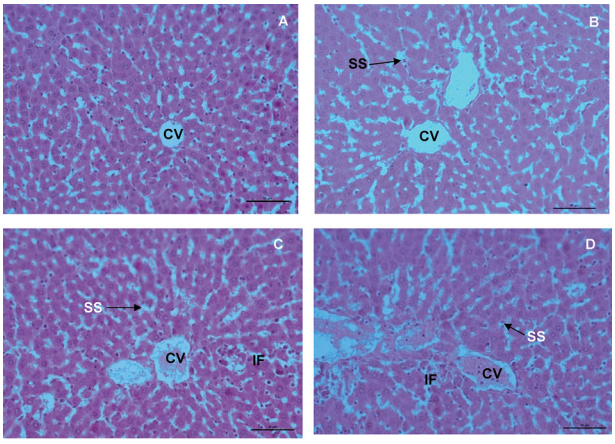


Figure 2. Morphologic changes of the liver in control and experimental groups are presented in A–D. (A): Photomicrograph of the normal liver showing the normal morphological architecture of the control group with Central Vein (CV) and surrounding Hepatocytes (H), Sinusoidal Space (SS). (B): 900 MHz exposed liver showing mild degenerative changes of hepatocyte in the periportal region (arrow). (C):1800 MHz exposed liver showing moderate hepatocyte degeneration around the central vein (arrow mark), a small amount of inflammatory cell infiltration in the periportal region; (D):2100 MHz exposed animals liver showing hepatic centrilobular mononuclear cell infiltration. A degenerative change of hepatocyte around the central vein was found followed by swelling of Sinusoidal Spaces (SS), irregular cord arrangement, and the onset of hepatic cell necrosis with inflammatory infiltration (IF) (X 100).

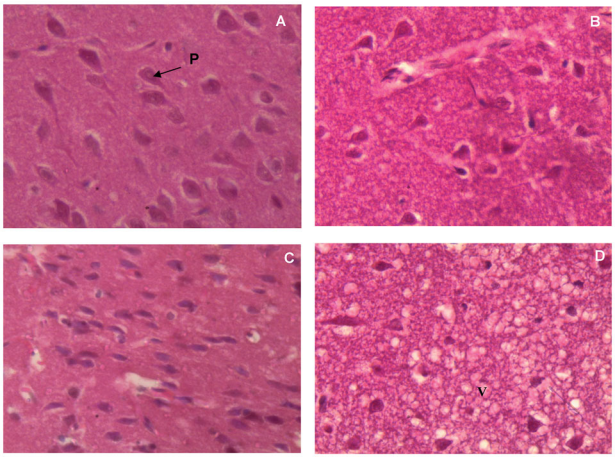


Figure 3. (A) Cerebral cortex of the control group showing normal cytological characteristics of the neurons. It also showed glial cells in the resting stage, normal healthy capillaries. (B): EMR exposed group showed an irregular arrangement of neurons in the neurocortex with degenerated nuclei in pyramidal neurons (Py). (C): EMR exposed group at 1800 MHz frequency showed pyknotic nuclei. (D): EMR exposed group at 2100 MHz frequency depicted severe vacuolization (V) and pyknosis in the neuron cells (X 100).

Histopathology

The biochemical study was further corroborated by histopathological observations. Light microscopic observation of various tissues was assessed through hematoxylin and eosin staining procedures. Figure 2 (A–D) is showing the images of liver tissue . Histopathology of the control rat liver showed normal hepatocytes with regularly arranged hepatic chords, clear central vein (CV), and sinusoidal spaces (SS) Figure 2(A). After one month of EMR exposure, liver sections of exposed rats showed frequency-dependent hepatic injuries such as the disintegration of hepatic cords, central vein congestion, eosinophilic cytoplasm, dilated sinusoidal spaces, proliferation, and infiltration of Kupffer cells around the central vein Figure 2(B–D).

Coronal sections of the brain were studied for cytological changes in the cerebral cortex and hippocampus region showed in Figure 3. Brain sections of control rats showed well-formed, regularly arranged healthy neuron layers in the cerebral cortex with a large number of the normal healthy neuron **(**Figure 3(A)**).** EMR exposure shunts the normal features of the brain after 30 days of exposure. Hematoxylin and eosin staining clearly showed an increased number of degenerated cells in the brain of exposed rats. The cerebral cortex of exposed animals showed an irregular arrangement of neuronal layers with loss of nerve filament (Figure 3(B–D)). Similarly, the hippocampus region was also assessed where it is found that one-month exposure to EMR caused pyknosis in the molecular (M), granular (G), and polymorphic cell layer (Po) of the DG region (Figure 4(B–D)), whether the control group animals showed normal features in all the layers of the DG region (Figure 4(A)).

The cerebellum of the brain is responsible for coordinating voluntary movements. It is also responsible for several functions including motor skills such as balance, coordination, and posture. Sections of control group animals showing normal neurons in molecular, Purkinje, and the granule cell layer. Healthy stellate and Purkinje neuron cells were present with branched dendrites in the midsagittal section of the brain **(**Figure 5(A,B)**).** The exposed animal showed degenerated Purkinje neurons showing a less number of Purkinje cells with short dendritic processes in a frequency-dependent manner. The neuronal degeneration was confirmed with increased perivascular spaces, and a pyknotic, shrunken cell nucleus in the Purkinje layer **(**Figure 5(C–H)**)**.

Discussion

Mobile phone usage is currently one of the fastest advances in technology. The proximity of communication devices to the brain and abdominal organs has raised concerns about EMR's biological interactions and

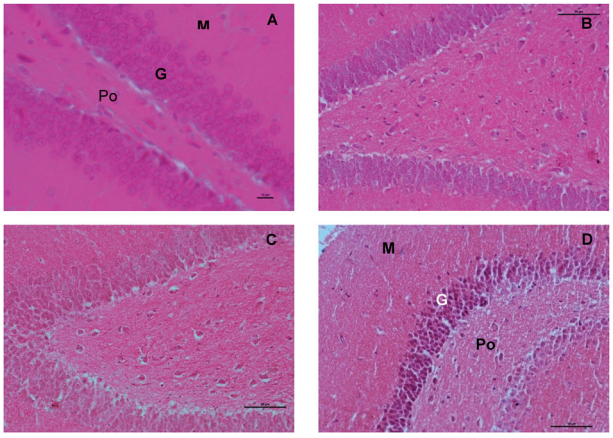


Figure 4. (A) Brain (Hippocampus region) of control rat showing well-formed neurons in the superficial molecular layer (M), granular layer (G), and the polymorphic cell layer (Po) in the DG region. (B):900 MHz exposure of EMR showed very few numbers of pyknotic nuclei. (C): 1800 MHz exposure depicted loosely packed, darkly stained, shrunken granular cells with an increase in pericellular spaces. (D): Exposure of EMR at 2100 MHz showed the cells of Pyknotic nuclei in the granular and polymorphic layers (Po), intense damage with significantly activated microglia (Mg) cells in polymorphic layer (X 100).

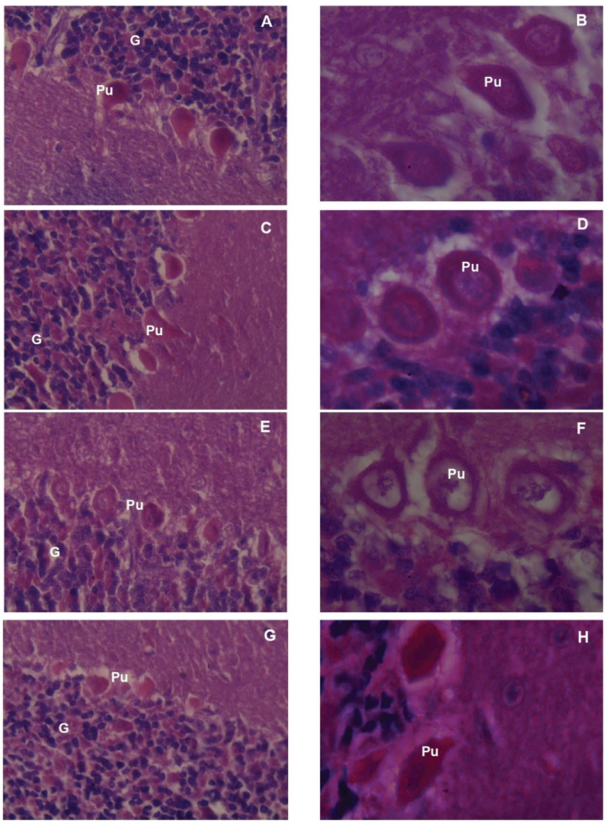


Figure 5. (A,B) Brain of a control rat showing cerebellum region with healthy neurons in the molecular layer (M) and Purkinje cell layer (Pu) with branched dendrites. (C, D): 900 MHz exposure of EMR showed mild changes in the nuclei of the Purkinje neurons. (E–F): 1800 MHz exposure depicted darkly stained, shrunken cytosol of the Purkinje neuron cells with an increase in pericellular spaces. (G–H): Exposure of EMR at 2100 MHz showing neuronal degeneration along with loss in the number of Purkinje cells, clumped nuclei with short dendritic processes, and empty perivascular spaces (X 400 & 1000).

consequences [36,37]. An increase in body weight is associated with many health issues, the bodyweight showed a considerable increase in the exposed group of animals. These results were further substantiated by Gerardi and the group, they observed that the EMR group showed increased body weight due to substantially increased blood glucose levels in young animals [38]. Another study confirmed that chronic electromagnetic exposure leads to increases in body weight in a frequency-dependent manner. Increased bodyweight does not always imply a risk to one's health directly, rather it is suggested that electromagnetic fields can affect metabolic processes [39].

Systematic circulation depends on hematological components carrying nutrition and oxygen, so these are important for health. Exposure of electromagnetic frequencies showed a drop in the red blood corpuscles [40]. In addition to this decreased percentage of hemoglobin and hematocrit were assessed and further verified by Omar and the group showed a declining number of platelets that is a sign of various kinds of anemia and leukemia [41,42].

EMR exposure leads to alter the prevailing incidence of metabolic disorders involving altered glucose metabolism. A possible reason behind the increased glucose level might be the interaction of EMR frequencies to the pancreas, which may alter glucose metabolism [43]. EMR-induced oxidative stress is also a contributing factor in the impairment of glucose metabolism [44].

Serum transaminases (AST and ALT) are well-defined liver enzymes. EMR-exposed animals showed a significant surge in the serum transaminases that may be due to damage to the cell membrane indicating hepatic damage. Kula and the group also confirmed the elevated AST and ALT levels in the steelworkers exposed to EMR [45]. Our finding also depicts the significant surge in serological triglycerides and cholesterol in exposed animals. These are the markers of liver function that indicates the damage in hepatic tissue [46]. Serum albumin and bilirubin level is the key indicator of a healthy liver. Decrease levels of serum albumin by EMR exposure may be an indication of weakening the function of the liver, probably due to hepatocellular damage [47]. The reason for the increased bilirubin may be because of damaged cell membrane that causes

their leak into circulation after EMR exposure [48]. Urea and creatinine are the key parameters to assess kidney functioning [49]. This study showed an increase in urea and creatinine level. Urea is an end product of protein catabolism, excessive catabolism of the protein with enhanced amino acid deamination may cause increased serum urea concentration. Another reason for the elevated concentration of serum urea may be RBC degradation [50,51]. EMR exposure caused a condition of hyperuricemia, which means excessive uric acid levels in the blood. Overproduction of uric acid might be associated with frequent blood cell lysis in EMR-exposed animals [52].

The study showed excessive free radical production, expressed in terms of TBARS content in exposed rats, a potent indicator of lipid peroxidation. Accumulation of the lipid peroxidation products could be a result of the gradual degradation of hepatic as well as neural tissue. Brain tissues are highly sensitive to EMR because of the excessive lipid peroxidation than the liver [53].

In combat against free radicals, GSH is the first line of body defense mechanism. The level of GSH has been significantly declining in both the liver and brain [54]. GSH protects against EMR associated damage in two ways, it can either detoxify species of activated oxygen, such as H_2O_2, or reduces lipid peroxides [55]. A decline in GSH level could directly diminish a number of reactive oxygen species (ROS) and is oxidized to GSSG in this process. The enzymes responsible for conversion of GSSG to its reduced form (GSH) are glutathione peroxidase, glutathione reductase, glutathione-s-transferase, and G6PDH. The study showed a significant decrease in these enzymes following EMR exposure. Decrease in these enzymes is directly associated to EMR-induced oxidative damage and leads to hepatological and neurological damage [5].

SOD and catalases are the potential targets of EMR induced toxicity and their alteration may be a compensation for the decreasing activity of glutathione. EMR exposure increased the production of ROS. Therefore, the increase in SOD and catalase may also be attributed to the defense mechanism against an increased level of H_2O_2 [15]. The elimination of most free radicals relies on the oxidation of endogenous antioxidants, which were shown by the depletion of antioxidant catalase and SOD activity [56].

A previous study found the connection between oxidative stress and AChE activity in various brain regions [57]. The data of the current study exhibited a significant decrease in AChE activity due to electromagnetic frequencies in the brain [19]. The result of the current study allowed us to hypothesize signal transmission and antioxidant competence of brain tissue due to EMR exposure.

An imbalance between free radicals and antioxidants plays a major role in persuading histopathological damage [58]. Silva and the group reported that chronic exposure of 2 G and 3 G frequencies resulted in histopathological alteration and DNA damage in the chick embryo liver [59]. Another study was also in support and found that EMR exposure was associated with severe liver damage [60]. According to Chauhan and colleagues, 35 days of exposure of 2450 MHz frequency showed an increase in histopathological modifications in the liver tissue [58]. The intensity of damage caused by radiofrequency radiation varies with the exposure time as well as the frequency of use [17].

The study indicated that the brain tissues are more prone to EMR than the liver. Here, the question comes that why the brain has a higher percentage of damage than the liver. A possible reason is the mobile phones close approximately to the head, as brain cells are metabolically active that makes it cells more susceptible to EMR [61]. As the liver and brain's functional status are related, proper brain activity necessitates a well-functioning liver. An accepted aspect of this connection is the hepatic failure associated with the behavioral disorder in the case of hepatic encephalopathy [62]. ALT and AST are liver damage markers that have been linked to coronary and metabolic disorders, as well as identified as a risk factor for Alzheimer's disease and cognitive impairments [63]. Thus, this study applied biochemical, histopathological analysis, and the connectivity of liver and brain damage to examine the structural and functional integrity in EMR exposed animals.

Conclusion

Our findings documented that the frequency of EMR exposure is a significant factor associated with its toxicity, the extent of toxicity is increased with a simultaneous increase in the frequency of exposure. In addition, this study confirms that EMR exposure initiates hepatic and neural injury, but the potency of damage was more prominent in the brain. Our results will provide support for future studies in the analysis of electromagnetic radiation and its associated systemic injury.

Ethics approval

No human subjects were used in the study. Experiments were conducted on laboratory animals

(*Wistar* rats). Animals were maintained with recommended guidelines (1854/GO/Re/S/16/CPCSEA/IAEC/JU/24). The study was ethically approved by the Jiwaji University according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Consent to participate

All authors meet the qualifications for authorship and had an opportunity to read and comment on the manuscript.

Author contribution

Sharma Anjali and Shukla Sangeeta designed the study. Sharma Anjali carried out the experimental work and drafted the manuscript. Shrivastava Sadhana was involved in the compilation and analysis of data. All authors read and approved the final manuscript.

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Disclosure statement

The authors report no conflict of interest.

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Data availability statement

This manuscript has no associated data

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