Molecular Biology Reports (2025) 52:144
https://doi.org/10.1007/s11033-025-10217-8

ORIGINAL ARTICLE

Short-term exposure of 2.4 GHz electromagnetic radiation on cellular ROS generation and apoptosis in SH-SY5Y cell line and impact on developing chick embryo brain tissue

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Received: 9 September 2024 / Accepted: 2 January 2025 / Published online: 21 January 2025
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Abstract

Background Electromagnetic radiation (EMR) from wireless technology and mobile phones, operates at various frequencies. The present study analyses the major impact of short-term exposure to 2.4 GHz frequency EMR, using the two model systems chick embryos and SH-SY5Y cell lines. We hypothesized that exposure to this frequency would induce oxidative stress and apoptosis in neurons.

Methods and results Chick embryos were exposed continuously to 2.4 GHz EMR for 4 h each day over a 5-day period, and comparisons were made with a control group. At the end of the exposure, brain tissues were dissected for histopathological analysis, antioxidant assays, and reactive oxygen species (ROS) detection. Additionally, SH-SY5Y cells were exposed to 2.4 GHz EMR to assess cell viability, DNA damage, and apoptosis. Our results showed that exposure to 2.4 GHz EMR induces oxidative stress in both chick embryos and the SH-SY5Y cells, though no significant tissue-level impact was observed. In SH-SY5Y cells, ROS production increased after 4 h of exposure, accompanied by moderate DNA damage and early markers of apoptosis, such as upregulation of the *Bax* gene. Furthermore, we observed that antioxidants, such as NAC and Mito-TEMPO, helped mitigate the cytotoxic effects of EMR in both the study models.

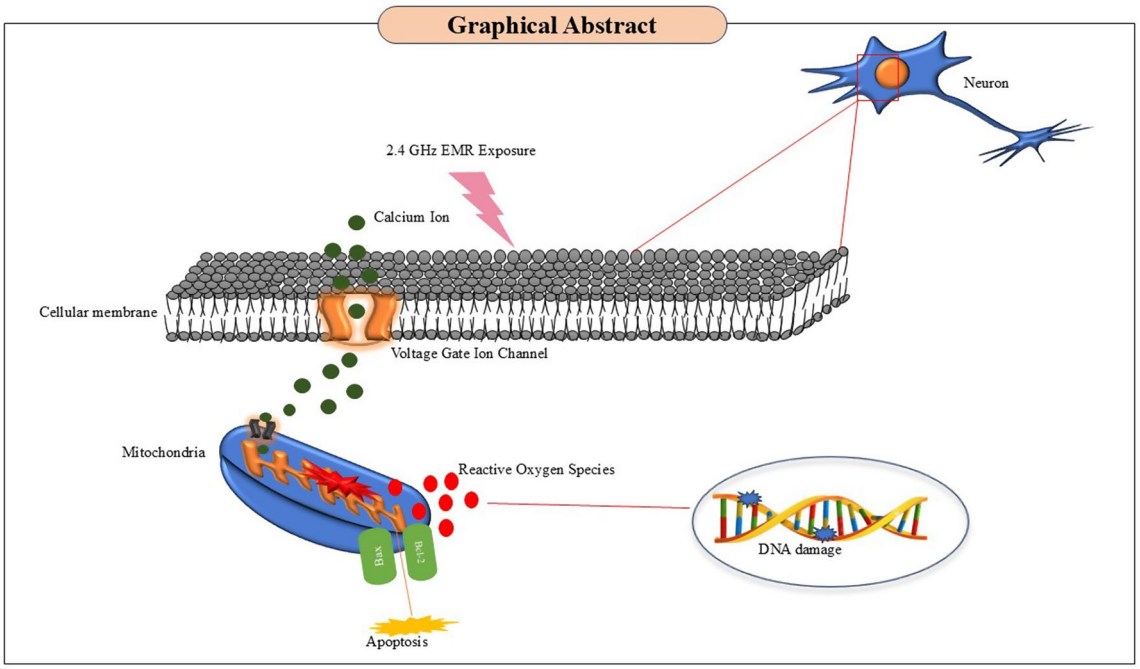
Conclusion In conclusion, short-term exposure (4 h) to 2.4 GHz EMR induced moderate cellular and molecular changes, primarily oxidative stress. The oxidative stress was reduced by antioxidants, which suggests potential benefits in preventing EMR-induced cytotoxicity. Extended exposure to EMR beyond 4 h may pose adverse health risks to humans, endorsing further investigation.

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Graphical Abstract



Keywords Electromagnetic radiation · Calcium ion · Oxidative stress · Apoptosis · SH-SY5Y

Abbreviations

EMR	Electromagnetic Radiation
ROS	Reactive Oxygen Species
NAC	N-Acetyl cysteine
Mito TEMPO	Mitochondrial-targeted antioxidant to scavenge superoxide
CM H2DCFDA	5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate
DHE	Dihydroethidium
SEM	Scanning Electron Microscopy
PBS	Phosphate Buffer Saline
FBS	Fetal Bovine Serum
PI	Propidium Iodide
SOD	Superoxide Dismutase
CAT	Catalase
FTIR	Fourier transform infrared spectroscopy
AO	Acridine Orange

Introduction

The communication devices such as Wi-Fi, Bluetooth, and cordless phones that aid in faster data transfer up to a range of several megabits per second, are of benefit to people, but they are proven to cause the emission of 2.4 GHz frequency radiation which as to be considered neither a benefit nor a health risk [1, 2]. The advancements in the rapid growth of technology hail a new horizon towards the improvements in the lifestyle of the people [3]. Several researchers have studied the impact of EMR demonstrated the impact of EMR on the biological and physiological factors causing oxidative stress, DNA damage and neurological effects, which are being induced by communication devices [4, 5]. In this current study, we have experimentally tried to prove how short-term exposure to 2.4 GHz influences immediate cellular response, including oxidative stress and gene expression. Additionally, the study focuses on the effects of antioxidants in controlling oxidative stress induced by the 2.4 GHz frequency and would pave the way towards addressing a much-needed gap, furnishing new perspectives on the overall study of the effects of communication devices.

The brain is distinguished as the most sensitive organ highly exposed to electromagnetic radiation and could cause deleterious effects such as behavioural changes affecting sleep, anxiety problems, and impairment in cognitive functions [6, 7]. The brain consists of approximately 86 billion neurons, the reactive oxygen species arising from the neuronal structure, interact with pathophysiological factors that can impede numerous essential processes of cellular functions such as inflammation, rapid cellular growth, differentiation and neuronal activity. These interfering molecules arise from various sources like the modification of

biochemicals and signalling processes or through the induction of oxidative stress causing harm to DNA, RNA, proteins, or fatty acids. Furthermore, ROS can also exert an effect on the cell cycle and protein expression processes [8, 9]. Calcium is a known modulator of ROS production which involves two pathways intracellular calcium-dependent pathway and the calcium-independent pathway, both pathways play a role towards the excessive production of ROS. In the calcium-dependent pathway, calcium plays a major role in regulating ROS production, whilst calcium overload alters mitochondrial membrane potential and the activation of several calcium-dependent protein kinases [10]. On the contra, calcium was not involved in the secretion of excessive ROS within the cells in the calcium-independent pathway. Besides the calcium-independent pathway may play a significant role in mitochondrial dysfunction, NADH oxidase activation, oxidative stress amplification and endoplasmic reticulum stress [11–13].

The initial research finding indicates that EMR could result in abnormal brain activity [14]. Exposure to EMR induces intracellular stress, leading to the expression of stress-related genes and their corresponding proteins [15]. Furthermore, the excessive production of ROS caused by oxidative stress is altered due to EMR exposure, resulting in DNA damage within brain cells [16, 17].

The study reported by [18] signifies that 2.4 GHz frequency radiation could obstruct memory and learning functions in the brain, as well as induce heightened anxiety and behavioural disorder changes, which come into being when exposed to 2.4 GHz frequency, radiation for a period of 4 h per day. Alongside the molecular level, it has been discerned that it transpires an overexpression of certain miRNA, namely the rno-miR-181a-5p, phosphatidylserine (PS), and triacylglycerol (TAG). Besides, prolonged exposure to this frequency radiation has been shown to induce neurodegenerative diseases [19–21]. However, exposure to electromagnetic radiation at 2.45 GHz frequency radiation did not have any discernible effects at cellular and molecular levels [22].

The study investigates the effects of 2.4 GHz electromagnetic radiation, given exposure for a shorter duration of time for 4 h per day. The study was conducted using the chick embryo and SH-SY5Y cell line. The results of Histopathological examinations, measurements of ROS generation, evaluating antioxidant levels, apoptosis, and *Bax/Bcl2* gene expression levels obtained through this study have comprehended the effects of 2.4 GHz electromagnetic radiations. Finally, the study on the NAC and Mito-TEMPO antioxidants validates the effects of antioxidants in reducing oxidative stress examined using the model system, chick embryo brain and the SH-SY5Y cell line exposed to 2.4 GHz frequency radiation.

Materials and methods

Chicken embryo model and 2.4 GHz EMR exposure

The experiments were performed using the chicken embryo model, ensuring that the embryos were below the period of the 14th day of gestation to avoid the need for animal ethical clearance [23, 24]. Eggs were kept for incubation at 37 °C with 5% humidity and continuously monitored using a digital thermal hygrometer. The eggs were exposed to 2.4 GHz electromagnetic radiation for 4 h each day from the 5th day to the 10th day of incubation. Two mobile phones were used for EMR exposure one placed outside the incubator and the other inside. Both phones were set to airplane mode and transmitted data to each other, emitting radiation. Control and exposed groups were kept under identical conditions in separate incubators, isolated from different sources of electromagnetic radiation. The experiment included 30 eggs (15 eggs in each group), with the control group not exposed to [25]. After 5 days of exposure, the embryo's brains were dissected for experimental studies.

Hematoxylin and eosin (H&E) staining

Hematological studies were done using a standard protocol [26]. Brain tissue samples were done for fixation using 10% formalin. Tissues were sectioned (5-µm thickness) and stored as paraffin-embedded tissue, the tissue samples were stained using hematoxylin and eosin (H&E) to visualize, a cellular framework of the brain. The tissues were analysed using a light microscope (Amscope, USA) and the results were validated using ImageJ software (https://imagej.nih.gov/ij).

Scanning electron microscopy (SEM) analysis

Brain tissue samples were dissected and obtained from the chicken embryos. Ultra-structural images of the brain tissue were obtained using the SEM analysis as mentioned previously [27]. The chick embryo brain tissue was washed using the 10% sucrose solution, the tissue was dipped with 10% glutaraldehyde, and it was kept overnight. The brain sections were sectioned using the cryotome (10 µm), and it was processed to remove any water molecules and, finally, analysed by scanning electron microscope (NST Quanta 250FEG).

Nissl bodies - PI staining

To visualize the Nissl bodies present in the brain tissue, the propidium iodide (PI) fluorescence staining was performed [28]. The tissue sections underwent deparaffinization and

rehydration using xylene and with decreasing concentrations of ethanol. Sections were incubated for 30 min at room temperature with 0.1 mg/ml PI in 0.01 M phosphate-buffered saline (PBS) pH 7.2, under dark conditions. The slides were washed with PBS and were mounted using a mounting medium thereby examined under a fluorescence microscope (AmScope, USA) with a red emission filter.

Brain tissue total ROS generation

The total ROS generation in brain tissue was measured using the CM-H2DCFDA (5-(and-6)-chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate) (Invitrogen) [29]. Following exposure, the brain was homogenized in PBS, and incubated in the CM-H2DCFDA for 30 min in a 96-well plate. Fluorescence intensity (Excitation/Emission: 490/520 nm) was measured using a multimode microplate reader (Synergy H1MFG, BioTek, USA).

Tissue antioxidant enzyme activity

Brain tissues were homogenized with 0.1 M Tris–HCl buffer (pH 7.4) by creating a homogenate 20% (w/v). The centrifugation was done at 560 × g for 10 min at 4 °C the supernatant collected was measured for superoxide dismutase (SOD) and catalase (CAT) activity by spectrophotometric assays [26]. The SOD activity was measured by the inhibition of colour intensity developed in n-butanol measured at 510 nm against a butanol blank. CAT activity was assessed by measuring the consumption of H_2O_2 (Hydrogen Peroxide) with chromogen development measured at 620 nm.

Fourier-transformed infrared spectroscopy (FTIR)

Brain tissue was done for Fourier transform infrared spectroscopy (FTIR) to study metabolic fingerprinting study [30]. Brain tissue was homogenized vibrating mode (iRuptJr, Neuation) at 4 °C and potassium bromide (KBr) pellets were prepared with the dehydrated homogenate. The FTIR spectrum between wavelengths was recorded between 400 and 4000 cm^{-1} to assess biomolecular absorbance patterns (Shimadzu, Japan) [31].

Culturing of SH-SY5Y cells

The neuroblastoma cell line SH-SY5Y was obtained from the National Centre for Cellular and Sciences (NCCS) in Pune, India. The cells were maintained in a 1:1 mixture of DMEM and Ham's F12 containing 10% Fetal Bovine Serum (FBS) with 1% Penicillin/Streptomycin. and maintained with 5% CO_2 at 37˚C in a humidified atmosphere.

Cell viability assay

Cell viability was assessed by the resazurin assay [32]. After exposure, cells were incubated with resazurin solution (80 µM) for various duration at 37º C. The fluorescence intensity (Excitation/Emission: 540/590 nm) was measured with a multimode microplate plate reader (Synergy H1MFG, BioTek, USA). The relative percentage viability of the cell was calculated by comparing the fluorescence intensity unit (a.u) values by considering the control cells as 100% viable.

COMET assay

DNA damage was examined through COMET assay (alkaline method) [33]. After EMR exposure, cell suspensions of SH-SY5Y were mixed and plated onto slides with a low-melting agarose. The slides were then subjected to lysis, electrophoresis and neutralization before staining with ethidium bromide (EtBr). The fluorescence microscopic images were analyzed using COMET scoring software, following the formula:

$$\text{Olive moment} = (\text{tail mean-head mean}) \times \%\ \text{of DNA in the tail})$$

Intracellular calcium imaging in SH-SY5Y cell line

The intracellular calcium levels were measured using the calcium-sensitive fluorochrome Fura 2 AM [34]. After EMR exposure, cells were observed under confocal microscopy (Olympus 2300 model) after incubating with 1 µM Fura 2 AM for 30 min.

Impact of antioxidants on cellular oxidative stress

The activity of antioxidants towards EMR-induced oxidative stress was investigated by pre-treating SH-SY5Y cells with 1 mM N-acetyl cysteine (NAC, antioxidant) or 20 µM Mito-TEMPO (mitochondrial-targeted antioxidant to scavenge superoxide) for 2 h. After exposure, ROS levels, superoxide generation, and lipid peroxidation were measured using 10 µM CM-H2DCFDA (Invitrogen) (Ex/Em: 485/530 nm), 5 µM dihydroethidium (DHE) (Sigma Aldrich) (Ex/Em: 510/590 nm), and 1 µM BODIPY 581⁄591 C11(Invitrogen) (Ex/Em 580/610), respectively [35–39]. The relative fluorescence intensity was measured in arbitrary units (a.u.) through a multimode microplate reader (Synergy H1MFG, BioTek, USA).

Total ROS imaging

Total ROS generation from cells was evaluated through CM-H2DCFDA, a fluorescence imaging dye. After exposure, the SH-SY5Y cells were incubated with the CM-H2DCFDA and examined under confocal microscopy (Olympus 2300 model). Fluorescence intensity was analyzed using ImageJ software and indicated as a relative fluorescence unit.

Effect of electromagnetic radiation on apoptotic gene expression

The total RNA was isolated from SH-SY5Y cells using the TriZol reagent (Sigma-Aldrich). cDNA was synthesized from the RNA, and the real-time quantitative PCR (RT-qPCR) was done using SYBR™ Green Universal Master Mix (Applied Biosystems). Primer sequences for forward primer (FP) and reverse primer (RP) were as follows: (Bcl2) FP: 5′GAACTGGGGGAGGATTGTGG 3′ and RP: 5′ GCC GGTTCAGGTACTCAGTC 3′; (Bax) FP: 5′CCCCCGAGA GGTCTTTTTCC 3′ and RP: 5′CCTTGAGCACCAGTTT-GCTG3′; (Beta-Actin- internal reference gene) FP: 5′ GA CCTCTATGCCAACACAGT 3′ and RP: 5′ AGTACTTG CGCTCAGGAGGA 3′ were used to assess apoptotic gene expression. Relative fold changes were calculated using the $2^{\Delta\Delta Ct}$ method, and products were verified by agarose gel electrophoresis [26].

Autophagy evaluation in cells – AO staining

Autophagy in SH-SY5Y cells was assessed using Acridine Orange staining. After exposure, cells were incubated using AO (1 µg/ml) for 15 min, the autophagic vesicles were visualized under a fluorescence microscope (Amscope, USA). Green fluorescence indicated normal cells, while red fluorescence signified autophagic cells [40].

Evaluation of apoptosis – annexin V/PI staining and flow cytometry

Early and late apoptotic cells were detected using the annexin V/PI staining. SH-SY5Y cells were exposed to 2.4 GHz EMR and incubated with annexin V/PI. Apoptotic changes were visualized using fluorescence microscopy (Olympus 2300 model).

Statistical analysis

SPSS software was used to perform the statistical analysis. The statistical significance differences between the two groups were assessed by a t-test, while the one-way ANOVA was used for comparisons between more than two groups. A p-value of <0.05 was considered statistically significant, and $p>0.05$ indicated no significant difference.

Results

Histopathological Observation

The light microscopic visualization of H&E staining of chick embryo brain tissue shows that no pathological and morphological changes were observed in the 2.4 GHz exposed group by comparing to the control group (Fig. 1A).

Brain surface morphology – SEM images

Scanning electron microscopy images of brain tissue obtained from 10th -day chick embryos were illustrated in (Fig. 1B). The results show moderate surface morphology changes observed in the 2.4 GHz exposed group, in the 10th -day chick embryo brain tissue when compared with the control.

Effect on nissl bodies of neurons

The fluorescence microscopic images (Fig. 1C and D) indicate the PI-positive signals of Nissl bodies in the neuronal cytoplasm. The result indicates that the number of Nissl bodies/area was not significant ($p>0.05$) in the 2.4 GHz treated group compared to the control group.

Infrared spectroscopic and macromolecular changes of the brain tissue

The FTIR results show the shifts in wave number, as the indicator of qualitative changes in macromolecular composition (proteins, fatty acids, polysaccharides and nucleic acids). The (Fig. 2D) indicates the representative FTIR spectrum of control and exposed brain tissues. Table 1 represents the corresponding wave number of functional groups as important macromolecules. The shift in wave number indicates the changes due to 2.4 GHz exposure when compared with the control group. The average wave numbers from two independent experiments were obtained and compared for further evaluation.

Tissue total ROS generation

The effect of 2.4 GHz radiation on brain tissue and the total ROS generation is illustrated through the DCF fluorescence activity assay (Fig. 2 C). The results indicate a significant p-value ($p<0.05$) and an elevation of total ROS generation,

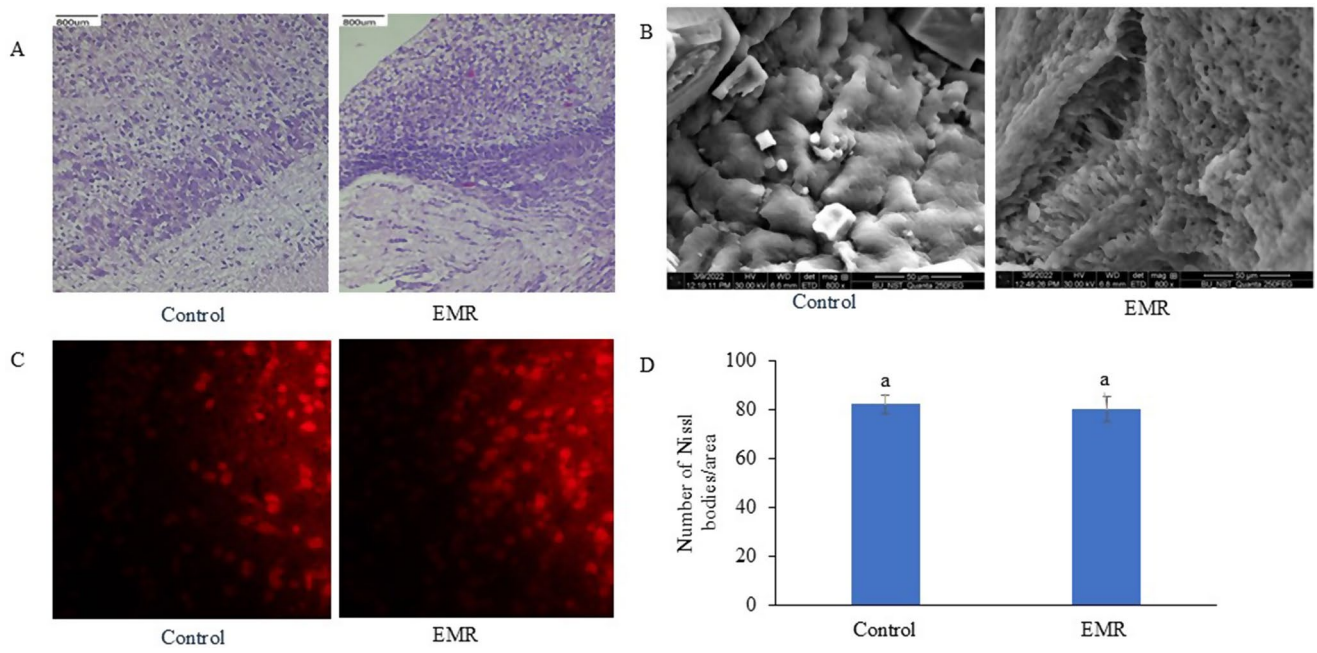


Fig. 1 Effect of EMR on brain tissue architecture. Effect of EMR on histology (H&E) (**A**), morphology (SEM) (**B**) and nissel bodies (**C** & **D**) of chicken embryo brain tissue. The data represented are mean ± SD of six identical experiments. Values are labelled with distinct letter indicates statistically significant difference with each other ($P < 0.05$)

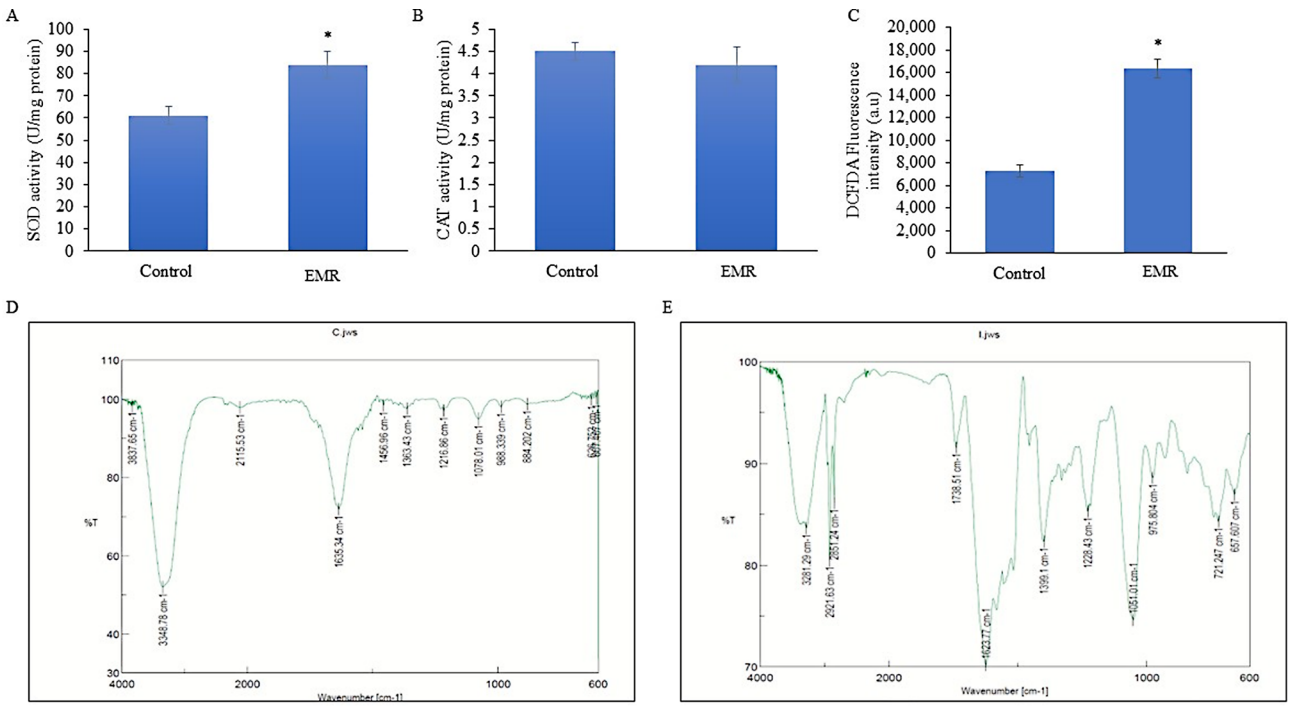


Fig. 2 Effect of EMR on brain tissue antioxidants. Effect of EMR on tissue antioxidants, SOD (**A**) and CAT (**B**), total ROS generation (DCFDA) (**C**) and representative FTIR of control and exposed tissue (D & E). The data represented are mean ± SD of six identical experiments. Values are labelled with * indicates statistically significant difference with each other ($P < 0.05$)

Table 1 Spectral band assignment for the FTIR spectra of brain tissue

S.No	Control	Exposed	Spectral assignment	Macromolecule
1	3348.78	3281.29	Amide A: mainly N–H Stretching; O–H stretching	Proteins with the little contribution from polysaccharides
2	1635.33	1623.77	Amide I: Protein C=O stretching	Protein
3	1363.43	1399.1	COO^- symmetric stretching	Fatty acids polysaccharides, amino acids
4	1216.86	1228.43	PO^-2 asymmetric stretching:	Phospholipids and nucleic acids

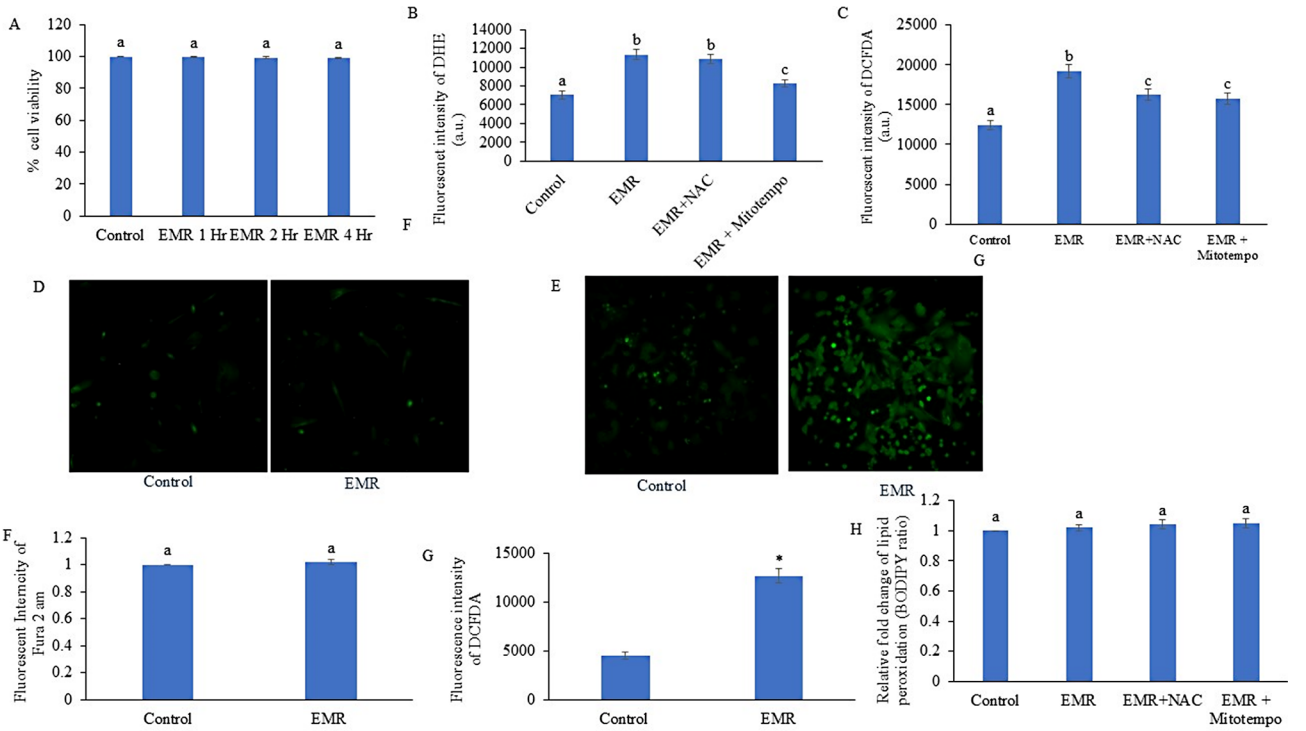


Fig. 3 Oxidative stress effect of EMR on SH-SY5Y cell lines. The time dependent effect of EMR on cell viability (**A**). The EMR effect and influence of antioxidants (NAC and Mito-TEMPO) on cellular superoxide generation (**B**), total ROS production (**C**) and lipid peroxidation (**H**). The representative confocal illustration of total ROS generation (**E** & **G**) and intracellular calcium (**D** & **F**). Data represented are mean ± SD of three identical experiments. Values labeled with distinct letter and * indicates statistically significant difference with each other ($P < 0.05$)

at 2.4 GHz radiation on brain tissue when compared with control.

Tissue antioxidant enzyme activities

The (SOD and CAT) antioxidant enzymes activity within the brain tissue were presented in Fig. 2A & B respectively. The SOD activity showed a significant p-value ($p < 0.05$) in the exposed group when compared to the control group. On the other hand, there was no significant p-value ($p > 0.05$) seen in the CAT activity during 2.4 GHz exposure in the exposed group when compared to the control group.

Effect of exposure time on cell viability

The time-dependent impact of 2.4 GHz exposure on cell viability is shown in Fig. 3A. The current study illustrated that there was no significant p-value ($p > 0.05$) decrease in the cell viability test when observed up to 4 h compared with control.

Effect on intracellular calcium accumulation

The intracellular fluorescence intensity of Fura 2 AM indicated by 340/380 ratio is illustrated in Fig. 3D and F. The 4 h period exposure radiation of 2.4 GHz in the SH-SY5Y cell line does not induce any significant p-value ($p > 0.05$)

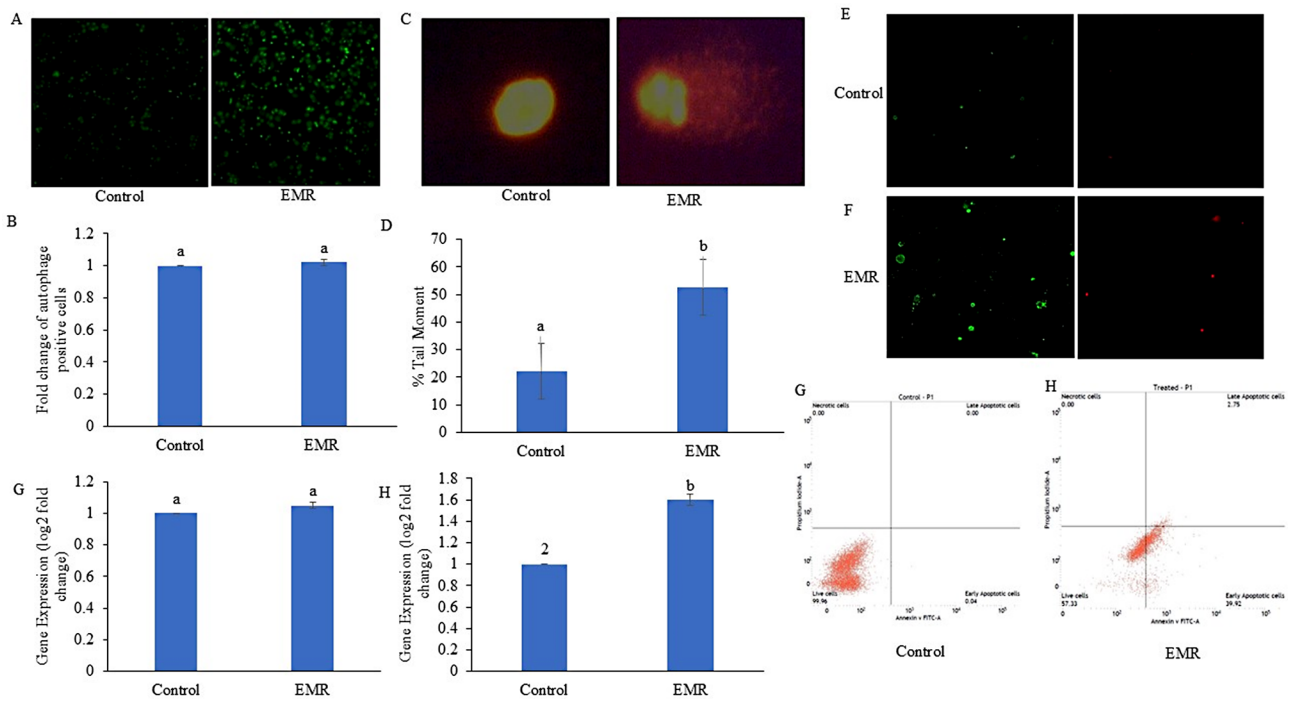


Fig. 4 Effect of EMR on SH-SY5Y cell lines. The effect of EMR on autophagy (**A** & **B**), DNA damage (**C** & **D**), annexin V/PI (**E**, **F**, **G** & **H**), and the relative fold change in gene expression (**G** & **H**). Data represented are mean ± SD of three identical experiments. Values labeled with distinct letter and * indicates statistically significant difference with each other ($P < 0.05$)

fold changes in intracellular calcium level when compared to the control group.

Cellular total ROS generation

The effect of 2.4 Ghz radiation on cellular total ROS generation is illustrated through the DCFDA4 fluorescence activity assay (Fig. 3E). The results indicates a significant ($p < 0.05$) elevation of total ROS generation during 2.4 GHz radiation exposed on SH-SY5Y cell line when compared with control.

Effect on cellular ROS generation and influence of antioxidants

The impact of 2.4 GHz radiation exposure in cellular superoxide and total ROS generation along with the influence of antioxidants (NAC and Mito-TEMPO) was illustrated in Fig. 3B & C. The results indicated that 2.4 GHz radiation exposure in SH-SY5Y cells significantly elevated the superoxide (DHE fluorescence) and total ROS (DCF fluorescence) when compared with the control. While focusing on the impact of antioxidants, Mito-TEMPO pretreatment significantly suppresses the superoxide and total ROS generation showing to be significant p-value ($p < 0.05$). On the other hand, NAC exposure shows a significant p-value ($p < 0.05$) and thus inhibits total ROS level without influencing the superoxide generation.

Effect on lipid peroxidation and influence of antioxidants

The effect of 2.4 GHz radiation exposure on cellular lipid peroxidation (Oxidation ratio of BODIPY C11) and the influence of antioxidants is illustrated in Fig. 3H. The result shows that 2.4 GHz radiation exposure does not significantly increase the lipid peroxidation level when compared to the control group. In addition, there was no influence of antioxidants (NAC and Mito-TEMPO) being observed.

Effect on DNA damage

The fluorescence images (Fig. 4C & D) of the COMET assay results illustrate that a significant p-value of ($p < 0.05$) alongside an increase in the tail moment (DNA damage) was observed in the 2.4 GHz exposed group when compared with the control.

Effect on autophagy

The acridine orange staining (Fig. 4A & B) of SH-SY5Y cells revealed that 2.4 GHz electromagnetic radiation exposure does not induce a significant change in the number of

autophagy positive cells (AVO-positive) when compared to the control.

Effect on early and late apoptosis

The effect of 2.4 GHz radiation on cellular apoptosis is illustrated through the flow cytometry scatter plot (Fig. 4F). The results indicate that 2.4 Ghz radiation prominently increased early apoptosis with initiation of late apoptotic positive cells. The above observation was further validated with AnnexinV-FITC stained fluorescence images (Fig. 4 E).

Gene expression (Bax and Bcl2)

The differential gene expression of Bax and Bcl2 is shown in Fig. 4 G and H respectively. The results indicate that the gene expression of Bax was elevated and no significant ($p \geq 0.05$) change was observed for Bcl2 with respect to 2.4 GHz electromagnetic radiation exposure when compared to the control.

Discussion

In the near period times, people are exposed to and surrounded by electromagnetic radiation which is emitted from various sources like mobile phones, and wireless communication devices. This study specifically focuses on the widespread use and exposure to wireless communication devices operating at 2.4 GHz frequency, and the potential effects caused by this frequency radiation, particularly on neurons present in the brain [1].

The study results shows, Histopathology examination was used as a preliminary study to identify tissue damage, it is reported that no alterations were observed in the hippocampal region of chick embryo brain tissue after inducing electromagnetic radiation for 4 h per day, at a period of 5 consecutive days. Similar studies have also shown that the hippocampal region did not exhibit significant changes in brain tissue upon exposure to 2.4 GHz [6]. In contrast, a study on long-term exposure to EMR for 2 h per day over a time period of 35 days revealed that neuronal cells and vesicles with standard arrangements in the rat brain's hippocampal region exhibited clear nuclei and nucleoli. The cerebral cortex region showed a reduced density of pyramidal cells and signs of neuronal degeneration [17]. In the interim, the 2400 MHz electromagnetic frequency exposed at two different timings such as 40 min/day and 60 min/day revealed significant effects. The 40 min exposure unveiled that pyramidal cells in CA1, CA2, and CA3 regions, granule cells showed signs of degeneration, whereas, the 60 min exposure exhibited contracted morphology [41]. Subsequently, in our current ultrastructural study (Scanning Electron Microscopy), moderate surface morphology changes were observed in chick embryo brain tissue after exposure to electromagnetic radiation for 4 h per day over 5 days, whereas, in another study, there was inflammation of neurons being observed in 14-day-old chick embryos through SEM analysis [42].

Furthermore, after 4 h of exposure to 2.4 GHz frequency, the ROS production level was increased in brain tissue and the current results of our study showed increased superoxide dismutase (SOD) levels with no significant changes in catalase (CAT) levels in chick embryos that were exposed to 2.4 GHz frequency for 4 h for 5 days compared to the control group. Similarly, a previous study suggested that EMR significantly increases the SOD and CAT activity after 4 h of exposure [7, 43]. This may be due to the fluctuation in the SOD and CAT anti-oxidant enzymes, which are to protect the cells against toxic ROS levels.

The ROS generation normally occurs in cells through physiological metabolic pathways [44], whereas, environmental and chemical exposure increases the production of ROS which causes oxidative stress [30]. The present in vitro study reveals that 4 h of exposure significantly increased ROS generation in the SH-SY5Y cell line as the EMR induces mitochondrial dysfunction by disrupting electron transport chain components, leading to superoxide generation. This cascade contributes to oxidative stress and triggers the apoptotic pathways [15]. Moreover, a previous study has demonstrated that a time-dependent manner of, 2.4 GHz electromagnetic radiation exposure caused ROS generation and DNA damage in the SH-SY5Y cell line [45]. The occurrence of oxidative stress resulting from the influence of electromagnetic radiation (EMR) has been postulated to initiate DNA damage. Our investigation, however, observed a notable extent of DNA damage within a mere timeframe of 4 h, after subjecting the cells to EMR exposure. In this manner, an early study has been reported in which 2.4 GHz frequency induces DNA damage [46], even though, few investigations illustrate the absence of DNA damage in tissues [47].

Based on our oxidative stress results, we evaluated the impact of general antioxidants such as NAC and mitochondria targeting SOD mimic antioxidant (Mito-TEMPO) to reduce oxidative stress during EMR exposure. The Mito-TEMPO passes through the phospholipid bilayer and accumulates in mitochondria where it reduces oxidative stress, and mitochondrial dysfunction [48]. The NAC ameliorates oxidative stress-induced damage and also attenuates hepatotoxicity and brain damage [49]. Our findings showed that NAC and Mito-TEMPO act as potential anti-oxidants to reduce oxidative stress from 2.4 GHz electromagnetic radiation exposure and triggers protective effects. The corroborated antioxidative

roles of NAC and Mito-TEMPO abate EMR-induced oxidative stress. However, their mechanisms need further investigation. NAC probably replenishes glutathione that is stored in cells to confer more resistance against ROS. On the other hand, Mito-TEMPO targeted action inside the mitochondria makes it a good anti-oxidant for therapeutic interventions, protecting the mitochondrial integrity against EMR-induced stress. The study results open new strategies for antioxidant-based therapies against oxidative stress-related neuronal damage [50]. Although previous studies reveal that the time-dependent cell viability alterations were changed by exposure to electromagnetic radiation, similar studies have found that various exposure periods decreased the cell viability in the SH-SY5Y cell line [15, 45]. In the present study, no alterations in SH-SY5Y cell viability have been found after 4 h exposure to 2.4 GHz electromagnetic radiation.

In a previous study on EMR exposure, calcium has a major role in generating oxidative stress and programmed cell death has been reported [43, 51]. The study has shown a significantly increased level of calcium after 2450 MHz frequency exposure [52]. However, our findings showed that 4 h exposure to 2.4 GHz electromagnetic frequency does not affect the intracellular calcium ion level.

The EMR exposure in the present study showed an alteration in the early apoptosis in the SH-SY5Y cell line. Additionally, a previous study suggested that time-dependent early and late apoptosis occurred after EMR exposure [53]. Thereafter, autophagy is assumed to play a pivotal function in cellular adaptation, eradication of intracellular organisms, and prevention of ageing [54]. There have been contradictory accounts regarding the influence of EMR on the induction of autophagy. Early detection of alteration in the autophagy marker 1 A/1B-light chain 3 (LC3) gene after exposure to EMR with different time intervals has already been reported [45]. In our investigation, electromagnetic radiation did not show any notable effect on autophagy. However long-term exposure to EMR showed Bax and Bcl-2 gene expression after 4 h in the hippocampal region [55]. Conclusively, the Bax and Bcl-2 genes showed alterations after 900 MHz for 2 h of exposure [56]. As expected, 2.4 GHz electromagnetic radiation-induced early apoptosis. Corroborating these data, the apoptosis marker genes, Bax, showed an elevated expression without affecting Bcl-2, indicating that 2.4 GHz frequency initiates early apoptotic events without showing notable necrotic cells.

Conclusion

This study demonstrates the intricacy of the influential impacts of short-term exposure to 2.4 GHz electromagnetic radiation (EMR) at the cellular and tissue levels, thus shedding light on its potential biological implications. Even though there were no significant structural changes detected in histopathological studies of chick embryo brain tissues, results of scanning electron microscopy revealed micro-impacts after short-term exposure of 2.4 GHz, which induced moderate surface modifications. Similarly, antioxidant enzyme activities displayed differential responses where the levels of SOD increased, and on the other hand, CAT activity remained the same. These observations thus emphasise the tight regulation of oxidative stress responses to EMR exposure. In neuronal cell, SH-SY5Y, a marked increase in ROS generation was accompanied by DNA damage and upregulation of apoptotic markers, especially the Bax gene. These findings show that EMR causes oxidative stress and triggers the activation of apoptosis-related pathways stating that mitochondria have a role in these events. Interestingly, the antioxidant protection by NAC and Mito-TEMPO shows a potential area of intervention for the abrogation of oxidative stress due to EMR.

Acknowledgements D.K would like to thank Dr. Manjunatha M V, Associate Professor, Department of Neurovirology, NIMHANS, Bengaluru for providing the cell line (SH-SY5Y) for this study. Also, like to thank the Central Instrumentation Center, Bharathiar University, Coimbatore for providing the confocal microscopy facility.

Author contributions The manuscript's concept was conceived by S.R, J.M & D.K. S.R, J.M, D.K, & G.B.M arranged the project and oversaw the research. The manuscript was written by D.K and G.B.M. All authors read and approved the manuscript.

Funding No funding was provided for this project.

Data availability No datasets were generated or analysed during the current study.

Declarations

Ethical approval Ethical approval is not applicable to this study (Short-term exposure of 2.4 GHz electromagnetic radiation on cellular ROS generation and apoptosis in SH-SY5Y cell line and impact on developing brain tissue).

Competing interests The authors declare no competing interests.

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