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A Prolonged exposure to Wi-Fi Radiation Induces Neurobehavioral Changes and Oxidative Stress in Adult Zebrafish

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Abstract
The rapid development and use of wireless technologies have raised concerns about the possible harmful effects of long-term exposure to non-ionizing electromagnetic radiation (EMR). Wi-Fi routers are the most commonly used devices at households, offices, hotels, hospitals, airports, train stations etc., to provide wireless internet access to multiple devices. Therefore, prolonged exposure to EMR warrants thorough scientific investigation and risk assessment. Herein, we evaluated the impact of 2.45GHz Wi-Fi radiation exposure for 4 hours daily over a consecutive 30-day period on neurobehavioral and brain oxidative stress parameters in adult zebrafish. Post-exposure assessments revealed significant neurobehavioral impairments, altered locomotion, decreased neurotransmitter levels (AChE), and increased oxidative stress. Further studies are needed to better understand the molecular mechanisms involved and to assess the broader implications for vertebrate neurobiology and public health.

Keywords: Wi-Fi radiation, Neurobehavior, Locomotion, Oxidative stress, AChE

Introduction

Wireless Fidelity (Wi-Fi) networks enable short-range data communication between access points and a variety of personal electronic devices, including computers, printers, televisions, gaming systems, etc. These networks predominantly function within the 2.45GHz frequency band, emitting radiation during active data transmission. Consequently, individuals in proximity to these devices experience continuous low-intensity exposure to electromagnetic fields (EMFs), particularly during extended use. The growing dependence on these wireless technologies has prompted considerable concern about their possible biological effects, particularly regarding prolonged exposure (Vignera et al., 2012). Few earlier reports indicate that radiofrequency (RF) radiation may induce various cellular disturbances, both reversible and irreversible, such as interference with the cell cycle, alterations in gene expression patterns, and activation of programmed cell death pathways (Li et al., 2007; Nazıroğlu et al., 2013). It has been hypothesized that some of these cellular damages occur due to an imbalance between the generation of reactive oxygen species (ROS) and the capacity of the antioxidant defense system, leading to excessive ROS accumulation (Akdag et al., 2007; Repacholi & Greenebaum, 1999). Elevated ROS are known to cause oxidative harm to biomolecules including lipids, proteins, carbohydrates, and nucleic acids. Among these, ROS-induced damage to DNA is proposed as a fundamental molecular pathway involved in the initiation of cancer in humans (Ongel et al., 2009).

Studies under exposure conditions similar to public exposure from household Wi-Fi devices found no effect on gross morphology development and survival in general (Prlić et al., 2022). However, studies on other species like Drosophila melanogaster and rodents, have shown that chronic exposure to RF-EMFs can cause oxidative stress, DNA damage, altered gene expression, and behavioral abnormalities (Cappucci et al., 2022; Vafaei et al., 2020). These impacts raise valid concerns about the long-term ecological and health consequences of our increasingly wireless world not just for humans, but also for the diverse species that share our environment.

While the deleterious effects of EMR from mobile phones have been extensively studied, there remains a paucity of comprehensive data regarding the biological impact of Wi-Fi based EMR, despite its increasing prevalence (Atasoy et al., 2013; Avendano et al., 2012). Experimental evidence in animal models has demonstrated that exposure to static magnetic fields can trigger oxidative imbalances linked to metabolic disturbances (Elferchichi et al., 2010; Ghodbane et al., 2011). However, investigations specifically focusing on RF-EMR exposure have yielded inconsistent findings. Some studies report enhanced ROS production and depletion of antioxidant reserves following mobile phone EMR exposure (Çelik et al., 2016; Özorak et al., 2013; Salah et al., 2013), while others observe no significant perturbations in oxidative stress markers such as malondialdehyde (MDA) (Atasoy et al., 2013). These discrepancies suggest that the extent of oxidative damage may depend on the magnitude and duration of ROS generation after EMR exposure (Brydon et al., 2001).

In various animals, including zebrafish and rats, RF-EMF exposure has resulted in locomotor activity changes, which are frequently associated with neurobehavioral impairment in rodents (Narayanan et al., 2019). In this context, the present study systematically examined the neurobehavioral and certain biochemical consequences of prolonged exposure EMR (2.45GHz, 4 hours/day for 30 consecutive days), emitted by indoor Wi-Fi device in adult zebrafish. Anxiety-like behavior and locomotor performance were assessed using established behavioral paradigms, including the novel tank test, locomotion analysis, and scototaxis assay. Concurrently, neurochemical assessments were conducted by quantifying AChE activity and evaluating oxidative stress markers such as lipid peroxidation (LPO), reduced glutathione (GSH), and catalase (CAT), to elucidate the interplay between RF-EMR exposure, redox imbalance, and cholinergic disruption.

Material and methods

Animal maintenance and feeding

For the present study, animals approximately three months of age were procured from a certified vendor and were acclimatized to laboratory conditions for a period of 10 days. Sex differentiation was carried out based on morphological characteristics and the males and females were separated into two distinct holding tanks. Each tank was maintained with 5 liters of dechlorinated system water. Water temperature was regulated at 28.5 ± 2 °C, and the pH was maintained at an alkaline level of 7.5 to ensure optimal physiological conditions. A controlled photo period of 14 hours' light and 10 hours' dark was implemented consistently. Tanks were cleaned every alternate day. The animals were fed a formulated powdered pellet diet containing 60% protein content, administered twice daily in appropriate quantities.

Experimental design

After a short adaptation period, the separated male and female fish were randomly assigned to control and experimental groups in separate tanks. 15 fish in were maintained in each tank. The Wi-Fi device (CP Plus 4G model, 2.45GHzWi-Fi signal) was placed in the center of a platform and the fish tanks were kept at a distance of 20 cm away from it. The schematic design of the device and the position of tanks used for radiation exposure in this study are shown in Figure 1. The experimental group were continuously exposed to EMR originated from this Wi-Fi device for 4 h/day for 30 consecutive days. The exposure system was designed in a way as to apply equal condition to all 15 animals at the same time during the experiment. On the other hand, zebrafish in the control group were kept under identical environmental conditions with no exposure to EMR (Control group). On day 30, a video camera (Sony handycam) was used to record the neurobehavioral outcome for both the groups. Subsequently, all zebrafish were euthanized, and tissues were dissected out for oxidative stress estimations. The video analysis was done by using Panlab SMART v3.0 software free version (Harvard Apparatus, United States).

Ethical statement

Animal handling was done as per the guidelines of the "Committee for the Purpose of Control and Supervision of Experiment on Animals (CCSEA)," Government of India, with Institutional Animal Ethics Committee IAEC, approval, Department of Zoology, The Maharaja Sayajirao University of Baroda, Vadodara, Gujarat, India (Approval No. 827/GO/Re/S/04/CCSEA).

Neurobehavior Analysis

Locomotion activity

Locomotor behavior plays an important role in the feeding, social, and defensive activities of the zebrafish throughout its lifespan. On day 30, individual zebrafish from each group were carefully subjected to the novel dive tank test and their movement was tracked for 6 min from the top and lateral side of the aquarium.

Novel Tank Test

The zebrafish novel tank test is used to assess anxiety and locomotion. For this, individual zebrafish from each group was carefully subjected to the novel dive tank and their locomotion was tracked for 6 mins using the protocol of Fontana et al., 2022. The behavioral endpoints like time spent in bottom, total distance travelled, duration of freezing bouts and number of entries to upper half were used to measure the anxiety.

Scototaxis

Scototaxis was performed in a tank divided into equally sized partitions, where half of the tank area was uncovered, and the rest area was covered with black paper to avoid light exposure. The fish is introduced in the center of the tank, it's movements were recorded for 6 mins to examine the total time spent in different areas (Mezzomo et al., 2016). The same procedure was followed for all the control and exposed fish.

Acetylcholinesterase activity

A 10% brain homogenate was prepared in ice-cold 0.1M phosphate buffer (pH 8.0) and centrifuged at 800

0 rpm for 20 minutes at 4 °C. In a 96-well plate, 50 µl of acetylcholine iodide and 50 µl of the supernatant were added and incubated for 15-20 minutes at room temperature. The reaction was stopped by adding 100 µl of Ellman's reagent (DTNB), and absorbance was measured at 412 nm using an ELISA reader (Ellman et al., 1961).

Oxidative stress parameters

Sample preparation

After recording neurobehavioral outcomes, zebrafish were euthanized to isolate the brain, washed with ice cold saline to remove blood. Brains of six zebrafish were pooled in each sample. Each sample was gently homogenized in 50 mM phosphate buffer saline solution of pH 7.4 with homogenizer. The homogenate was centrifuged at 700 rpm for 5 min at 4˚ C in cooling centrifuge. The resultant pellets were discarded, and supernatants were used for estimation of CAT activities and LPO, GSH levels (Nirwane et al., 2016).

Catalase activity

The reaction mixture consisted of 2 ml phosphate buffer (pH 7.0), 0.95 ml of hydrogen peroxide (0.019 M) and 50 µl of sample supernatant in a final volume of 3 ml. Absorbance was recorded at 240 nm every 10 sec for 1 min. The results were expressed as units of catalase (CAT) activity/mg protein.

Lipid peroxidation

100 µl of supernatant was mixed 2.5 ml TBA (0.7% in 30% Glacial acetic acid), incubated at 95˚ C for 1 hr. After cooling the mixture was centrifuged at 4000 rpm for 10 min at 4˚ C. The absorbance was read at 532 nm against the blank. The extent of lipid peroxidation (LPO) was expressed as nmol of malondialdehyde/mg of protein.

Reduced Glutathione

A volume of 200 µl tissue supernatant was mixed with 0.6 ml of 0.2 nmol/L Tris-EDTA buffer and 50 µL of 0.01 mol/L DTNB, followed by the addition of 2.5 mL methanol. The mixture was incubated at 37 °C for 30 minutes with occasional shaking and then centrifuged at 4000 × g for 10 minutes at 4 °C. The supernatant was collected, and absorbance was measured at 412 nm using an ELISA microplate reader.

Statistical analysis

All values are expressed as mean ± SEM. Statistical analyses were performed using GraphPad Prism 8 software for Windows (GraphPad Software, San Diego, CA, USA). The differences between the groups were analyzed with t-test. Statistical significance was considered at $p < 0.05$.

Results

Locomotion activity

The group exposed to Wi-Fi radiation exhibited reduced locomotor activity, as indicated by a shorter total distance traveled and lower velocity compared to the control group (Figure 2A and 2B).

Novel Tank Test

In the 6 min novel dive test, the group exposed Wi-Fi EMR spent significantly more time in the bottom half of the tank and exhibited increased freezing duration and a higher number of freezing bouts compared to the control group (Figure 2C and 2D).

Scototaxis

In the Scototaxis or light dark preference test the results revealed that Wi-Fi exposed group spent significantly more time in dark compared to control group which could be an indication of stress like behavior (Figure 2E).

AChE activity

The acetylcholinesterase (AChE) activity in the brain was monitored in control and Wi-Fi exposed zebrafishes. As presented in Figure 3 there was a significant decrease in AChE activity in exposed fishes when compared with the control group.

Oxidative stress parameter

Malondialdehyde (MDA) levels, a marker of lipid peroxidation (LPO), were significantly elevated in the brains of Wi-Fi-exposed zebrafish, indicating oxidative stress. Catalase activity, crucial for decomposing H_2O_2 and maintaining redox balance, was significantly reduced in the exposed group compared to controls. Conversely, reduced glutathione (GSH) levels were significantly increased following Wi-Fi exposure (Figure 4).

Discussion

Electromagnetic fields (EMFs) are invisible forms of energy, produced by both natural sources (Earth's magnetic field) and human made technologies, including Wi-Fi, mobile phones and various other electrical utilities. With the rapid expansion of wireless communication networks and electronic devices, environmental EMF exposure has become increasingly widespread. Despite the widespread environmental presence of Wi-Fi radiation, limited studies exist which have systematically explored its effects on living organisms. This research addresses this gap, providing important insights into the potential risks of prolonged Wi-Fi originated EMF exposure.

The potential effects of RF-EMF on human and various other animal models is being well document for the past few years. Khira and Uggini (2024) demonstrated that exposure of 4G mobile phone with 1.13 W/Kg SAR and 1800 MHz frequency altered locomotion pattern in larval zebrafish. Bhattacharya et al. (2022) showed that mobile phone radiation (900 MHz) reduced locomotor activity and enhanced anxiety-like behaviour in adult zebrafish. A previous study by Daniels et al., demonstrated reduction in locomotor activity, increased grooming and freezing behavior in rats upon EMR exposure in the mobile phone range (Daniels et al., 2009). Similarly, Sokolovic et al. (2012) demonstrated that microwave radiation exposure induced anxiety-related behaviors in rodent models. Spandole-Dinu et al. (2023) reported that mice subjected to long term exposure to 2.45GHz Wi-Fi radiation exhibited increased locomotor activity and decrease in global DNA methylation while there were no significant changes in brain structure. Aligning with these observations, the present study found that adult zebrafish exposed to 2.45GHz Wi-Fi radiation exhibited typical induction of anxiety-like behavior, as assessed by the novel tank diving test. Specifically, exposed fish demonstrated a significant increase in the time spent in the bottom half of the tank, an elevated number and duration of freezing episodes, reduced distance traveled and lower mean swimming velocity collectively indicating enhanced stress and anxiety responses following Wi-Fi radiation exposure.

Acetylcholinesterase (AChE) plays a pivotal role in regulating cholinergic neurotransmission by catalyzing the hydrolysis of acetylcholine at synaptic clefts, thereby terminating neuronal signaling. Our result showed a reduced AChE level in Wi-Fi exposed group when compared to control. Recent studies suggest that exposure to radiofrequency electromagnetic fields (RF-EMFs), including Wi-Fi radiation, can significantly alter AChE activity, potentially leading to synaptic dysfunction and behavioral disturbances by hampering cholinergic response in the organism (Sharma and Shukla, 2020).This observation aligns with a study done by A study conducted by Obajuluwa et al. (2017) observed a significant decline in AChE levels along with elevated anxiety in rats exposed to 2.45 GHz Wi-Fi for durations of 4, 6, and 8 weeks. Megha et al. (2015) reported a marked decrease in AChE activity in the cerebral cortex of rats subjected to chronic 2.45GHz Wi-Fi radiation, attributing these effects to EMF-induced oxidative damage and inflammation. A similar study demonstrated reduced hippocampal AChE activity in Wistar rats following prolonged RF-EMF exposure, accompanied by cognitive deficits and histopathological alterations (Narayanan et al., 2019). Supporting these findings, Kim et al. (2017) observed AChE inhibition in cortical neurons of mice exposed to 835 MHz mobile phone radiation, correlating with signs of neuronal hyperactivity and demyelination.

Furthermore, Sharaf et al. (2017) found that prolonged Wi-Fi radiation exposure 24 hours daily for six months led to heightened anxiety and significantly reduced levels of dopamine, AChE, and serotonin in specific brain regions of rats.

Free radicals, notably reactive oxygen species (ROS) (Brieger et al., 2012), are generated by nature during normal metabolic processes and play critical roles in cellular signalling and immunological control (Bergendi et al., 1999). However, changes in intracellular ROS concentrations caused by radiofrequency (RF) exposure may be dependent on both the frequency and amplitude of the signal (Pooam et al., 2022). Several studies have found elevated amounts of malondialdehyde (MDA), a critical biomarker of lipid peroxidation, and protein carbonyls, indications of protein oxidation, in the brains of animals exposed to electromagnetic radiation (EMR). Specifically, exposure to 2.45GHz EMR has been shown to elevate lipid peroxidation levels and diminish the activity of antioxidant enzymes responsible for mitigating oxidative damage in tissues (Gumral et al., 2009; Türker et al., 2011). Atasoy et al. (2013) reported a significant drop in catalase (CAT) activity in rats exposed to Wi-Fi signals, indicating compromised antioxidative defense mechanisms. This decrease in CAT activity, which is essential for neutralizing hydrogen peroxide and superoxide radicals, could be due to an excess of reactive species (Kono & Fridovich, 1982). Furthermore, Ceyhan et al. (2012) discovered an increase in glutathione peroxidase (GSH-Px) levels in skin tissues following 2.45GHz EMR exposure (1 hour per day for 28 days). Collectively, these findings indicate that RF-EMR exposure can enhance ROS generation, leading to oxidative stress and subsequent DNA damage (Durdik et al., 2019; Jin et al., 2021; Santini et al., 2018). In agreement with these previous reports, our findings revealed a significant decrease in catalase activity alongside increased lipid peroxidation (LPO) and glutathione (GSH) levels in the Wi-Fi exposed group. Consistent with these observations, Sharma and Shukla (2020) demonstrated that 90 days of RF-EMF exposure (1–4 hours per day, 5 days per week, 0.231 W/kg) resulted in elevated ROS levels, suppression of antioxidant enzymes such as superoxide dismutase (SOD) and CAT and increased pro-inflammatory cytokine expression.

Overall, our experimental findings provide compelling evidence that continuous exposure to Wi-Fi radiation induces significant oxidative stress, likely mediated by excessive generation of reactive oxygen species (ROS). Supporting this biochemical disturbance, we observed altered neurobehavioral responses, including impaired locomotor activity, increased anxiety-like behavior in the novel tank diving test, and altered light/dark preference in the scototaxis assay indicating functional disruptions in the sensory-motor system. Furthermore, a notable reduction in acetylcholinesterase (AChE) activity was detected, which is indicative of excess cholinergic activity as a consequence of anxious behavior. potential cholinergic dysfunction and impaired synaptic signaling under EMR exposure. These cholinergic and behavioral changes are likely linked to the oxidative burden imposed on neural tissues. However, further experiments are required to demonstrate the underlying mechanism of EMR with different frequencies on various tissues.

Conclusion

The present study demonstrates that prolonged exposure to electromagnetic radiation (EMR) emitted by wireless devices (Wi-Fi) significantly compromises the antioxidant capacity of blood, indicating a systemic oxidative imbalance. Also, notable neurobehavioral alterations lead to stress and anxiety like behavior. These findings highlight the potential health risks associated with chronic Wi-Fi exposure, particularly in domestic and occupational settings, and underscore the importance of minimizing unnecessary exposure where feasible. This study may stimulate future helpful research in the development of new protective approaches. Furthermore, a detailed understanding of the precise molecular mechanisms and specific sites of action involved in continuous exposure to electromagnetic radiation (EMR) emitted by wireless devices is warranted.

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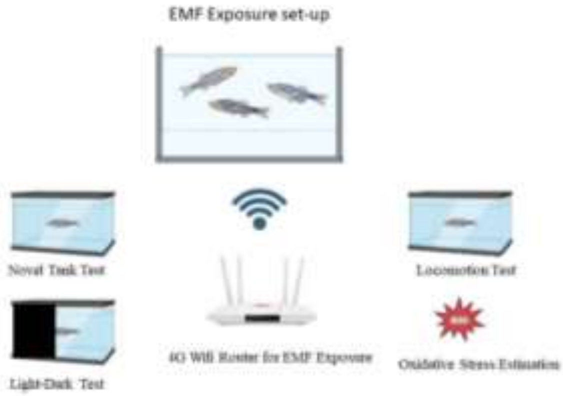

Figure 1: Exposure set up.

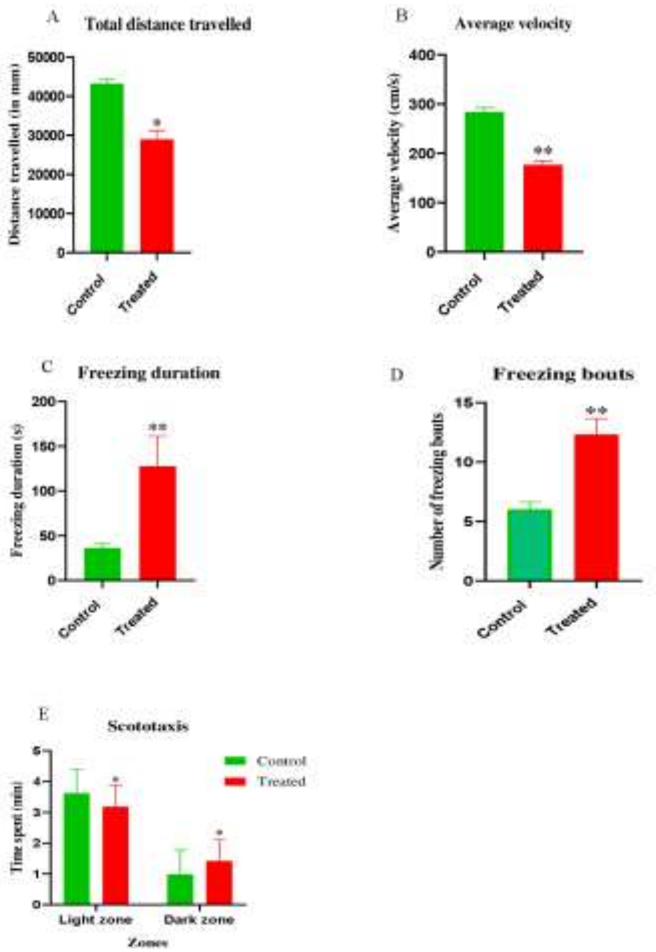

Figure 2: Neurobehavior Analysis on 30th day, where; A- Total distance travelled, B- Average velocity, C- Freezing duration, D- Freezing bouts and E- Scototaxis

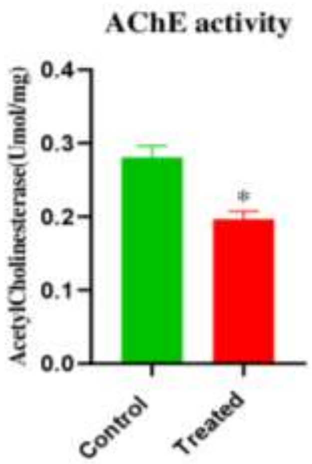

Figure 3: Acetyl Choline Esterase Activity.

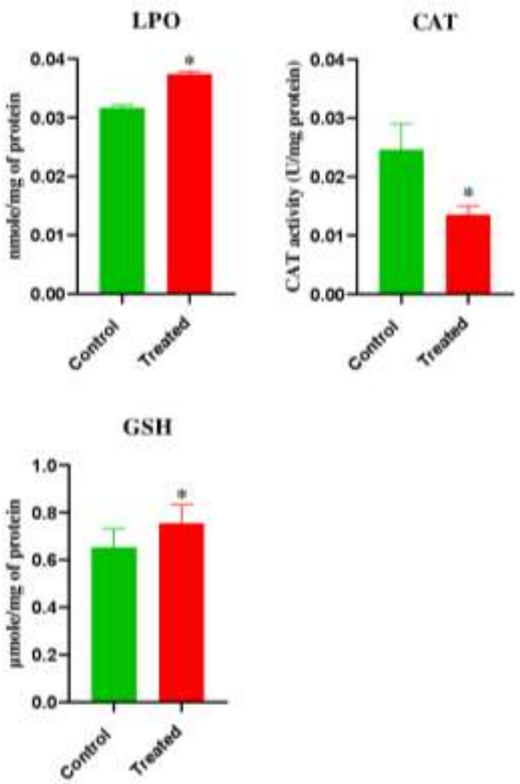

Figure 4: Oxidative stress Analysis, where; A- Lipid peroxidation, B- Catalase, C- Reduced glutathione.