Original Research Article

DNA Damage in Zebrafish Induced by Low-Frequency Electromagnetic Fields: Insights from Comet Assay

ABSTRACT

In our modern era marked by rapid technological advancements, the widespread use of electronic devices has led to an unprecedented increase in exposure to electromagnetic fields (EMFs) across various frequencies. Among these, low-frequency electromagnetic fields (LF-EMFs) have emerged as a topic of significant interest due to their pervasive presence in everyday environments and their potential interactions with biological systems. Despite extensive research on the biological effects of high-frequency EMFs, such as those emitted by mobile phones and Wi-Fi routers, our understanding of the impact of LF-EMFs, particularly on DNA integrity, remains limited. This study seeks to address this gap in knowledge by investigating the effects of LF-EMFs on the DNA integrity of zebrafish, a valuable model organism with well-characterized genetic pathways. Leveraging the Comet Assay, a powerful tool in genetic toxicology, we aimed to assess DNA damage at the cellular level. Our findings reveal a significant increase in both tail length and DNA content within the comet tail region of zebrafish following exposure to LF-EMFs, indicative of heightened DNA damage compared to unexposed controls. These results underscore the potential genotoxic effects of LF-EMFs on zebrafish DNA integrity, raising concerns about the risks associated with EMF exposure. This study contributes to our understanding of the biological effects of electromagnetic radiation, particularly LF-EMFs, and emphasizes the need for further research to comprehensively evaluate the potential risks posed by EMF exposure.

Keywords:Comet assay, DNA, Low-frequency electromagnetic field, Zebrafish.

1. INTRODUCTION

In an era of rapid technological advancement, the spread of electronic devices has exponentially increased electromagnetic field (EMF) exposure across various frequencies. Low-frequency electromagnetic fields (LF-EMFs) have garnered significant scientific interest due to their pervasive presence in everyday environments and potential interactions with biological systems. While the biological effects of high-frequency EMFs, like those emitted by mobile phones and Wi-Fi routers, have been extensively studied, the impact of LF-EMFs, particularly on DNA integrity, remains relatively underexplored. Despite DNA's inherent stability as the fundamental molecule of life, it remains vulnerable to damage from various environmental stressors, including radiation and chemical agents. LF-EMFs, though non-ionizing, have been associated with cellular responses, leading to speculation about potential genotoxic effects.

The Comet Assay, a cornerstone technique in genetic toxicology, offers a precise means of evaluating DNA damage at the cellular level. The Comet Assay, also known as single-cell gel electrophoresis (SCGE) initially developed by Ostling and Johanson in 1984 as a "micro-electrophoretic study. This early assay version provided a method to evaluate DNA strand breaks at the single-cell level under neutral conditions (pH = 10), laying the foundation for subsequent advancements. A significant improvement came with the introduction of alkaline conditions (pH > 13) [1], allowing for better optimization of DNA denaturation and the detection of single-stranded DNA breaks and alkaline-sensitive lesions [1]. An assay further refined by coining the term "comet assay" for its ability to identify genotoxic agents under alkaline conditions [2-3]. This alkaline version of the assay became the gold standard for detecting DNA damage in eukaryotic cells due to its enhanced sensitivity and specificity [4] and for exploring the genotoxic repercussions of environmental factors, including LF-EMFs [1, 5-8]. During the Comet Assay procedure, cells subjected to an electric field exhibit fragmented DNA migration from the nucleus, forming a comet-like tail whose length and intensity correlate with the extent of DNA damage.

In recent years, the use of the comet assay in cell culture analysis has significantly broadened, covering a wide range of cellular parameters. A studyshowcased the assay's versatility in evaluating critical aspects such as cell viability, proliferation, apoptosis, and micronucleus frequency [9]. This comprehensive approach equips researchers with a powerful toolkit to assess cellular responses to various stimuli, offering insights into both genotoxic and cytotoxic effects. Additionally, the comet assay has been modified to detect increased levels of oxidized DNA bases, allowing for the differentiation between DNA damage from direct chemical binding and that induced by chemical-mediated oxidative stress. The specialized application was highlighted, emphasizing its significance in understanding the complex mechanisms behind DNA damage and oxidative stress responses [10].

This adaptability and robustness make the comet assay invaluable for exploring the genotoxic impacts of various environmental factors. Recent research has increasingly utilized the comet assay to investigate DNA damage induced by LF-EMFs, an emerging environmental concern. As EMFs are recognized as significant environmental pollutants, understanding their biological effects has become more critical. Studiesexemplified this ongoing research, highlighting the importance of comprehending the potential genotoxic effects of EMF exposure on biological systems [11-13].

However, amidst these advancements, a critical research gap persists regarding the limited exploration of the impact of EMF on DNA damage in zebrafish utilizing the comet assay. While studies in other animal models such as earthworms [14], rainbow trout [15], and rodents [16] have been conducted, investigations in zebrafish remain conspicuously scarce. This gap underscores the necessity to acknowledge and address the shortage of research about the specific effects of EMF on DNA damage in zebrafish.

Zebrafish, with their well-characterized genome and highly conserved molecular pathways, serve as a valuable model for investigating the biological consequences of environmental stressors such as LF-EMFs. Their rapid development from embryo to adulthood and transparent embryos make them particularly amenable to real-time observation and analysis of cellular processes, including DNA damage and repair mechanisms.

This research aims to bridge the gap in our understanding of LF-EMF effects on DNA integrity by specifically focusing on zebrafish, an organism poised at the intersection of environmental and molecular biology. By leveraging the Comet Assay as a powerful tool for genotoxicity assessment, this study represents a comprehensive investigation into the impact of LF-EMFs on zebrafish DNA integrity. Through meticulous examination at the cellular level, this research endeavors to contribute to our understanding of the biological effects of electromagnetic radiation.

2. MATERIAL AND METHODS

2.1. ZEBRAFISH AND THEIR MAINTENANCE

Adult zebrafish (*Danio rerio*) were obtained from a commercial supplier and kept in well-maintained glass tanks containing dechlorinated water. The tanks provided a stable environment with a pH range of 7.5 to 7.8 and a temperature maintained between 25°C and 28°C. To ensure proper acclimatization, the zebrafish were kept in these conditions for one week before the start of the experiments. They were fed Instincts® Spirulina brine shrimp once daily. The zebrafish selected for this study had not been previously used in any experiments. All procedures strictly followed the protocols and guidelines established by The Zebrafish Information Network (ZFIN) and the Ethical Committee of Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, for animal handling and experimentation.

2.2. EXPOSURE

A LF-EMF device was constructed based on the Helmholtz coil theory, with a frequency range of 0 to 150 Hz. The experimental group of zebrafish was exposed to a consistent LF-EMF set to a frequency of 50 Hz and an intensity of 300μT for one hour daily over 90 days (N = 6 for each group). This exposure regimen was based on previous studies [17-19]. Control zebrafish were kept without any EMF exposure to serve as a baseline for comparison.

2.3. COMET ASSAY

At the end of the exposure period, zebrafish were anesthetized with appropriate protocol [20] and their brains were carefully dissected. The comet assay with necessary modifications was used to detect DNA damage [1]. SYBR Green dye was used to stain the DNA, enhancing the assay's visualization and sensitivity. Fluorescence microscopy was employed for detailed imaging of DNA damage within individual cells.
Parameters such as tail length and the percentage of DNA in the comet head and tail were measured using ImageJ OpenComet software to quantify DNA damage. Each sample was analyzed by examining 40 comets to assess DNA integrity. Statistical analysis was conducted using the student t-test to determine the significance of the observed differences between the control and experimental groups. This rigorous analysis provided insights into the biological effects of LF-EMF exposure on zebrafish DNA.

3. RESULTS AND DISCUSSION

3.1. RESULT

The analysis comparing tail length in zebrafish between the control and LF-EMF exposed groups revealed a significant difference. Zebrafish exposed to LF-EMF had a markedly longer tail length (27.27 ± 3.12) compared to the control group (13.62 ± 3.81), indicating a substantial increase in tail length due to LF-EMF exposure ($p < 0.0101$) (shown in Figure 1). In the context of a comet assay, tail length refers to the distance that fragmented DNA travels from the nucleus toward the comet tail during electrophoresis, serving as an indicator of DNA damage. Longer tail lengths signify greater DNA fragmentation and potentially more severe damage. Therefore, the increased tail length in the LF-EMF exposed group suggests elevated DNA damage in these zebrafish compared to the control group.

Additionally, when examining DNA content in the head and tail regions of zebrafish, the LF-EMF exposed group displayed significantly higher Tail DNA content (74.035 ± 5.001) compared to the control group (33.99 ± 3.36) (shown in Figure 3). Conversely, the control group had higher Head DNA content (66.003 ± 3.36) compared to the LF-EMF exposed group (25.96 ± 5.001) (shown in Figure 2). These findings indicate that the observed increase in tail length in the LF-EMF exposed group is due to higher DNA content in the tail region, providing strong evidence that LF-EMF exposure affects the DNA integrity of zebrafish.

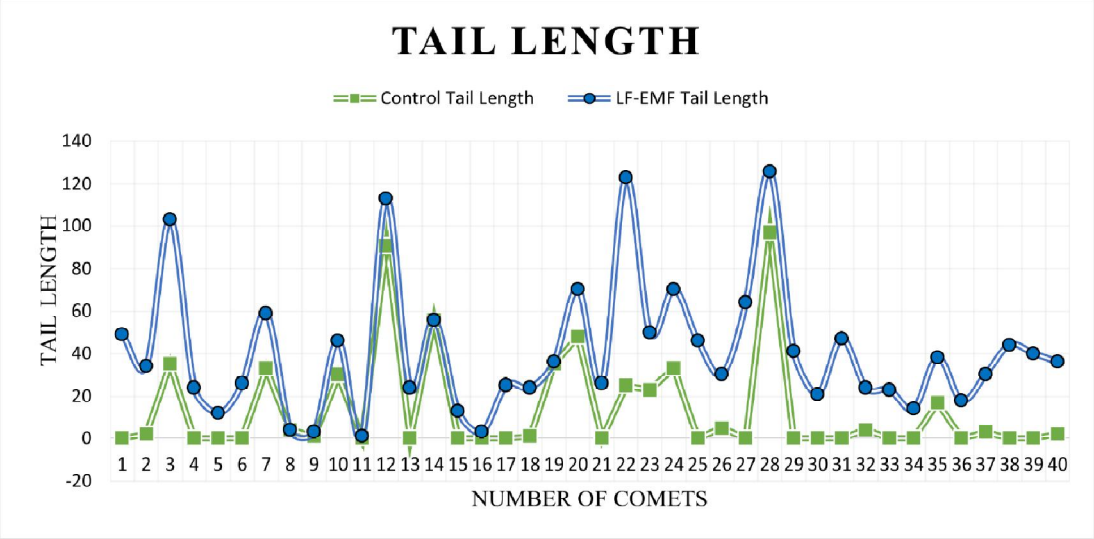

Figure 1: Tail DNA Length in Zebrafish Exposed to LF-EMF (40 comets). The analysis of tail DNA length in zebrafish exposed to LF-EMF and control groups. LF-EMF-exposed zebrafish exhibited a significantly longer tail DNA length (27.27 ± 3.12) compared to the control group (13.62 ± 3.81) ($p < 0.0101$).

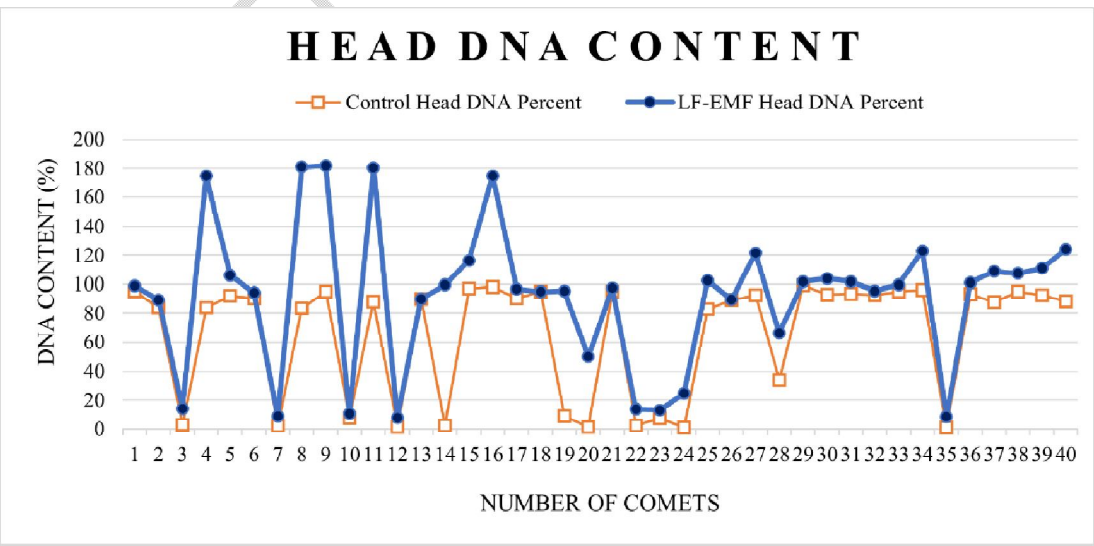

Figure 2: Head DNA Content in Zebrafish Exposed to LF-EMF (40 comets). Comparison of head DNA content in zebrafish exposed to LF-EMF and control groups. The control group showed higher head DNA content (66.003 ± 3.36) compared to the LF-EMF exposed group (25.96 ± 5.001).

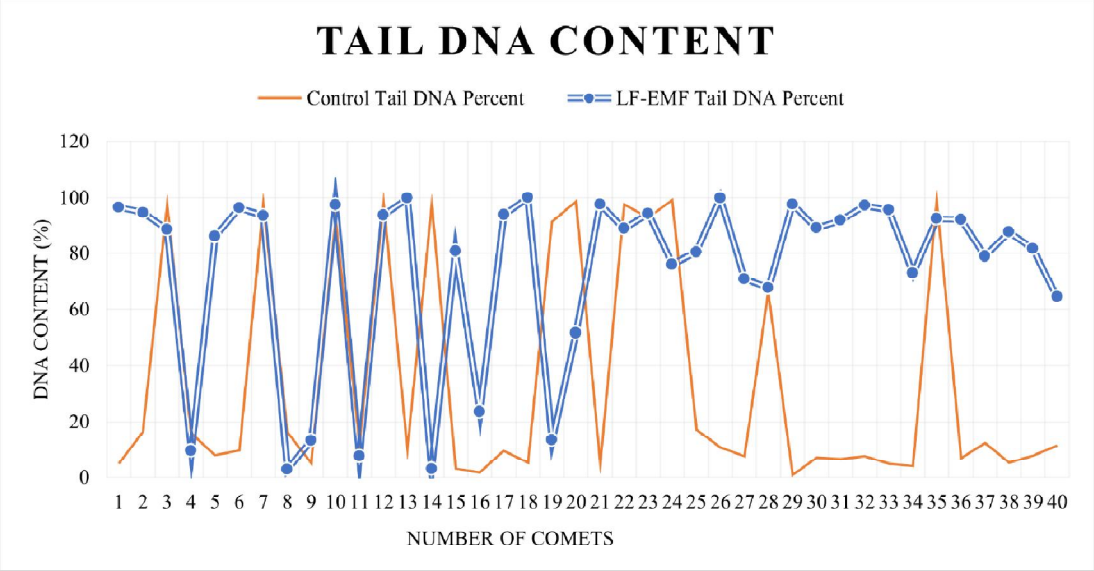

Figure 3: Tail DNA Content in Zebrafish Exposed to LF-EMF(40 comets). Examination of tail DNA content in zebrafish exposed to LF-EMF and control groups. The LF-EMF exposed group exhibited significantly higher tail DNA content (74.035 ± 5.001) compared to the control group (33.99 ± 3.36) (p = 0.05).

3.2. DISCUSSION

The investigation into the impacts of EMFs on DNA and the potential health hazards linked to EMF exposure has garnered considerable attention within the scientific community. EMFs are widespread in contemporary environments, originating from power lines, electronic devices, and wireless networks. To comprehend the possible genotoxic effects of EMFs, the comet assay is employed, which measures DNA damage by identifying anomalies like single- and double-strand breaks in DNA structures. This method is essential for evaluating the contribution of EMFs to genetic instability and associated health concerns. The comet assay provides crucial parameters, including the average percentage of tail DNA, which indicates the extent of DNA damage in cell populations, and the Olive tail moment, which represents the migration extent and pattern of DNA damage in individual cells. These metrics are fundamental in assessing genotoxic effects and understanding DNA repair mechanisms [21-22].

A notable DNA strand was discovered that breaks in rat lymphocytes exposed to both FeCl2 and magnetic fields (MF), as shown by comet assay analysis [23]. This discovery highlights the potential for certain chemical and MF interactions to cause significant DNA damage. Exposure to 1800 and 2100 MHz low-intensity microwave radiation from cell phones

significantly increased tail intensity, indicating oxidative stress and DNA damage [24]. These findings emphasize the harmful effects of microwave radiation on genetic integrity. Additionallyincreased DNA damage in chick embryo brains after exposure to 2G and 3G cell phone radiation was reported [25]. Comet assay analysis showed significant increases in various indices, such as mean comet length, tail length, percentage of DNA in the tail, and tail moment. These results highlight the increased susceptibility of developing brain tissue to radiofrequency electromagnetic fields (RF-EMF).

A significant DNA damage was observed in earthworms (*Eisenia fetida*) following exposure to 900 MHz radio frequency microwave radiation [26-27]. This observation indicates that EMFs can affect organisms at various ecological levels, suggesting broader ecosystem implications. A study investigated the effects of EMFs on *Allium cepa* bulbs, a model used to evaluate ecotoxicity and genotoxicity [28]. The study revealed increased DNA damage in root meristems due to EMF exposure, as shown by changes in comet assay indices like the percentage of head DNA and tail DNA, and olive tail moment. Additionally, the degree of damage was positively correlated with the frequency of EMF exposure, indicating potential cytotoxic and genotoxic effects on plant tissues. These studies collectively highlight the extensive and varied impacts of EMFs on living organisms across different ecosystems.

The low-frequency EMFs (LF-EMFs) caused significant genotoxic and cytotoxic effects in aquatic species like Rainbow trout (*Oncorhynchus mykiss*), common ragworm (*Hedistediversicolor*), and the Baltic clam (*Limecolabalthica*), indicating potential risks to aquatic ecosystems from EMFs [15]. The impact of EMFs on DNA is further highlighted by studies involving zebrafish. Anincreasein the percentage of DNA in the comet tailwas observed, particularly in zebrafish larvae exposed to higher doses of gamma radiation, suggesting a dose-dependent relationship between radiation exposure and DNA damage in zebrafish [29]. Similarly, RF-EMF exposure in zebrafish embryos led to increased oxidative stress and activation of apoptotic and autophagic processes, indicating cellular stress and damage due to EMFs exposure [30]. Similar to these, our study showed that LF-EMF exposure significantly increased tail length and DNA content in the tail region, suggesting DNA damage. These findings collectively enhance the understanding of the adverse effects of EMFs on DNA integrity in zebrafish.

Exposure to ELF-EMF caused DNA damage in various cell lines [31], while it was found that magnetic field (MF) exposure in the human B lymphoblastoid (TK6) cell line reduced cell sensitivity to mutagens, as evidenced by decreased tail intensity [32]. Further research into human exposure has shed more light on potential risks. For instance, a prolonged ELF-EMF exposure among thermal power plant workers and found a significant increase in comet assay indices, such as tail DNA percent and tail factors, indicating increased DNA damage in the exposed group [33]. Similarly, high-frequency mobile phone-specific EMFs at 1950 MHz significantly increased DNA damage in buccal cells, providing further insight into the potential hazards of mobile phone use [34].

However, not all research indicates that EMFs present significant risks to DNA. Some studies found no statistically significant variances in DNA damage when exposing human diploid fibroblasts and hamster cells to LF-EMFs [35-36]. Similarly, no significant effects in Jurkat cells were found when exposed to LF-EMFs [37], and no DNA damage was observed in human blood cells exposed to LF-EMFs in vitro [38]. These results suggest that certain types of EMFs may not cause DNA damage under specific circumstances. High peak-power pulsed electromagnetic fields (HPPP-EMFs) induced DNA damage in frog erythrocytes (*Xenopus laevis*) only when accompanied by elevated temperatures, indicating the potential influence of other factors in EMF-induced DNA damage [39]. These studies underscore the complexity of the relationship between EMFs and DNA damage, emphasizing the importance of considering various factors when evaluating their potential effects.

The intricate and sometimes conflicting results emphasize the complex connection between EMFs and DNA damage. While some studies indicate possible risks, especially linked to extended EMF exposure or high-frequency RF/MW EMFs, others do not corroborate these

findings. This diversity in research outcomes emphasizes the necessity for a thorough examination of the factors affecting EMF-induced DNA damage and underscores the significance of considering different variables such as exposure length, EMF frequency, and experimental setups.

This study addressed a significant gap in current knowledge by investigating the impact of low-frequency LF-EMFs on zebrafish DNA. Before this research, there had been limited exploration into the effects of LF-EMFs on zebrafish DNA. Our findings demonstrate that LF-EMFs can induce DNA damage in zebrafish, with the comet assay proving to be a valuable tool for detecting and analyzing such damage. This study provides deeper insights into the potential adverse effects of LF-EMF exposure on genetic integrity in zebrafish, contributing to the understanding of the broader implications of EMFs exposure in aquatic organisms.

4. CONCLUSION

In conclusion, this study has offered valuable insights into the potential genotoxic effects of low-frequency LF-EMFs on the integrity of zebrafish DNA. Through the utilization of the comet assay, a robust tool renowned for its precision in assessing DNA damage, we observed a notable increase in both tail length and DNA content within the comet tail region of zebrafish specimens following LF-EMF exposure, contrasting with unexposed controls. These findings vividly illustrate the capacity of LF-EMFs to induce significant genetic damage in zebrafish, thereby underscoring concerns surrounding the potential risks associated with EMF exposure.

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