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

ABSTRACT

Increased exposure to low-frequency electromagnetic fields (LF-EMFs; 3 Hz–300 kHz) from sources like power lines, appliances, and industrial equipment has raised concerns about their potential biological effects. While high-frequency EMFs have been extensively studied, the impact of LF-EMFs, particularly on DNA integrity, remains poorly understood. Given the role of DNA damage in genomic instability and disease, this study investigates the genotoxic effects of LF-EMFs on Zebrafish DNA using the Comet Assay, a reliable tool for assessing genetic damage.

Our findings reveal a significant increase in tail length (27.27 ± 3.12 µm in exposed fish, 13.62 ± 3.81 µm in controls, $p < 0.01$), tail DNA content (74.035 ± 5.001% in exposed fish, 33.99 ± 3.36% in controls), and a corresponding decrease in head DNA content (25.96 ± 5.001% in exposed fish, 66.003 ± 3.36% in controls), indicating elevated DNA fragmentation. The increased DNA damage may be attributed to LF-EMF-induced oxidative stress, which leads to excessive reactive oxygen species (ROS) production. ROS can attack DNA molecules, causing strand breaks and base modifications. Additionally, LF-EMFs may impair DNA repair mechanisms, including base excision repair and non-homologous end joining, reducing the cell's ability to fix damaged DNA. Furthermore, alterations in ion channel activity and intracellular calcium homeostasis caused by LF-EMF exposure may contribute to oxidative stress, further compromising genomic stability.

These results highlight the genotoxic potential of LF-EMFs, emphasizing the need for further research to assess their risks and biological consequences.

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

1. INTRODUCTION

Electromagnetic fields (EMFs) have become an integral part of modern life due to the widespread use of electrical devices, many of which contribute to the artificial overlay of EMFs onto the Earth's natural magnetic field. Extremely low-frequency EMFs (ELF-EMFs), operating within the 50–60 Hz range, are classified as non-ionizing radiation and do not have enough energy to disrupt molecular bonds or induce significant thermal effects in biological tissues. However, ELF-EMFs interact with cells by generating weak electrical currents, potentially influencing biological processes. The implications of these interactions for human health remain an area of active research, as the long-term effects of ELF-EMF exposure are not yet fully understood.

Occupational exposure to EMFs presents both direct and indirect risks. Direct effects include the induction of electric currents in biological tissues, while indirect effects primarily involve interference with medical devices such as pacemakers and defibrillators. The potential for

long-term adverse outcomes, including carcinogenesis, remains inconclusive due to insufficient epidemiological and mechanistic evidence [1]. Given these uncertainties, systematic health monitoring of EMF-exposed workers is essential. Surveillance programs aim to detect acute physiological effects and device malfunctions, particularly in individuals with implanted medical devices. While established exposure limits reduce immediate risks, the absence of diagnostic tools to assess long-term EMF-related health effects underscores the need for continued research [2].

Recent studies have explored the potential therapeutic applications of ELF-EMFs, particularly in oncology. Research suggests that ELF-EMFs may modulate apoptosis, a key cellular process involved in tissue homeostasis and cancer therapy [3]. Since impaired apoptosis is a hallmark of cancer, restoring or enhancing apoptotic pathways is a major objective in anticancer treatments. ELF-EMFs alone appear to have minimal effects on apoptosis, but when combined with external stressors—such as chemotherapeutic agents or ionizing radiation—both enhancement and inhibition of apoptosis have been observed. These variations suggest a complex interaction between ELF-EMFs, cellular stress responses, and apoptotic regulation, necessitating further investigation to clarify the underlying molecular mechanisms [3].

In addition to apoptosis, ELF-EMFs have been found to influence other cellular functions, including proliferation and differentiation. The effects of ELF-EMFs on cell growth appear to be highly dependent on exposure parameters and cell type. Some studies report that ELF-EMFs inhibit cell proliferation, potentially due to oxidative stress and an increase in intracellular reactive oxygen species (ROS). In contrast, other findings suggest that ELF-EMFs may enhance proliferation, contributing to tissue regeneration, bone formation, and wound healing. ELF-EMFs also appear to modulate membrane stability and metabolic activity, which has implications for potential therapeutic applications such as weight regulation and disease management [4]. These diverse biological effects highlight the complexity of ELF-EMF interactions and emphasize the need for further research to determine exposure conditions that distinguish between beneficial and harmful outcomes.

DNA, while structurally stable, remains susceptible to damage from environmental stressors, including electromagnetic radiation. Although ELF-EMFs lack ionizing properties, their potential to induce genomic instability has drawn increasing scientific attention. The Comet Assay, a widely used technique in genetic toxicology, serves as a sensitive method for detecting DNA strand breaks and oxidative damage. Originally developed by Ostling and Johanson (1984), the assay was later refined under alkaline conditions to improve the detection of DNA lesions. This advancement has enabled precise evaluation of the genotoxic effects of ELF-EMFs, providing insight into the extent and nature of DNA damage [5-12].

The Comet Assay has been applied across multiple cell culture and animal models to assess genotoxicity and cytotoxicity. Beyond DNA damage detection, it has been used to evaluate cell viability, proliferation, apoptosis, and micronucleus formation [13]. Modifications of the assay have also enabled the detection of oxidized DNA bases, allowing differentiation between direct chemical-induced damage and oxidative stress-mediated lesions [14]. Recent research utilizing this technique has focused on ELF-EMF-induced DNA damage, raising concerns about the potential biological consequences of long-term exposure [15-17].

Despite these advancements, limited studies have examined the effects of ELF-EMFs on DNA integrity in *Danio rerio* (Zebrafish), an established model organism in environmental and molecular biology. While research has been conducted on ELF-EMF genotoxicity in species such as earthworms [18], rainbow trout [19], and rodents [20], studies involving Zebrafish remain scarce. Given their genetic similarity to higher vertebrates, transparent embryonic development, and well-characterized molecular pathways, Zebrafish provide a valuable model for investigating the biological impact of ELF-EMF exposure.

This study aims to address this gap by systematically evaluating ELF-EMF-induced DNA damage in Zebrafish using the Comet Assay. This approach will contribute to a more

comprehensive understanding of ELF-EMF effects on genomic stability, cellular stress responses, and potential health risks. Expanding knowledge in this area is critical for assessing the broader implications of electromagnetic exposure and its impact on biological systems.

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 [21-23]. 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 using an approved protocol [24], and their brains were carefully dissected to isolate the brain tissue. The Comet Assay was performed on single-cell suspensions extracted from the dissected tissue [5]. The cells were embedded in agarose gel and subjected to an alkaline electrophoresis procedure to allow DNA strand breaks to manifest as tail migration. To enhance DNA visualization, SYBR Green dye was used, which specifically binds to DNA and fluoresces under UV light, making the fragmented DNA more easily detectable under a fluorescence microscope.

The cells were then subjected to electrophoresis in an alkaline buffer, which denatures the DNA and allows for the detection of both single-strand breaks and alkali-labile sites. When an electric field was applied, fragmented DNA migrated from the nucleus, forming a comet-like shape, where the length and intensity of the tail indicated the extent of DNA damage.

Fluorescence microscopy was employed to capture images of the comet-like structures. The DNA damage was quantified by measuring two primary parameters: the tail length (distance between the head and the tail of the comet) and the percentage of DNA in the comet head and tail. These measurements were carried out using ImageJ OpenComet software, which allowed for precise and automated analysis of the comet images.

To ensure reliability, 40 comets from each sample were analyzed, providing a representative sample for each treatment group. Statistical analysis was performed using the student t-test to determine if significant differences in DNA damage existed between the control and experimental groups.

3. RESULTS AND DISCUSSION

3.1. RESULT

The analysis comparing tail DNA length between Zebrafish in the control and LF-EMF exposed groups revealed a significant difference. Zebrafish exposed to LF-EMF exhibited a markedly longer tail DNA length (27.27 ± 3.12 µm) compared to the control group (13.62 ± 3.81 µm) (p< 0.01) (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. 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, the LF-EMF exposed group showed significantly lower head DNA content (25.96 ± 5.001%) compared to the control group (66.003 ± 3.36%) (p< 0.01) (Figure 2). Conversely, the LF-EMF exposed group displayed significantly higher tail DNA content (74.035 ± 5.001%) compared to the control group (33.99 ± 3.36%) (p = 0.05) (Figure 3). These findings suggest 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 adversely affects the DNA integrity of Zebrafish.

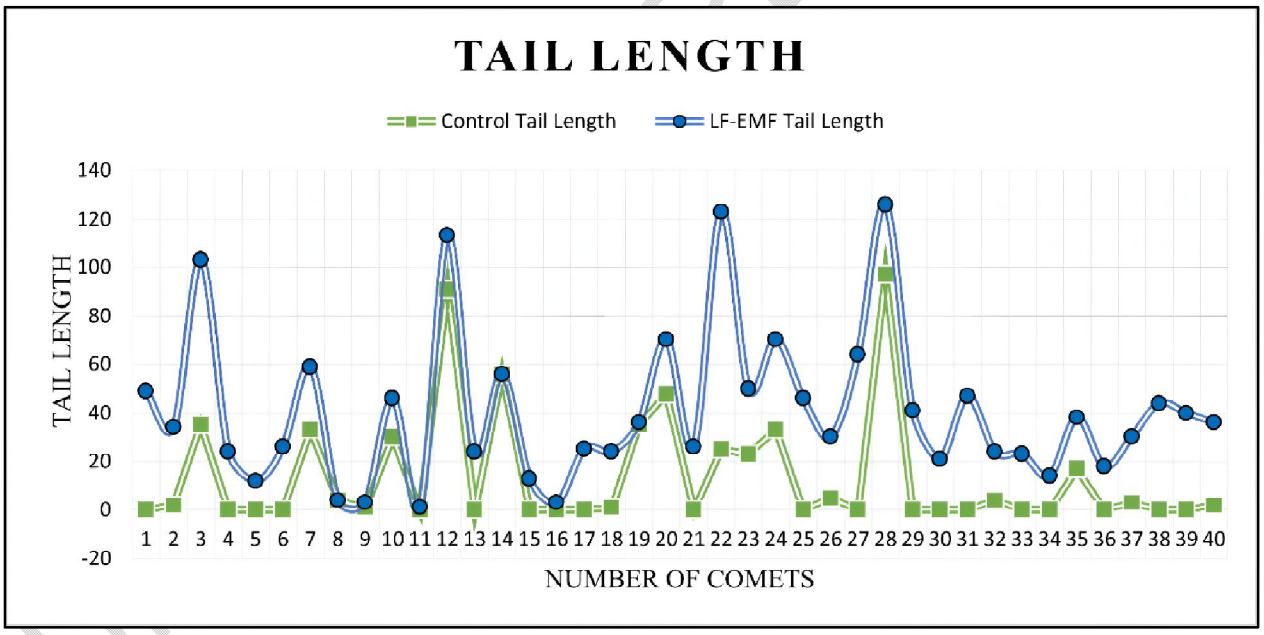

Figure 1: *Tail DNA Length in Zebrafish Exposed to LF-EMF (*Comparison of tail DNA length between Zebrafish exposed to LF-EMF and control groups. The exposed group showed a significantly longer tail DNA length (27.27 ± 3.12 µm) compared to the control group (13.62 ± 3.81 µm) (p< 0.01), indicating DNA fragmentation due to LF-EMF exposure)

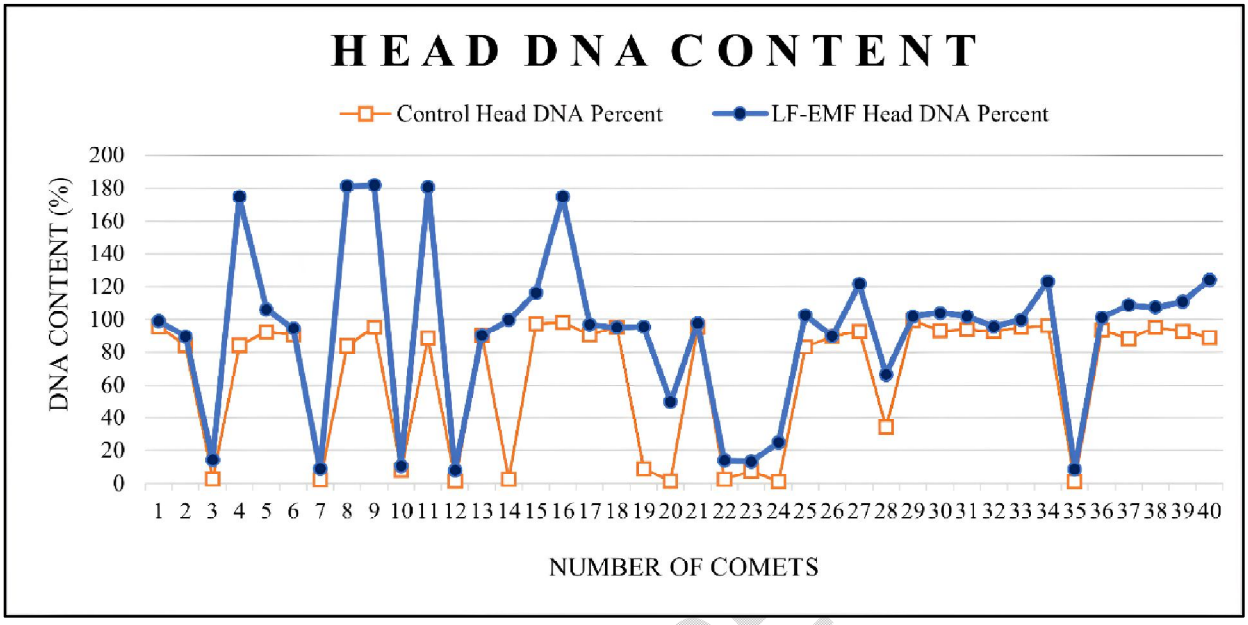

Figure 2: *Tail DNA Content in Zebrafish Exposed to LF-EMF*(Comparison of tail DNA content between Zebrafish exposed to LF-EMF and control groups. Exposed fish exhibited significantly higher tail DNA content (74.035 ± 5.001%) compared to the control group (33.99 ± 3.36%) (*p*< 0.01), suggesting elevated DNA fragmentation in the exposed group)

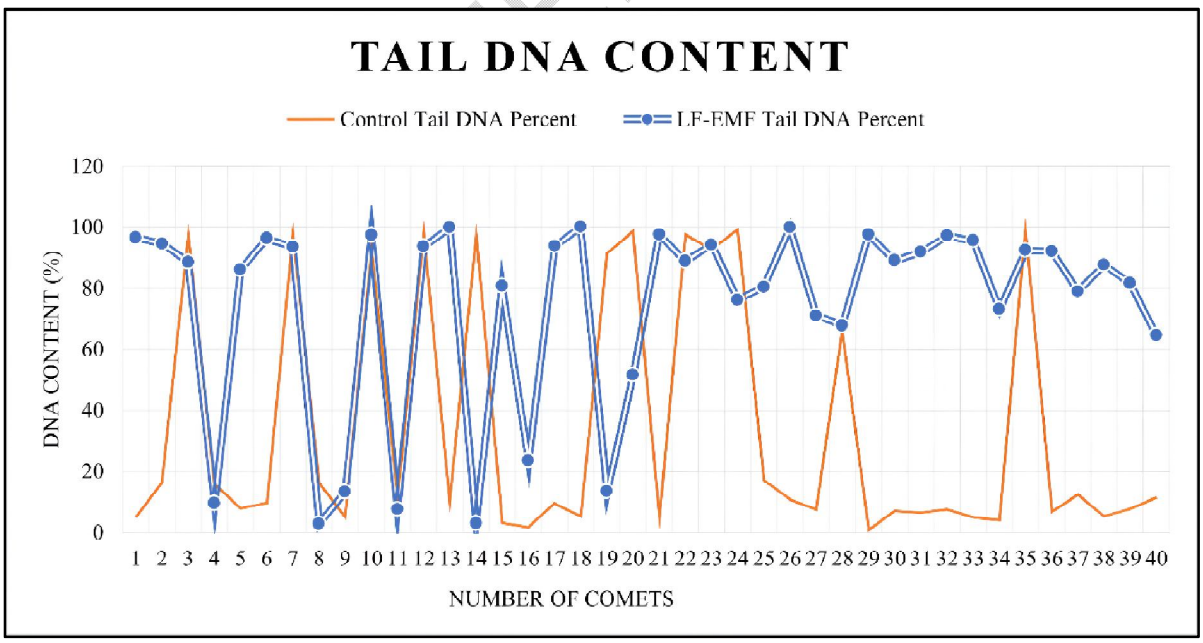

Figure 3: *Head DNA Content in Zebrafish Exposed to LF-EMF*(Comparison of head DNA content between Zebrafish exposed to LF-EMF and control groups. The control group exhibited significantly higher head DNA content (66.003 ± 3.36%) compared to the exposed group (25.96 ± 5.001%) (*p*< 0.01), indicating that LF-EMF exposure

resulted in reduced head DNA content and increased DNA fragmentation in exposed fish)

3.2. DISCUSSION

The investigation into the impacts of electromagnetic fields (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 [25-26].

A notable DNA strand break was discovered in rat lymphocytes exposed to both $FeCl_2$ and magnetic fields (MF), as shown by comet assay analysis [27]. 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 [28]. These findings emphasize the harmful effects of microwave radiation on genetic integrity. Additionally, increased DNA damage in chick embryo brains after exposure to 2G and 3G cell phone radiation was reported [29]. 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).

The brain is frequently used in Comet Assay studies due to its high sensitivity to oxidative stress, limited DNA repair capacity, and vulnerability to environmental exposures like electromagnetic fields (EMFs) [30]. As a highly oxygen-dependent organ, the brain is particularly susceptible to reactive oxygen species (ROS)-induced DNA damage, which the Comet Assay can effectively detect [30-31]. Moreover, EMF exposure has been shown to alter blood-brain barrier integrity, further increasing neuronal susceptibility to DNA fragmentation [32]. Since neuronal DNA damage is closely associated with neurodegenerative diseases and cognitive decline, Comet Assay studies on brain tissue provide valuable insights into the biological effects of ELF-EMFs on neural integrity, apoptosis, and stress responses.

A significant DNA damage was observed in earthworms (*Eisenia fetida*) following exposure to 900 MHz radio frequency microwave radiation [33-34]. 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 [35]. 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 (*Limecola balthica*), indicating potential risks to aquatic ecosystems from EMFs [19]. The impact of EMFs on DNA is further highlighted by

studies involving Zebrafish. An increase in the percentage of DNA in the comet tail was 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 [36]. 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 [37]. 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.

The increasing exposure to electromagnetic fields (EMFs) from electronic devices raises concerns about their effects on biological systems. EMFs contribute to oxidative stress, impacting reproductive health, cardiovascular function, brain activity, behavior, and DNA integrity. Studies have shown that EMFs can cause genetic damage, which is particularly highlighted by comet assay findings. Exposure to EMFs also affects behavior, leading to altered activity levels, stress responses, and cognitive functions in various species. Beyond human health, EMFs pose a potential threat to aquatic ecosystems, disrupting migratory patterns, reproduction, and biodiversity. In species such as Zebrafish, LF-EMFs have been shown to induce oxidative stress, DNA damage, apoptosis, and behavioral changes, significantly affecting survival and reproduction [38].

LF-EMFs may induce DNA damage and apoptosis in Zebrafish through a mitochondrial-dependent pathway, where oxidative stress plays a central role. Exposure to LF-EMFs is known to increase reactive oxygen species (ROS) levels, leading to mitochondrial dysfunction. The mitochondria, which are the primary sites of ROS generation, are particularly vulnerable to damage under oxidative stress. This damage can compromise the mitochondrial membrane potential, releasing pro-apoptotic factors like cytochrome c, triggering apoptosis. Additionally, ROS-induced DNA damage may exceed the repair capacity of the cell, further contributing to cellular dysfunction and cell death. The inhibition of Protein Kinase C alpha (PKCα) has been shown to enhance mitochondrial dysfunction and apoptosis in various cell lines, suggesting that LF-EMF exposure may similarly affect cellular processes through PKCα modulation, ultimately leading to mitochondrial damage and genetic instability [39].

Exposure to ELF-EMF caused DNA damage in various cell lines [40], 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 [41]. Further research into human exposure has shed more light on potential risks. 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 [42]. 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 [43].

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 [44-45]. Similarly, no significant effects in Jurkat cells were found when exposed to LF-EMFs [46], and no DNA damage was observed in human blood cells exposed to LF-EMFs in vitro [47]. 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 [48]. 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 EMF exposure in aquatic organisms.

4. CONCLUSION

In conclusion, this study provides compelling evidence of the genotoxic effects of low-frequency electromagnetic fields (LF-EMFs) on Zebrafish DNA integrity. Using the comet assay, we observed a significant increase in both tail DNA length and DNA content in the comet tail region in LF-EMF exposed Zebrafish compared to unexposed controls, indicating substantial DNA fragmentation and genetic damage. These key findings suggest that LF-EMF exposure can disrupt the structural integrity of DNA, highlighting the potential for such fields to induce genetic mutations and cellular damage. The implications of these results are considerable, as DNA damage can lead to a range of adverse biological effects, including compromised cellular functions, potential developmental defects, and increased risk of diseases such as cancer. Given the ubiquity of LF-EMFs in modern life—emanating from sources such as mobile phones, household electronics, and industrial machinery—there is growing concern about the cumulative effects of exposure, particularly in sensitive populations. Future research should aim to explore the underlying molecular mechanisms driving the DNA damage induced by LF-EMFs and investigate the long-term consequences of chronic exposure. Additionally, studies on the potential generational effects of LF-EMF exposure would be critical in assessing the broader environmental and health risks. Expanding research to include other animal models and human cell lines will provide a more comprehensive understanding of the genotoxicity of LF-EMFs across species, paving the way for developing protective measures or regulatory guidelines to mitigate the risks associated with prolonged exposure.

Ethical Approval:

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.

Disclaimer (Artificial Intelligence)

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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