Chapter-4

Effects of Extremely Low-frequency (50 Hz) Electromagnetic Fields on Vital Organs of Adult Wistar Rats and Viability of Mouse Fibroblast Cells

4.1 Introduction

The existence of extremely low frequency pulsed electromagnetic field (ELF-PEMF) has increased multifold due to the industrialization and increasing use of electronic appliances (Aerts et al., 2017; Gajšek et al., 2016; Hansson Mild et al., 2023; Swanson and Kaune, 1999). Several studies have been conducted to establish a link between the increase of electronic appliances in occupational and household environments and associated biological impact (Ahlbom et al., 2000; European Commission. Directorate General for Health and Consumers., 2015; Feychting et al., 2005; Feychting and Ahlbom, 1994; Feychting and Alhbom, 1993). ELF-EMF exposure is reported to induce neurological disorders, cardiovascular diseases, and, in some cases, cancer as well (Ebrahim et al., 2016; Hu et al., 2016; Repacholi, 2012; Veiga et al., 2005; Zhou et al., 2016). The electromagnetic fields generated by mobile phones and televisions are significantly less but receive more attention than other frequencies. The primary motivation behind ELF-EMF exposure assessment is to observe the significance of biological impact. Previous scientific evidence indicated that EMFs (> 0.4μT) might raise the risk of leukemia (Ahlbom et al., 2000; Feychting and Ahlbom, 1994; Kheifets et al., 2011), but some scientific evidence also suggested no impact on lipid peroxidation, sperm count, blood hematochemistry, liver, kidney, and hematological system of rats (Akdag et al., 2006; Kulkarni and Gandhare, 2015; Lai et al., 2016b, 2016a; Margonato et al., 1995; Zhang et al., 2020). Based on the literature, biological effects are prominently dependent on ELF-MF parameters (flux density, frequency, and exposure duration); hence, there is no general agreement about the ELF-EMF effects on biological systems.

In our previous study (Tekam et al., 2023), we investigated 50 Hz ELF-PEMF (1-3 mT, 20 min (twice) with 4 h gap) on the C6 (Glial) cells and behavioral parameters (i.e., spatial memory, anxiety, and motor functions) of Wistar rats under *in vitro* and *in vivo* conditions, respectively. Interestingly, cortical cell counts and hippocampus slices of brain tissues did not show any

significant alterations. This study aims to obtain a thorough understanding of the possible health consequences of 50 Hz ELF-PEMF (1-3 mT) with duration (20 min (twice) with 4 h gap) on cell proliferation and morphology of mouse fibroblast (RFP-L929), biochemical and histopathological parameters of vital organs of the adult Wistar rats under *in vitro* and *in vivo* conditions, respectively. The findings may contribute to developing guidelines for the safe use of devices operating within the 50 Hz ELF-PEMF range, helping to establish exposure limits that mitigate any potential risks associated with this type of electromagnetic field.

4.2 Materials and methods

4.2.1 Test kits

We received the aspartate aminotransferases (AST) (Cat. No. 126019910920) and alanine aminotransferases (ALT) (Cat. No. 126019910023)-modified IFCC kits from TARA Clinical Systems, India. We obtained bilirubin assay kits (Cat. No. 108119910920), creatinine (Cat. No. 117599910920), and creatine phosphokinase-MB (Ck-MB) (Cat. No. 116419910921) assay kits from Coral Clinical Systems, India.

4.2.2 Reagents and materials

The National Center for Cell Sciences (NCCS), Pune provided the RFP-L929 cell lines. We received fetal bovine serum (FBS), ethylenediaminetetraacetic acid (EDTA), Dulbecco's modified Eagles medium (DMEM) high glucose, phosphate-buffered saline (PBS), paraformaldehyde, trypan blue, trypsin, glycerol, penicillin and streptomycin solution (100X) from HiMedia, India. We also utilized distilled water and absolute ethanol (99.9%) under *in vitro* studies.

4.2.3 Animals

We received the adult male Wistar rats (8 weeks old, 150-180 gm) from the Central Drug Research Institute (CDRI), Lucknow, India. The protocols for handling and caring for

laboratory animals comply with the criteria set forth by the Institute Animal Ethical Committee (IAEC.) Indian Institute of Technology (Banaras Hindu University) Varanasi, India (IIT(BHU)/IAEC/2022/073). We also followed the U.K. Animals (Scientific Procedures) Act, 1986 and associated guidelines and compliance with ARRIVE guidelines. We randomly divide the rats into four groups with sample size ($n = 6$ rats/group) determined as per the result of G*power analysis for statistical significance ($p < 0.05$). In the experiment, the standard high-quality propylene cages were used for housing the rats in a controlled environment with a 25±2˚C temperature and relative humidity of 45-55%. The rats were acclimatized in the light-dark cycle (12-hour light and 12-hour dark period) as per the previous guidelines followed for performing animal experiments (Prajapati and Krishnamurthy, 2021).

4.2.4 50 Hz ELF-PEMF exposure system

The monoaxial coil configuration, magnetic field direction, current characteristics, and experimental setup are mentioned in our previous study (Tekam et al., 2023). Table 4.1 includes the design specifications for the magnetic exposure system. Figures 4.1 (A-F) depicts the structural details of Helmholtz coils, schemas of the magnetic field exposure system (*in vivo* and *in vitro*), and photographs of the actual exposure chamber. We also maintained thermal equilibrium and field homogeneity (field fluctuations < ± 5%) throughout the exposure duration.

The wooden frame and foundation help to reduce eddy current losses and keep the total weight of the device as minimal as possible. We have also used 0.5 cm thick acrylic sheets for the exposure chamber with a temperature sensor and controller (tAPMAN 48 7E-1) to maintain 37±2 °C during *in vitro* studies. The relative humidity inside the exposure chamber was kept constant by the natural water evaporation in a petri dish. We did not install the CO_2 supply unit within the exposure chamber. The primary function of CO_2 supply is to maintain a stable

physiological pH in cell culture (Ashdown et al., 2020), and as far as CO_2 supply during exposure is concerned, the exposure duration/sample inside the exposure chamber is less than 30 min which is acceptable according to literature (Dubey et al., 2021).

Table 4.1. Design specification of 50 Hz ELF-PEMF exposure system.

Exposure coil	
Coil type	Monoaxial Helmholtz circular coils
Coil outer diameter	
Circular coil-1	0.530 m
Circular coil-2	0.530 m
Coil inner diameter	
Circular coil-1	0.490 m
Circular coil-2	0.490 m
Wire length	
Circular coil-1	898.668
Circular coil-2	898.668
Number of turns	540 (each)
Maximum current	
Circular coil-1	3 A
Circular coil-2	3 A
Maximum magnetic field	3.06 mT
Total resistance	23.3 Ω/coil
Minimum current	
Circular coil-1	1 A
Circular coil-2	1 A
Minimum magnetic field	1.08 mT
frequency	50 Hz
Exposure space	30 cm × 17 cm × 25 cm
Uniformity of field strength	< ± 5% deviation from center
Field direction and polarity	Horizontal, A.C. magnetic field

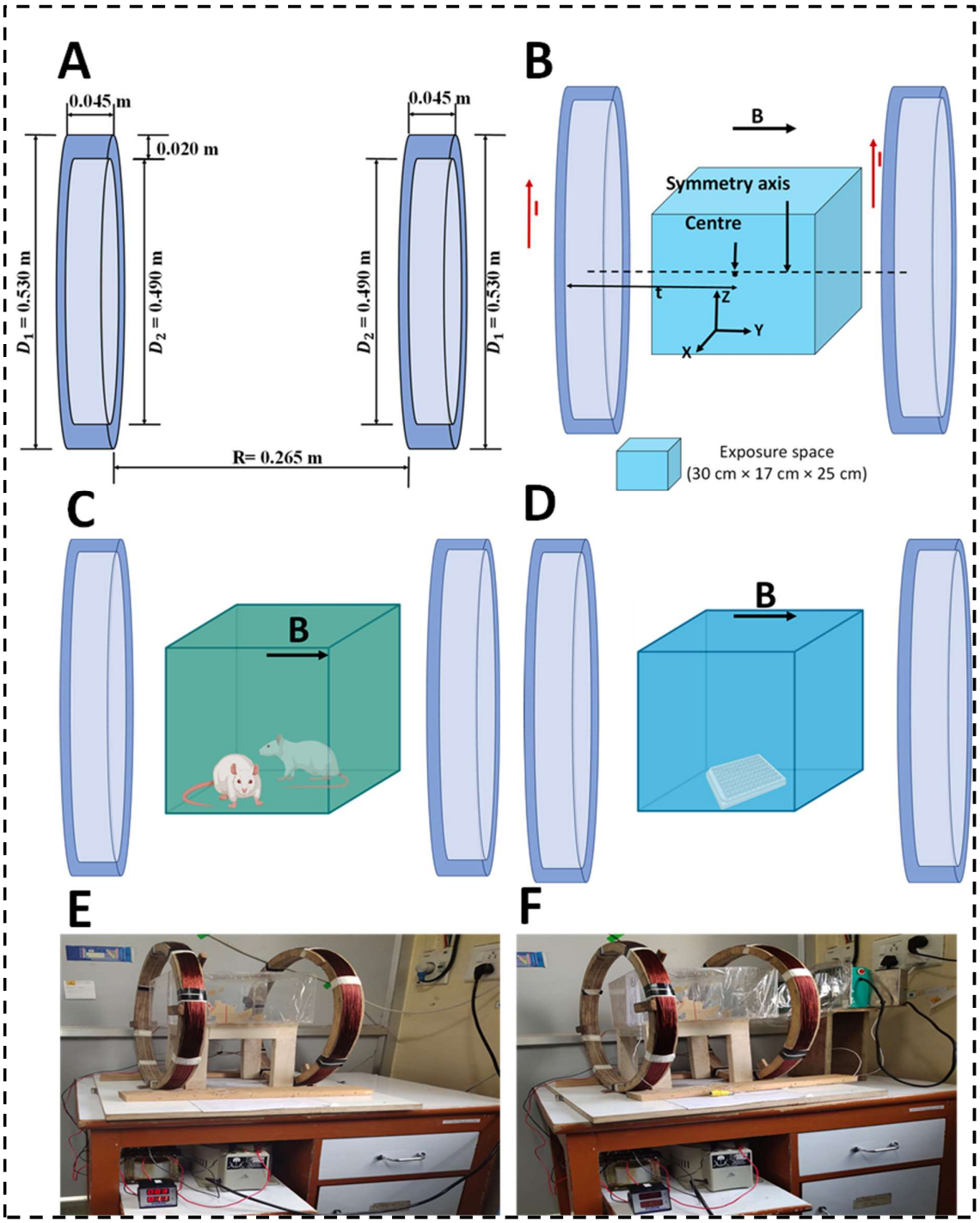

Figure 4.1. 50 Hz ELF-PEMF exposure system. (A) Schematic of horizontal magnetic field generating system, (B) Schematic for placement of exposure space (black arrow and red arrow indicate the magnetic field direction and current direction inside coils respectively), (C) Schematic representation of exposure system (*in vivo* model), (D) Schematic representation of PEMF exposure (*in vitro* model), (E) Prototype of horizontal magnetic field exposure system (*in vivo* model), (F) Prototype of horizontal magnetic field exposure system (*in vitro* model).

4.2.5 *In Vitro* analysis

4.2.5.1 RFP-L929 cell culture preparation

The red fluorescent protein (RFP-L929) is widely used for drug toxicity and phototoxicity assessment studies (Cannella et al., 2019; Ray et al., 2008). We used cell lines with a passage number not higher than 25 in all experiments. We have utilized a microscope (Nikon ECLIPSE Ti-U) to capture/examine the bright field images and fluorescence characteristic of genetically modified L929 cells—which glow red when stimulated at a green wavelength—to detect and track the growth of the cells following exposure to a magnetic field. Initially, RFP-L929 cells grown to sub-confluence were released by brief digestion with 0.5% trypsin/0.2% EDTA (trypsin/EDTA). We performed cell counting with the trypan blue method, and in this process, we used a mixture of trypan blue (25 µl), culture medium (15 µl), and cells containing medium (10 µl).

$$\text{Ratio} = \frac{\text{Total volume}}{\text{cell containing media}} \tag{4.1}$$

$$\text{Calculation formula} = \left(\frac{\text{No.of cells}}{4}\right) \times \text{dilution ratio} \times 10^4 \tag{4.2}$$

The cells were counted with a manual haemocytometer under an optical microscope and divided into groups for magnetic field exposure. After harvesting the cells from the culture dishes and rinsing with Ca^{2+} and Mg^{2+} free PBS, cell suspensions of RFP-L929 were prepared using DMEM and supplemented with FBS (10%) and antibiotics (1%). In culture plates, the cells were seeded at a density of 1×10^3 cells per well and kept in a 5% CO_2 incubator (Galaxy 170S, Eppendorf, Germany) for 24 hrs to ensure cell adhesion and stability.

The penetration depth of a magnetic field in L929 cells can be determined using a magnetic cell labelling method with unmodified colloidal magnetic nanoparticles (Nam, 2000). Additionally, fluorescent microscopy can detect changes in the stages of apoptosis in the

labelled cells. We could not perform the depth penetration studies due to COVID restrictions and the non-availability of experimental facilities. But, in the future, we will serve the studies to determine the penetration depth of 50 Hz magnetic field exposure.

4.2.5.2 Experimental protocol

Figure (4.2) depicts the experimental protocol for the *in vitro* studies. The cell cultures platform was divided into four groups: 1 mT, 2 mT, 3 mT, and control. The duration for magnetic field exposure is 20 min (twice) with a 4 h gap at a voltage range ($75 \text{ volts} \leq V \leq 190 \text{ volts}$) until the 5^{th} day or confluency (> 90%), whichever came first. The magnetic flux density used to observe the changes in cell proliferation and morphologies of RFP-L929 is sufficiently higher than in occupational and residential environments. The horizontal magnetic fields induce micro-currents in culture dishes to modify the electrochemical balance of the cell membrane and, consequently, whole cell function (Dj et al., 2000). All the experiments under identical conditions were repeated thrice.

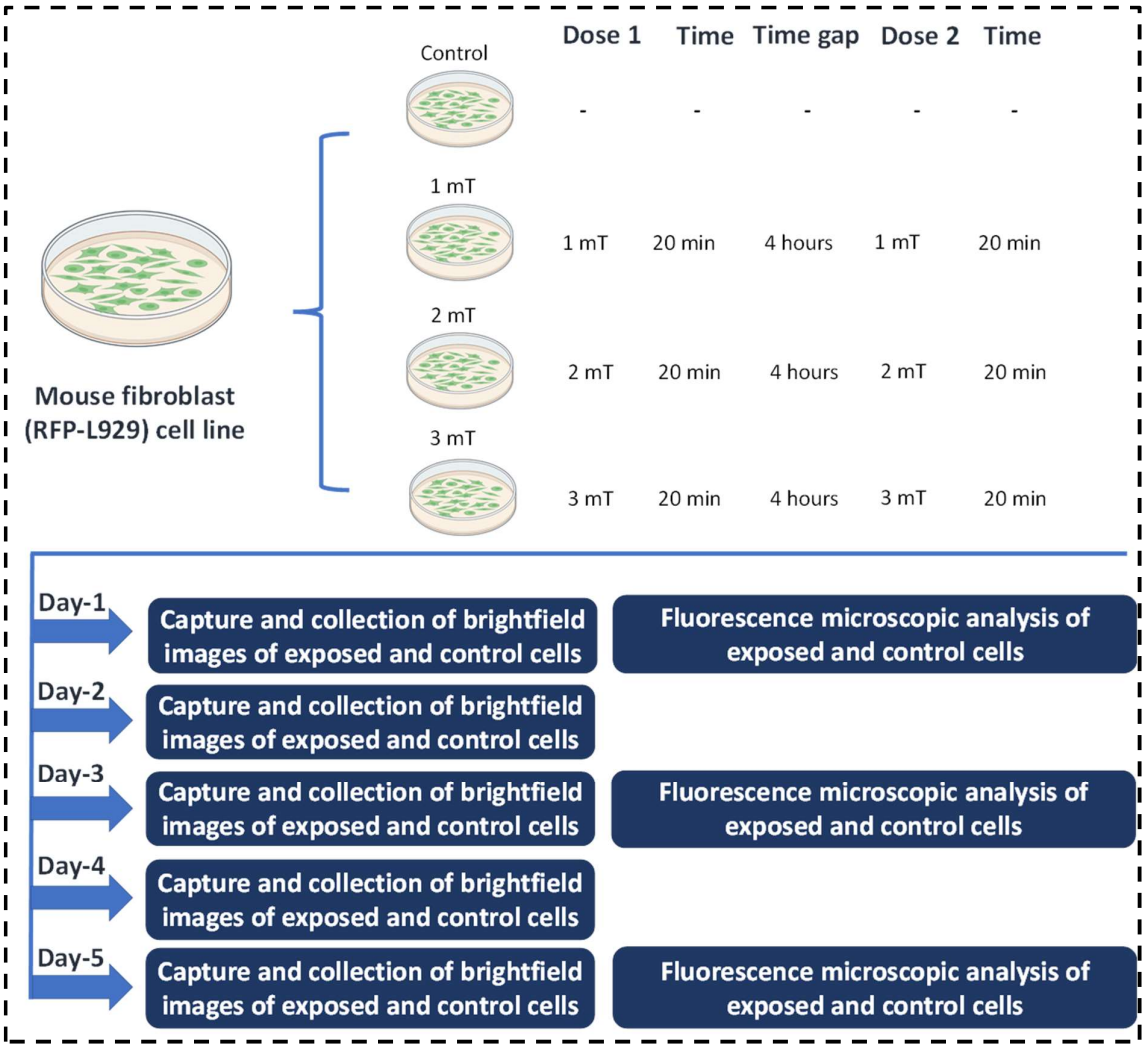

Figure 4.2. Schematic representation of repeated 5-day *in vitro* study. In this study, we exposed the cell culture to PEMF exposure (1-3 mT, 20 min (twice) with a 4 h gap) and observed any sign of a reduction in the proliferative activity of cells.

4.2.5.3 Cell proliferation and morphology analysis

The degree of cell viability served as a sign of healthy cells, and we utilized brightfield and fluorescent microscopic techniques to observe cell proliferation and morphologies throughout, as depicted in figure (4.2). The cell culture platform was exposed to 50 Hz ELF-PEMF (1-3 mT, 20 min (twice) with a 4 h gap) for 5 days or confluency (> 95%), whichever came first. We captured brightfield images under the microscope (Nikon ECLIPSE Ti-U) to observe any significant change in cell proliferation along with any signs of cell fragmentation and changes

in morphology from day 0 to day 5 of magnetic field exposure. We also captured fluorescent images of RFP-L929 cells under green light (λ= 495-570 nm) to detect any signs of cell fragmentation during magnetic field exposure. In contrast, the cell culture medium was changed every other day.

4.2.6 *In Vivo* analysis

4.2.6.1 Experimental protocol

We have performed the G*power analysis to determine the statistical significance of the acquired data. As per previous studies (Tekam et al., 2023), he effect size (0.30), α error probability (0.05), power (1-β err prob) (0.80), no. of groups (4), no. of measurements (5) and non-sphericity correction (€ = 1).The total sample size obtained was 24 ($n = 6$ rats/group) for statistical significance ($p < 0.05$) and to eliminate type I and II errors. The rats were then randomly divided into four groups: control,1 mT, 2 mT, and 3mT.

The analysis of variance's (E) degree of freedom was computed using the resource equation approach. If E (< 10), then the likelihood of significant results will grow with the number of animals, but if E (>20), the possibility of effective results will not increase with the number of animals (Charan and Kantharia, 2013; Festing and Altman, 2002).

$$\mathrm{E} = \text{Total number of animals} - \text{Total number of groups} \qquad (4.3)$$

In our study, we took 6 rats per group, and there were 4 groups. So, according to the formulae,

$$\mathrm{E} = (6 \times 4) - (4) \qquad (4.4)$$

and

$$\mathrm{E} = 20 \qquad (4.5)$$

According to the "resource equation method," $n = 6\, rats/groups$ can be considered an adequate sample size for our current investigation.

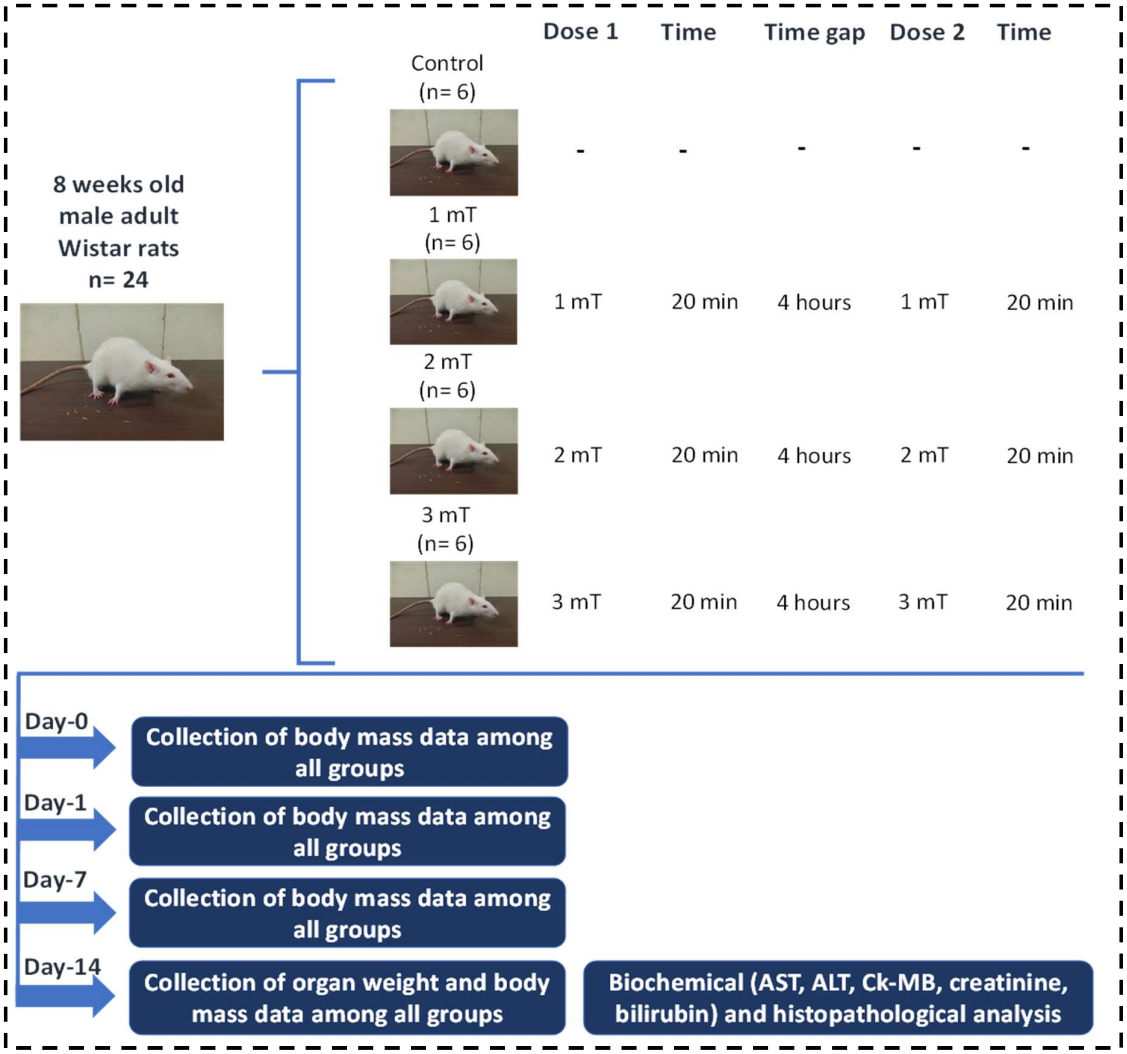

Figure 4.3. Schematic representation of repeated 14 days in vivo study. In this study, adult Wistar rats were exposed to 50 Hz ELF-PEMF exposure (1-3 mT, 20 min (twice) with a 4 h gap) and were observed for any sign of toxicity.

The experimental rats were then exposed to 50 Hz magnetic field (1-3 mT) for 20 min twice a day, with a 4 h gap, as illustrated in figure (4.3). We also maintained the 10-minute habituation interval before magnetic field exposure as mentioned in our previous study (Tekam et al., 2023). On day 14, the rats were decapitated by cervical dislocation, and tissue (liver, kidneys, and heart) and the blood was collected and stored in -80 °C for future analysis (Majumdar and Krishnamurthy, 2022; Prajapati et al., 2019).

4.2.6.2 Sample collection and data analysis

All rats were observed for general appearance of toxicity like changes in their fur texture, salivation, diarrhoea, convulsions, tremor, and body mass on days 0 (i.e., before starting the magnetic exposure), 7, and 14 of the experimental protocol. Besides, the rats were starved for overnight to reduce the impact of food intake on the clarity of serum and also to obtain baseline values before collecting blood and tissue samples (Prior et al., 2021). The blood was collected during the necropsy when the rats are under anaesthesia and stored in ethylenediamine tetraacetic Acid (EDTA)-coated blood collection tubes for biochemical analysis. The tissue samples (liver, kidneys, and heart) collected were weighted to measure the mass of organs of the low frequency-exposed rats and control group. We also followed Grubbs' suggestions for the statistical interpretation of data detection and treatment of outliers in the standard sample by excluding inappropriate organs (Piao et al., 2013). Based on the wet mass (absolute mass) and body mass, we calculated organ-to-body mass (relative mass) for different organs using the following formula:

$$\text{Organ coefficient} = [\frac{\text{Organ mass}}{\text{Total body mass}}] \times 100 \qquad (4.6)$$

4.2.6.3 Biochemical analysis

We performed the biochemical analysis in the blood serum to determine the effects of 50 Hz ELF-PEMF exposure on the functioning of vital organs. AST, ALT, total bilirubin (TBIL), serum creatine, and Ck-MB were measured to evaluate liver, kidneys, and heart functions.

4.2.6.3.1 AST/ALT and bilirubin

AST and ALT are biomarker enzymes for monitoring liver tissue integrity, aiding in diagnosing toxicity (Lagarto et al., 2011).The reference range for AST and ALT is 50-150 units/litre of

serum (U/L) and 10-40 U/L, respectively (Hasan et al., 2018). AST and ALT reportedly exist inside the mitochondria and cytosol of hepatocytes and cytosol, respectively (Lagarto et al., 2011). We clinically considered the significant alterations in transaminases as vital indicators of hepatocellular necrosis and hypertrophy of hepatocytes, respectively.

Bilirubin is a yellowish pigment produced in the spleen due to the breakdown of red blood cells (RBC). Its reference range is 0.1-0.7 mg/dl (2-15 µmol/L), and if it increases (> 17 µmol/L), it suggests the onset of liver disease (Manni et al., 2016). In the diseased conditions, bilirubin production surpasses the liver's metabolism, leading to increased circulating unconjugated bilirubin and obstruction of the bile ducts or damage to the hepatocellular structure followed by hyperbilirubinemia.

4.2.6.3.2 Ck-MB

CPK-MB test is also known as Ck-MB, a cardiac marker for diagnosing acute myocardial infarction (Majumdar and Krishnamurthy, 2022). The reference range of Ck-MB is 3 to 5% (percentage of total CK) or 5-25 IU/L(Cabaniss, 1990; Parikh and Pierce, 2024). The myocardium and skeletal muscles contain 15% and 1-3% Ck-MB isoenzyme, respectively (Glancy and Balaban, 2021; Kott et al., 2022; Mythili and Malathi, 2015). Higher value of Ck-MB indicates inflammation of the cardiac muscles (Latner, 2012; Singh et al., 2010).

4.2.6.3.3 Serum creatinine

Serum creatinine is considered as an essential parameter that gives information about the renal function, and elevation in its level in the blood indicates kidney dysfunction (Casal et al., 2019). As per the previous reports, the normal biochemical reference range of creatinine is 0.4-0.8 mg/dl (17.68-70.72 µmol/L)(Thammitiyagodage et al., 2020). The creatinine levels in the blood are also reported to elevate in cases of thyroid malfunction or muscular disorders (Da Silva et al., 2009; Kreider and Stout, 2021).

4.2.6.3.4 Histopathological analysis

The rat tissue samples (liver, kidneys, and heart) harvested at the end of 14th day of experimental protocol were fixed in a 10% buffered formalin solution. Then the sliced tissues (6 µm) were stained with haematoxylin (3 min) followed by eosin (1 min) and dehydrated in the graded ethanol solutions. The prepared slides were observed under the microscope (Olympus DS-52, Japan) at various magnifications to observe the microarchitecture of the tissues (Gupta et al., 2019; Khare et al., 2022).

4.2.7 Statistical analysis

All experimental data are presented as mean ± standard deviation (SD) and analyzed for statistical significance by Graph Pad Prism 5.0 software (RRID: SCR-002798). The organ coefficient and biochemical parameters were analyzed statistically by one-way ANOVA followed by Tukey's multiple comparison tests. At the same time, the total body mass of rats was analyzed statistically by two-way ANOVA followed by Bonferroni post hoc test. A $p < 0.05$ is the level of significance considered for all the tests performed.

4.3 Results

4.3.1 *In Vitro* analysis

4.3.1.1 No effect on RFP-L929 cell proliferation

Our study observed the effects of 50 Hz ELF-PEMF exposure among different exposed and control groups, as depicted in figure (4.2). The bright-field images and cell count percentages of RFP-L929 are illustrated in figures 4.4 (A& B), respectively. The effects of magnetic field exposure on the confluency of cells, as observed through the analysis of microscopic images, are depicted in figure 4.4 (A). No significant difference in the level of cell proliferation was observed from day 1 to day 5 among exposed and control groups ($p > 0.05$), as shown in figure 4.4 (B).

We also used fluorescence microscopy to detect any signs of cell fragmentation or change in the morphology of RFP-L929 cells during and after ELF-PEMF exposure. Figure 4.4 (C) shows no cell fragmentation among exposed and control groups. Moreover, the percentage of cell proliferation was also observed in exposed and control groups, as mentioned above.

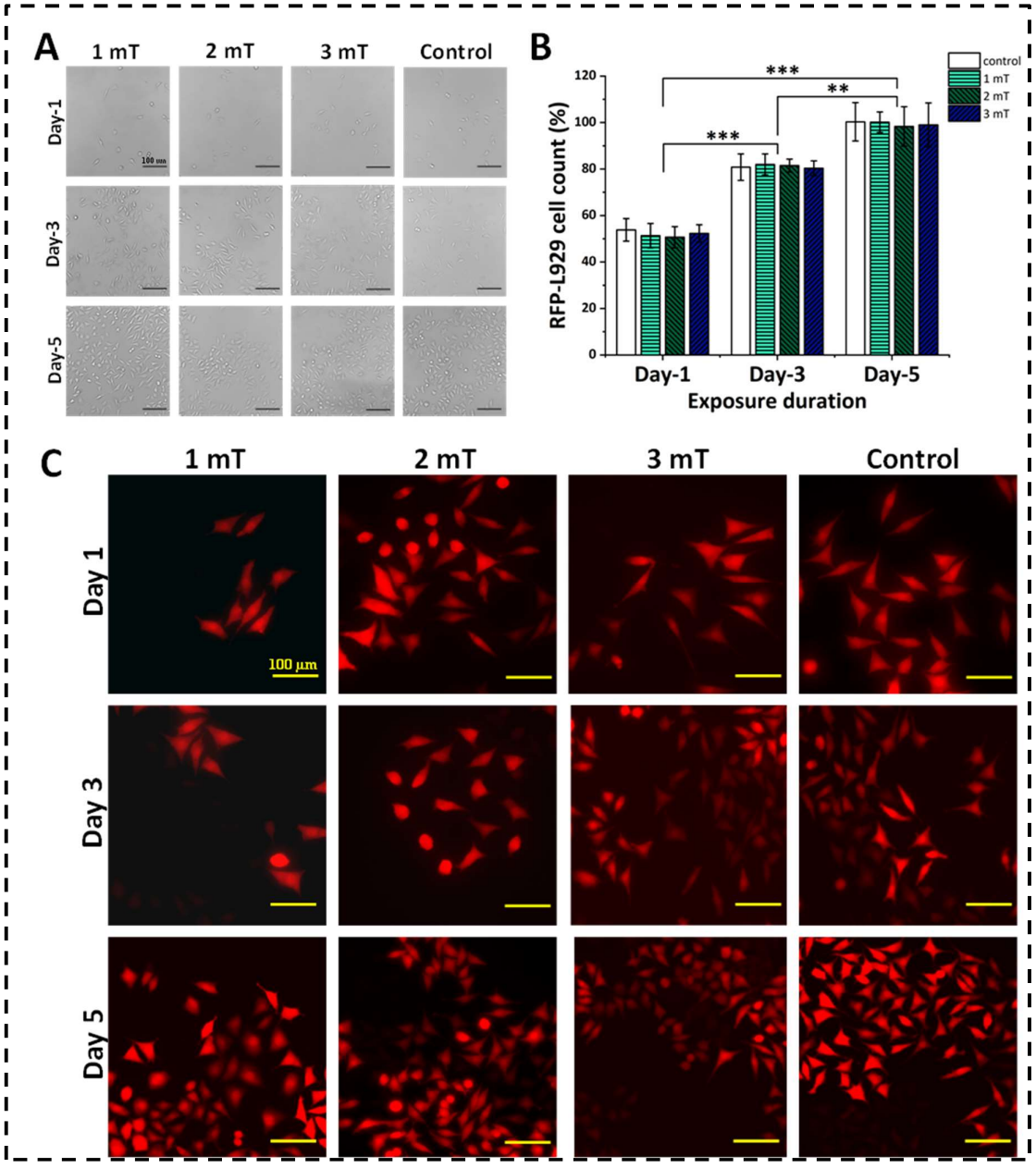

Figure 4.4. Effect of 50 Hz magnetic field (1-3 mT, 20 min (twice) with 4 h gap) on the proliferation and morphology of the RFP-L929 fibroblast cells. (A) Representative panel of brightfield images of RFP-L929 cells stimulated by the magnetic field compared to control, (B) RFP-L929 cell count at different magnetic field intensities over 5 days compared to control,

(C) Representative panel for fluorescent images of RFP-L929 exposed to magnetic field intensities compared to control. The initial seeding concentration was 1×103 cells/well and magnetic field with an exposure till the 5th day or confluency (90%). Scale bar: 100 μm for brightfield and fluorescent images. The values are expressed as mean ± S.D. (n = 6), and the level of significance is *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

4.3.2 *In Vivo* analysis

4.3.2.1 Effects on Organ Coefficients after the ELF-PEMF exposure

The organ coefficient is an early indicator of any pathological changes following a new drug or therapy administration. In the present study, the absolute mass of all the organs showed a slight increase in the 50 Hz ELF-PEMF-exposed groups. However, the organ-to-body mass ratio of the treatment rats did not change significantly among groups, as shown in figures 4.5 (A-C) ($p > 0.05$; Table 4.2). Therefore, it can be confirmed that the periodic exposure of 50 Hz ELF-PEMF exposure is safer and did not produce any harmful effects on the vital organs.

4.3.2.2 Effects on the total body mass after ELF-PEMF exposure

Body mass is essential for assessing the potential harmful effects that the ELF-PEMF exposure may produce on the animal health. Consequently, we employed the body mass measurement to ascertain the impact of periodic 50 Hz ELF-PEMF exposure on rats. We observed that the body mass of all rats gradually increased, indicating that the experimental rats did not suffer from any discomfort or pain, as illustrated in figure 4.5 (D). There were no discernible variations in the groups' total body mass according to the statistical analysis using two-way ANOVA [$F(3,80) = 9.94$; $p > 0.05$], time [$F(3,80) = 13.42$; $p > 0.05$], and their interaction [$F(9,80) = 0.031$; $p > 0.05$] (Table 4.3) under the present experimental conditions.

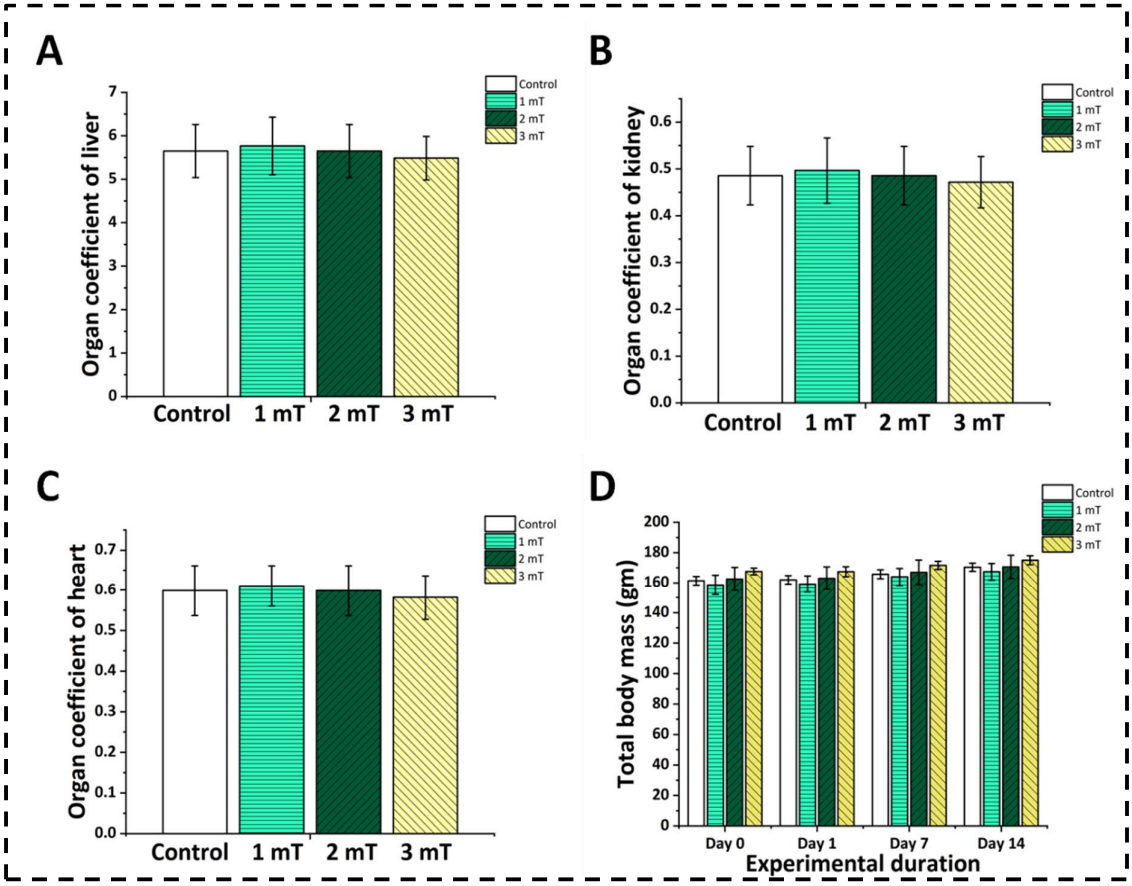

Figure 4.5. Effect of magnetic field (1-3 mT, 20 min (twice) with 4 h gap) on organ coefficient and total body mass. (A) Organ coefficient of the liver, (B) Organ coefficient of the kidney, (C) Organ coefficient of the heart, (D) Total body mass of rats. One-way ANOVA found significant differences. The values are expressed as mean ± S.D. (n = 6), and $p < 0.05$ is considered statistically significant.

Table 4.2. Effect of 50 Hz ELF-PEMF exposure (1-3 mT, 20 min (twice) with 4 h gap) on organ coefficients (liver, heart, and kidney) of adult Wistar rats.

	Liver coefficient (Mean ± SD)	**Kidney coefficient (Mean ± SD)**	**Heart coefficient (Mean ± SD)**	**Statistical significance**
Control	5.648 ± 0.610	0.485 ± 0.062	0.599 ± 0.062	N.S.
B = 1 mT	5.769 ± 0.660	0.496 ± 0.069	0.611 ± 0.050	N.S.
B = 2 mT	5.648 ± 0.610	0.483 ± 0.062	0.597 ± 0.062	N.S.
B = 3 mT	5.484 ± 0.500	0.471 ± 0.054	0.582 ± 0.054	N.S.

We could detect no significant difference between the 50 Hz ELF-PEMF-treated and control

groups. NS-Not significant

Table 4.3. Effect of 50 Hz ELF-PEMF exposure (1-3 mT, 20 min (twice) with 4 h gap) on total body mass of adult Wistar rats.

Total body mass (Mean ± S.D.) gm	**Control (gm) (Mean ± SD)**	**B = 1 mT (Mean ± SD)**	**B = 2 mT (Mean ± SD)**	**B = 3 mT (Mean ± SD)**	**Statistical significance among groups**
Day-0	161.4 ± 2.881	158.6 ± 6.505	162.6 ± 7.701	167.6 ± 2.19	N.S. (p > 0.05)
Day-14	170.4 ± 2.742	167.4 ± 5.505	170.6 ± 7.70	175 ± 2.915	N.S. (p > 0.05)

We could detect no significant difference between the 50 Hz ELF-PEMF-exposed and control groups. NS-Not significant

4.3.2.3 Effects on liver functions after the ELF-PEMF exposure

In the present study, serological examination of the enzymes (AST, ALT, and TBIL) depicting liver functioning were performed as shown in figure (4.6). We observed a significant difference in the AST, ALT, and TBIL level among the exposed and control groups, as illustrated in figures 4.6 (A-C). The results showed that 3 mT exposure induced the most significant alterations in AST ($p < 0.001$) serum level among ELF-PEMF exposed compared to control. On the contrary, 1 mT MF exposure caused a substantial change in ALT ($p < 0.001$; Table 4.4) level compared to 2 mT exposure ($p < 0.05$; Table 4.4) and 3 mT exposure ($p < 0.05$; Table 4.4). However, there were no significant differences in the AST level ($p > 0.05$; Table 4.4) of 2 mT exposed and ALT ($p > 0.05$; Table 4.4) under 2 mT and 3 mT exposure. Based on the results, the ELF-PEMF exposure effects can differ according to exposure intensity.

4.3.2.4 Effects on myocardial enzymes after the ELF-PEMF exposure

The heart is one of the vital organs primarily responsible for the oxygenated blood supply in the body by periodic contraction and relaxation of the myocardium, and obstruction in the flow leads to a clinical condition called myocardial infarction (Wu et al., 2019). Previous studies

have reported that after the myocardial infarction Ck-MB enzyme is released in the blood from the necrotic tissues immediately after 4-6 h, which reaches a peak value at 24-36 h and then declines rapidly (Grande et al., 1982). Hence, in the present study, the Ck-MB levels were estimated to detect whether the periodic 50 Hz ELF-PEMF exposure caused any myocardial tissue damage in rats. In the present study, we observed significant changes in the Ck-MB levels among the ELF-PEMF-exposed and control group ($p < 0.01$; Table 4.4), but the changes are considered normal according to existing literature (Cabaniss, 1990; Parikh and Pierce, 2024). Additionally, it was observed that the magnetic field exposure effects on the Ck-MB serum level among the ELF-PEMF-exposed groups were insignificant ($p > 0.05$) as depicted in figure 4.6 (D).

4.3.2.5 Effects on renal functions after the ELF-PEMF exposure

Kidneys are the highly perfused vital organs that are primarily responsible for eliminating metabolic wastes from the body, and damage to it can lead to the accumulation of nitrogenous waste in the body. Typically, creatinine levels in the blood can provide critical information about the renal functioning (Casal et al., 2019). In the present study, significant increases in the serum creatinine levels were observed in 1 mT ($p < 0.05$; Table 4.4), 2 mT ($p < 0.01$; Table 4.4), and 3 mT ($p < 0.001$; Table 4.4) exposed rats compared to the rats in control group as shown in figure 4.6 (E).

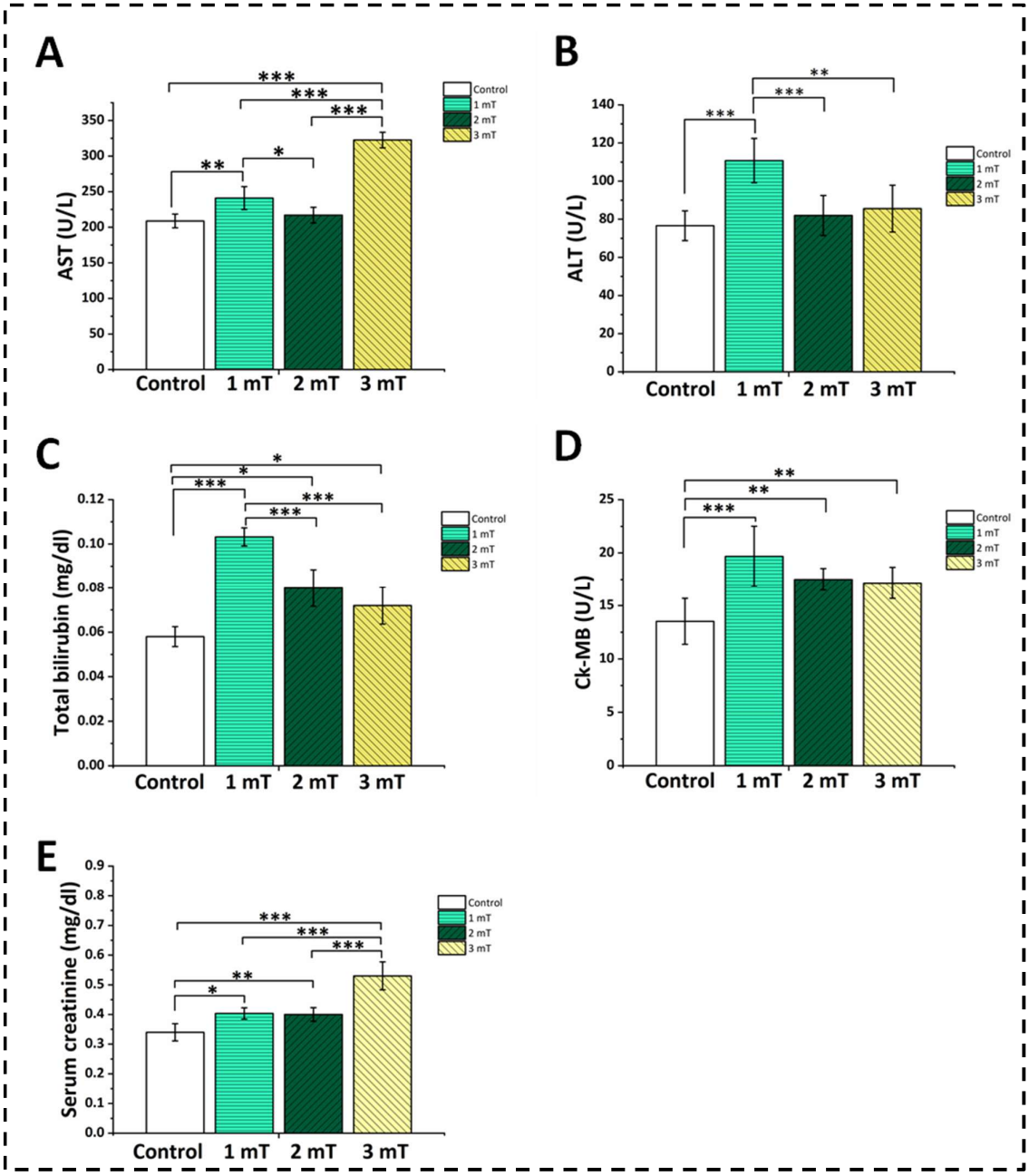

Figure 4.6. Effect of 50 Hz ELF-PEMF (1-3 mT, 20 min (twice) with 4 h gap) on blood serum concentration of (A) AST, (B) ALT, (C) Total bilirubin, (D) Ck-MB, (E) serum creatinine at the end of the experimental period. The values are expressed as mean ± S.D. (n = 6 male rats/groups), and the level of significance is *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (One-way ANOVA followed by Tukey's multiple comparison post hoc test).

Table 4.4. Effect of 50 Hz ELF-PEMF exposure (1-3 mT, 20 min (twice) with 4 h gap on serum enzymes of adult Wistar rats.

Biochemical parameters	Control (Mean ± SD)	B = 1 mT (Mean ± SD)	B = 2 mT (Mean ± SD)	B = 3 mT (Mean ± SD)	Statistical significance		
					1 mT	2 mT	3 mT
AST (U/L)	208.8 ± 9.628	241 ± 16.21	217 ±11.099	322 ± 10.96	**	*	***
ALT (U/L)	76.66 ± 7.82	110 ± 11.62	82 ± 9.5026	85.6 ± 12.259	***	NS	NS
TBIL (mg/dl)	0.06 ± 004	0.1 ± 0.004	0.08 ± 0.008	0.072 ± 0.008	***	*	*
DBIL (mg/dl)	0.03 ± 0.004	0.09 ± 0.01	0.06 ± 0.005	0.056 ± 0.005	***	*	*
IBIL (mg/dl)	0.026 ± 0.005	0.013 ± 0.012	0.02 ± 0.005	0.016 ± 0.005	***	*	*
Ck-MB (U/L)	13.52 ± 2.156	19.68 ± 2.83	17.5 ± 1.035	17.16 ± 1.487	***	**	**
Serum creatinine (mg/dl)	0.34 ± 0.029	0.39 ± 0.019	0.40 ± 0.0230	0.53 ± 0.0469	*	**	***

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; Ck-MB: creatine kinase-MB. The results are expressed as the mean ± standard deviation (n = 6). The blood serum parameters showed a wide range between the exposed and control groups. NS-Not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; (n = 6 male Wistar rats/group).

4.3.2.6 Histopathological examination

Liver, kidneys, and heart are the vital organs that control major bodily functions and damage to them can be proved fatal. Therefore, the histopathological examination is essential at the end of experimental protocol to ascertain the safety profile of the test compound. The present study shows that 50 Hz magnetic field exposure shows no evidence of changes in the microarchitecture of the tissue as well as in the morphology of liver, kidneys, and heart. It was observed that there were no histopathological alterations found in the liver tissue including the central vein and hepatocytes. There were round and centrally placed nuclei, with granular cytoplasm. Sinusoids of the control and the ELF-PEMF-exposed rats appeared normal, thus

highlighting the non-toxic effects on the periodic 50 Hz ELF-PEMF exposure. Similarly, the bowman's capsule, glomerulus, proximal and distal tubules of the rats exposed to the ELF-PEMF exhibited typical structure as the control rats. Further, it was observed that photomicrograph of the heart of rats exposed to 50 Hz ELF-PEMF exhibited normal myocardium tissue components including the muscle fibres, intercalated disks, central nuclei, and transverse striations as the control rats (depicted in figure 4.7).

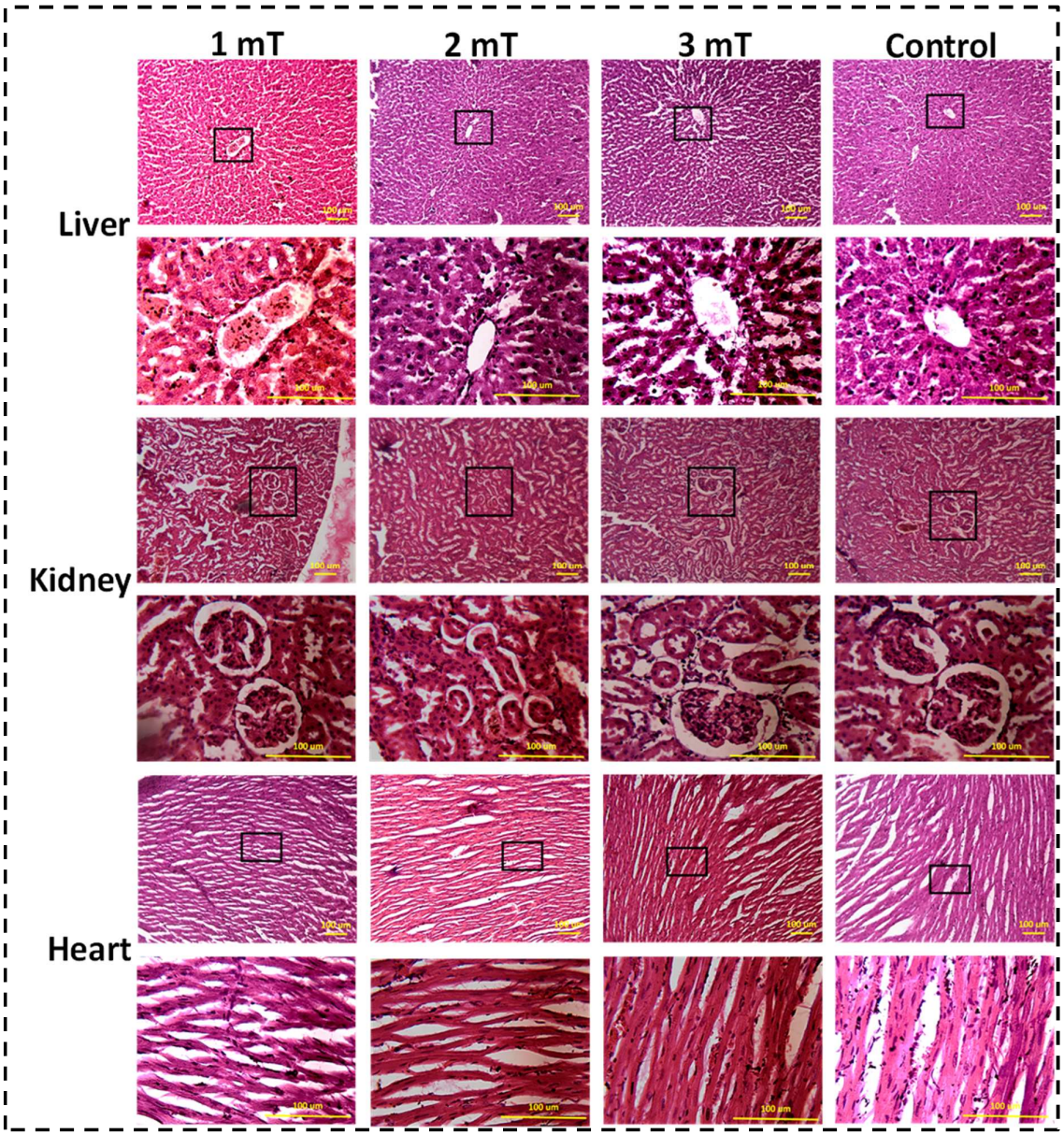

Figure 4.7. Effect of 50 Hz ELF-PEMF (1-3 mT, 20 min (twice) with 4 h gap) on organs like

liver, kidney, and heart tissue stained with hematoxylin and eosin.

Table 4.5. Summary of 50 Hz ELF-PEMF exposure (1-3 mT) effects on biochemical parameters, organ coefficient, and total body mass of rats.

	1 mT	2 mT	3 mT	Observation	Conclusion
Organ coefficient (gm)					
liver	$p > 0.05$	$p > 0.05$	$p > 0.05$	No significant changes were observed.	No significant changes in organ coefficient.
kidney	$p > 0.05$	$p > 0.05$	$p > 0.05$	No significant changes were observed.	
Heart	$p > 0.05$	$p > 0.05$	$p > 0.05$	No significant changes were observed.	
Total body mass (gm)					
Day-0	$p > 0.05$	$p > 0.05$	$p > 0.05$	No significant effects were observed.	No significant changes in body mass.
Day-14	$p > 0.05$	$p > 0.05$	$p > 0.05$		
Biochemical parameters					
AST (U/L)	$p < 0.01$	$p < 0.05$	$p < 0.001$	A wide range of post-exposure effects are observed. The elevation values of serum enzymes are under the mild category.	50 Hz ELF-PEMF exposure effects depend on field intensity, frequency, and duration.
ALT (U/L)	$p < 0.001$	$p > 0.05$	$p > 0.05$		
TBIL (mg/dl)	$p < 0.001$	$p < 0.05$	$p < 0.05$		
DBIL (mg/dl)	$p < 0.001$	$p < 0.05$	$p < 0.05$		
IBIL (mg/dl)	$p < 0.001$	$p < 0.05$	$p < 0.05$		
Ck-MB (U/L)	$p < 0.001$	$p < 0.01$	$p < 0.01$		
Serum creatinine	$p < 0.05$	$p < 0.01$	$p < 0.001$		

(mg/dl)	

ALT: alanine aminotransferase; AST: aspartate aminotransferase; TBIL: total bilirubin; DBIL: direct bilirubin; IBIL: indirect bilirubin; Ck-MB: creatine kinase-MB. The results are expressed as the mean ± standard deviation (n = 6). The blood serum parameters showed a wide range between the exposed and control groups. NS-Not significant; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; (n = 6 male Wistar rats/group)

4.4 Discussion

The ELF-MF exposure guidelines state that the ELF range (≤ 100 kHz), which includes power frequencies ($50/60$ Hz), is considered safe for household applications (Protection, 2010; Sciences and Health, 2002). Further, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) established exposure limits of 50 Hz, 100 μT for public, and 500 μT for occupational exposure (Akdag et al., 2009). Dasdag et al. studied the effects of occupational ELF-EMF exposure (0.10-0.25 mT) and found no adverse impact on the participants' hematological and immunological parameters (Dasdag et al., 2002). Moreover, the recent studies have prompted a re-evaluation of the occupational exposure limits for ELF-MF, leading to an increase in the recommended limit to 1 milliTesla (mT) (Choi et al., 2018; European Commission. Directorate General for Health and Consumers., 2015; Hansson Mild et al., 2023).These recommendations were based on the well-established data regarding the effects of acute exposure, and these recommendations shield the general public and employees from the harmful health impacts of electromagnetic field exposure. Numerous studies have also reported that cell membrane integrity was affected by electromagnetic field exposure, followed by the significant changes in biochemical parameters and enzymes (Buckner et al., 2018; Cucullo et al., 2005; Jeong et al., 2014; Pohling et al., 2023; Veiga et al., 2005; Yaghmaei et al., 2010). Besides, a significant amount of the studies are focused on magnetic field exposure with a duration of ≥ 1 h/day (Ibrahim et al., 2018; Kulkarni and Gandhare, 2015; Luo et al., 2017; Margonato et al., 1995; Zhang et al., 2020), but little evidence is available for the impact of 50 Hz magnetic field exposure with a duration of < 1 h/day on cell proliferation and biochemical

parameters. In our previous study (Tekam et al., 2023), we selected two weeks (*in vivo*) and 5 days (*in vitro*) exposure to study the effects of 50 Hz ELF-PEMF exposure on C6 cell proliferation and morphology and spontaneous activity of the rats. We reported that there were no alterations in the spontaneous alternation, anxiety-like behaviour, and changes in motor co-ordination in rats with no exhibited changes in the proliferation of C6 cells. Considering these reported findings, in the present study, we sought to learn more about the effects of 50 Hz magnetic field exposure with a duration < 1 h/day on different tissues and organs (liver, kidneys, and heart) for a two-week period in Wistar rats. Additionally, we also designed an *in vitro* experiment to investigate the 50 Hz magnetic field exposure effects on RFP-L929 cells. RFP-L929 cells are easily recognized by their adherent property, distinctive spindle shape, and the primary constituent of the extracellular matrix that contributes to the tissue architecture (Buckley, 2021; Dick et al., 2022). Moreover, it promotes healing and triggers inflammation in the event of injury. Numerous studies explored the influence of different magnetic field exposure on ornithine carboxylase activity (ODC), fibroblast growth, and metabolic activity and observed the difference in morphological clustering and orientation, unaltered ODC activity with altered metabolic activity, and cell response (Azadniv et al., 1995; Cress et al., 1999; Penafiel et al., 1997; Schuetz et al., 1985; Torricelli et al., 1998). Based on the studies, RFP-L929 cell lines seemed appropriate for the *in vitro* studies in our current investigation. Hence, we exposed RFP-L929 cells to 50 Hz ELF-PEMF (1-3 mT) for a duration of 20 min with a 4 h gap (twice daily) continuously for 5 days. The bright-field images demonstrated no discernible change in the RFP-L929 cells' ability to proliferate following the exposure, and fluorescent microscopy further revealed no sign of cell fragmentation and change in their morphology. Based on the above-results, we anticipate that 50 Hz ELF-PEMF exposure is non-toxic and consistent with earlier studies (Cress et al., 1999) and can be used safer for biomedical applications.

The selection of animal models for toxicity assessment studies plays a critical role in the accuracy of study findings. Wistar rats are commonly used in research due to their ease of handling, good fecundity, and familiarity with their spontaneous pathology. It consistently maintains a lower body mass and a significantly have higher survival rate than Sprague Dawley (SD) rats, which are the vital requirements for toxicity studies. The changes in organ mass is one of the most sensitive indicators of any drug toxicity which depends on the mass of the animal, age, sex, and experimental conditions (Gur and Waner, 1993). In our study, we observed a gradual increase in the body mass of rats throughout the experimental duration, indication no discomfort with proper organ functioning following the 50 Hz ELF-PEMF exposure. Similarly, after a comparative analysis of organ coefficients of different organs, we found no significant difference among the exposed and control groups, which is in agreement with previous literature (Piao et al., 2013). Therefore, it can be assumed that 50 Hz ELF-PEMF (1-3 mT) exposure is safer and did not affect the normal functioning of the vital organs.

Further, ELF-PEMF exposure is reported to affect the transaminase activity, and serum transaminases have been widely utilized as biomarkers for hepatocellular injury (Amara et al., 2006; Hashem and El-Sharkawy, 2009; Purushothaman et al., 2013). ELF-PEMF has also been reported to induce structural changes of the hepatocytes and mitochondria, which indicates cytotoxic effects (Hashem and El-Sharkawy, 2009; Parafiniuk et al., 1992; Salem et al., 2005). In our study, we observed significant differences in biochemical parameters among exposed and control groups ($p < 0.001$), as depicted in figures 4.6 (A-B). The considerable rise in aminotransferase levels can be alarming, but the observed elevated serum level of the enzymes can be considered mild as per the previous study (Nazeer et al., 2018). The 50 Hz ELF-PEMF exposure have been reported to induce significant changes in the cortisol, stress oxidative compounds, and, in some cases, hypoxia, which can be the reason behind the elevation in AST and ALT values (Yaghmaei et al., 2010). Moreover, a mild increase in ALT compared to AST

indicated that the in addition to liver, different muscles also contribute in rise of AST levels in blood (Sihem et al., 2006). We also observed significant changes in the TBIL levels in the serum of the field exposed compared to control groups ($p < 0.001$), but it was mild and within the reference range (Manni et al., 2016).

Previously, the renal impairment reported in animals have been attributed to the oxidative stress induced by the EMF exposure (Hanafy et al., 2010; Ozguner, 2005). Contrarily, the static magnetic fields do not affect serum creatinine levels in rats (Salem et al., 2005). Moreover, Gholampour et al. reported that 50 HZ ELF-EMF (1 mT, 24 h, 135 days) significantly affected creatinine and urea plasma concentration (Gholampour et al., 2011). Similarly, in the current study, the serum creatinine level increased considerably in the 50 Hz ELF-PEMF exposed groups ($p < 0.001$), but it is still considered within the normal accepted range (Thammitiyagodage et al., 2020). In agreement, we also observed no significant changes in the organ coefficient of the kidneys post-ELF-PEMF exposure for 14 days.

The primary reported mechanism of MF affecting the biological functions is changes in the velocities of flowing charges within the cell membranes (Adey, 1988). Additionally, ELF-PEMF-induced enzymatic activity was crucial in controlling the free radical mechanism (Adebayo et al., 2019; Ebrahim et al., 2016). Although there are significant differences in isoenzyme levels between the exposed and control groups in our investigation but these changes fall within the expected range (Cabaniss, 1990; Parikh and Pierce, 2024). The results we observed in figure 4.6 (D) suggesting the effects on intracellular charges are not significantly affected by 50 Hz ELF-PEMF exposure. Furthermore, there were no discernible variations in the heart coefficient between the groups.

In bio electromagnetics, the toxicological evaluation of ELF-PEMF exposure is not new, but identifying any notable pathological changes is crucial. Because visceral organs play a part in

metabolic and excretory processes, histological examinations have been done on them. These organs include the liver, heart, and kidney. Rats that were exposed to ELF-PEMF on a periodic basis showed intact tissue architecture and morphology in the histological sections of their liver tissues in the current investigation. When male Wistar rats were subjected to 50 Hz ELF-PEMF, their connective tissues revealed unaltered nuclei and hepatocytes without any indications of congestion or dilatation of the central vein or sinusoids, respectively (Figure 4.7). Hence, it indicates non-toxic nature of 50 Hz ELF-PEMF exposure on morphology and histology of liver (Erpek et al., 2007; Lagroye et al., 2011; Margonato et al., 1995). Additionally, mild effects on serum enzymes i.e., AST, ALT, bilirubin and non-significant effects on organ coefficient of liver also supports the above-mentioned results.

Kidneys are also vital components of metabolic and excretory mechanisms; hence they are vulnerable to numerous toxicants. In present study, the histopathological architecture of bowman's capsule and glomeruli are seemed unaffected by 50 Hz ELF-PEMF exposure as depicted in figure (4.7). The non-toxic effects of 50 Hz ELF-PEMF on kidney structure and morphology are also supported by previous evidences (Erpek et al., 2007; Margonato et al., 1995). Moreover, the organ coefficient data and mild effects on serum creatinine level in blood also supports the non-toxic nature of ELF-PEMF on kidney as illustrated in figures (4.5 & 4.6).

The effects of 14 days periodic ELF-PEMF exposure on myocardial tissues are also examined and it was observed that the structural components of heart tissues, like myofibrils, intercalated disks, central nuclei, and transverse striations, show no histological changes in exposed and control groups as depicted in figure (4.7). The non- toxic effects of 50 Hz ELF-PEMF exposure on rat myocardium is also consistent with previous studies (Söker et al., 2011; Zecca et al., 1998). Additionally, the elevation in Ck-MB levels are also found to be in the permissible range (< 50 UL) (Parikh and Pierce, 2024). Hence, it can be corroborated from histopathological examinations that 50 Hz ELF-PEMF exposure did not induce any toxic effects on vital organs

under present experimental conditions, which acts as supportive scientific evidence for safe use of 50 Hz ELF-PEMF exposure (Table 4.5).

4.5 Conclusion

The present study demonstrates the effects of 50 Hz ELF-PEMF using *in vitro* (RFP-L929 mouse fibroblast cells) and *in vivo* (adult male Wistar rats) models. The results reveal that an exposure duration of 20 min (each) with a 4 h gap is non-destructive for RFP-L929 cells and causes mild alterations in biochemical parameters but not in organ coefficient, tissue structure, and morphology of adult Wistar rats. The results demonstrated that 50 Hz ELF-PEMF exposure did not cause any significant cellular fragmentation and changes in the morphology of mouse fibroblast cells. We observed mild alterations in biochemical parameters of rats among magnetic field exposed and control groups. Conversely, histological analysis of the selected liver, kidney, and heart sections following ELF-PEMF exposure revealed no significant changes in tissue structure and morphology. Our efforts provide conceptual and experimental support to establish a link between 50 Hz magnetic field exposure in residential and occupational environments.

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