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Histopathological Effects Of 4g And 5g Electromagnetic Radiations On Liver Tissue

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Abstract

Objective: To compare the effect of EMF at 2100 MHz and 3500 MHz on the histomorphology and functions of a rat's liver.

Methods: Thirty male Sprague Dawley rats were divided into three groups with 10 rats in each group: a control group with no EMF exposure, a 2100 MHz exposure group, and a 3500 MHz exposure group. The rats in the exposure groups were subjected to EMF for 5h/day for 4 weeks. Histological examinations were performed to observe sinusoidal dilatation, congestion, and hepatocyte vacuolation in the classic hepatic lobule. Serum LFTS were assessed to evaluate liver function.

Results: The control group of rats exhibited no hepatocyte vacuolation, and only 10% showed sinusoidal dilatation and congestion. In contrast, 40% of the rats in Group B displayed moderate sinusoidal dilatation and congestion, whereas Group C had 20% with severe and 60% with moderate sinusoidal dilatation and congestion. Severe hepatocyte vacuolation was observed in 10% of the rats in Group C, while Group B had no rats with severe hepatocyte vacuolation. When comparing serum levels among the groups, ALT levels were 35.5 ± 4.7 in Group A (control), 42.4 ± 3.9 in Group B, and 49.8 ± 4.5 in Group C. Likewise, AST levels were 140.6 ± 4.3 in Group A, 263.5 ± 5.1 in Group B, and 295.3 ± 4.6 in Group C.

Conclusion: Electromagnetic field (EMF) exposure at frequencies of 2100 MHz and 3500 MHz has been found to impair liver structure and function in rats, with the severity of damage increasing with frequency. This indicates that higher frequencies, such as those used in 5G networks, may pose greater risks. This study underscores the potential risks associated with EMF exposure and indicates that further research is necessary to grasp its impact on human health.

Keywords: Electromagnetic Fields, Hepatocytes, Liver Function Tests, Vacuolation

Contributions:
A.H, S.A, U.U, S.F, T.F, T.Q - Conception of study
A.H, S.A, U.U, S.F, T.F, T.Q - Experimentation/Study Conduction
A.H, S.A, U.U, S.F, T.F, T.Q - Analysis/Interpretation/Discussion
A.H, S.A, U.U, S.F, T.F, T.Q - Manuscript Writing
A.H, S.A, U.U, S.F, T.F, T.Q - Critical Review

All authors approved the final version to be published & agreed to be accountable for all aspects of the work.

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Introduction

Mobile phones are now a fundamental aspect of daily life, with more than 192 million subscribers in Pakistan. Although these devices have transformed communication and connectivity, their extensive use has sparked concerns about the potential health effects of the electromagnetic field (EMF) they produce. The evolution of telecommunication technologies, from 2G to the forthcoming 5G, has introduced a range of frequencies from 800 to 2000 MHz, with 5G employing frequencies between 450 and 3800 MHz. This broadening spectrum of frequencies is becoming increasingly common globally.[1]

EMF has thermal and non-thermal effects on the human body. A small temperature increase can be compensated for by our body, but a large temperature increase can harm tissue and result in burns. The induction of electric current in the body affects membrane permeability by damaging ion channels, resulting in non-thermal effects.[2] Exposure to EMF results in oxidative stress due to the release of reactive oxygen species, which alter membrane conductivity by causing phospholipids in the membranes to peroxide. Living things include many defense mechanisms against reactive oxygen species (ROS). These processes are disturbed by the excessive generation of ROS by EMF, resulting in oxidative stress that damages the cell's ultrastructure and causes apoptosis(Figure 1).[3]

Exposure to EMF is commonly associated with a range of symptoms due to power absorption, including memory loss, behavioural changes, headaches, sleep disturbances, discomfort, depression, nausea, dizziness, irritability, loss of appetite, numbness, muscle spasms, tingling, and altered reflexes. Some individuals also report sensations such as buzzing in the head, light-headedness, heart palpitations, heat, cardiovascular issues, visual disturbances, agitation, nervousness, and respiratory problems. In more severe cases, exposure may lead to paralysis, seizures, stroke, and psychosis.[4] Even at lower intensities, electromagnetic fields (EMF) may induce cellular stress, increase the permeability of the blood-brain barrier, and cause genetic damage. Furthermore, the proximity of mobile phone antennas to abdominal organs, especially when devices are secured to belts, has raised concerns about potential effects on organs such as the kidneys and liver.[5]

EMF exposure affects the liver similarly to other toxicants; the reactive oxygen species (ROS) generated by EMF are mainly detoxified in the liver, contributing to liver injury and impaired hepatic function. The susceptibility of the liver to electromagnetic fields (EMF) is influenced by factors such as the Specific Absorption Rate (SAR) and the frequency of exposure. The international guidelines for safe EMF exposure levels are established by the International Commission on Non-Ionising Radiation Protection (ICNIRP). These guidelines are designed to minimise the potential harmful effects of EMF on human health, including liver function.[6–8]

N.A. Siddiqui observed fatty changes in the liver of white leghorn chickens exposed to EMF.[9] E.A. Adebayo detected distorted architecture and dilated sinusoids in the histological examination of liver sections in rats exposed to 1800 MHz. Sadhana and Anjali observed a significant surge in serum transaminases at 900MHz,1800MHz, and 2100MHz, which is attributed to damage to the cell membrane, indicating hepatic damage.[10]

This study aims to explore the impact of various EMF frequencies on liver health by analysing structural and cellular changes in a rodent model. Through this investigation, the study seeks to elucidate the potential mechanisms of liver damage associated with EMF exposure, compare its results with existing research, and provide insights that could inform public health policies and mobile phone safety guidelines.

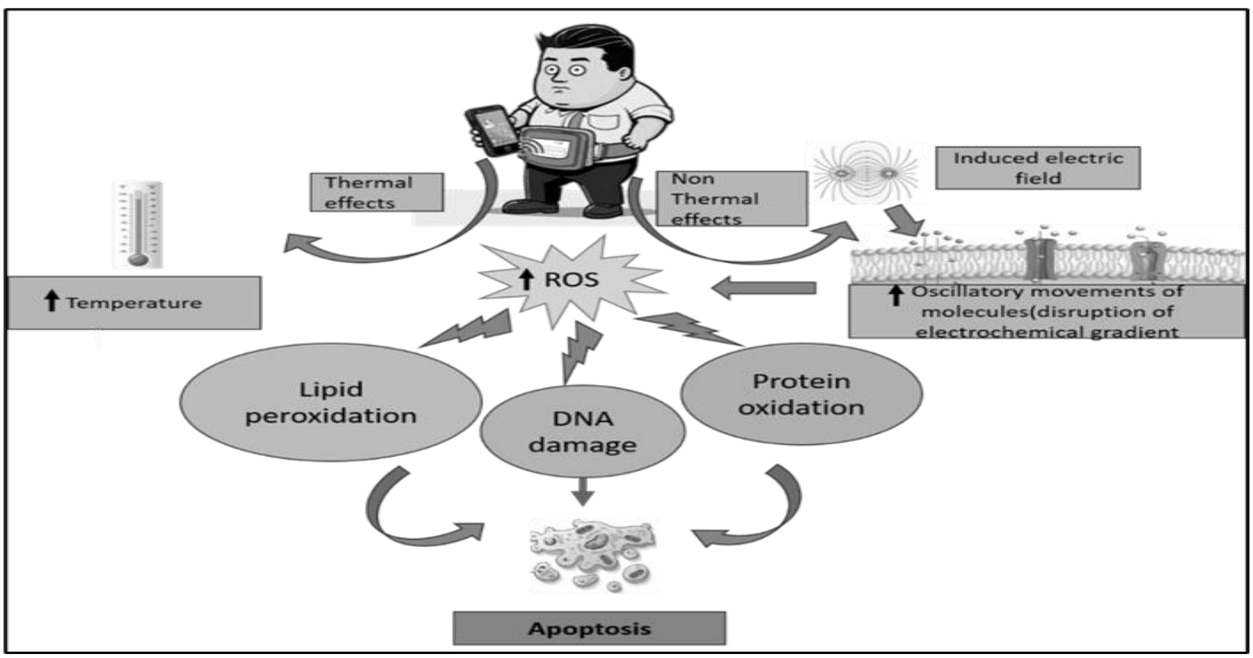

Figure 1: Figure showing thermal and non-thermal effects of RF EMR

Materials And Methods

A lab-based experimental study was conducted in collaboration with the National Institute of Health (NIH) and the Anatomy Department of Islamic International Medical College after approval by the Ethics Review Committee (Riphah/IRC/24/1059). The study lasted six months, from January 2024 to June 2024.

Thirty Sprague-Dawley adult male rats, each weighing between 200-250g and free from any visible abnormalities, were selected based on convenience.[11] Following sampling, the rats were randomly assigned to either the intervention or control group. They were housed in the Animal House at NIH, Chak Shehzad Islamabad, in cages with standard conditions: a temperature of $22 \pm 0.5^{\circ}C$,

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in an air-conditioned room with 50% humidity, and a 12-hour light/dark cycle. For seven days, the rats were given unrestricted access to food and water to acclimate. During the experiment, they were provided with rat pellets and water.

Control group (Group A)

10 adult male rats were kept on a standard diet orally throughout the experiment for up to 4 weeks. Rats in the control group were not exposed to any electromagnetic radiation.

Experimental group (Group B)

10 adult male rats were exposed to 2100 MHz EMF for 5h/day for 4 weeks.[12]

Experimental group (Group C)

10 adult male rats were exposed to 3500 MHz EMF for 5h/day for 4 weeks.[12]

The electromagnetic radiation exposure setup utilises the Analogue Devices Inc. ADALM-PLUTO (Plutosdr) module, which is effective at frequencies of 2100 MHz and 3500 MHz. This module features an AD9363 transceiver, providing full duplex operation, a 20 MHz bandwidth, and frequency generation capabilities ranging from 0.325 GHz to 3.8 GHz. To enhance performance, the original antenna was substituted with a 10 cm 2276 Trainer antenna from Scientech Technologies, which met the necessary specifications and permitted precise adjustments within the experimental cage. Rats housed in 40 × 40 × 60 cm cages were exposed to electromagnetic radiation, with far-field calculations establishing distances of 14 cm for 2100 MHz and 24 cm for 3500 MHz between the antenna and the cages to comply with exposure standards. MATLAB software is employed to control the ADALM-PLUTO, adjusting the antenna gain to 0 db for the experiments and subsequent analysis. This setup allows for a detailed investigation of electromagnetic effects on the subjects within the controlled environment of the experimental cages.

Two milliliters of blood were drawn via intracardiac sampling using disposable syringes from each animal. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were quantified using commercial kits designed for use with the Merck Micro lab 200® system. This process ensured the accurate measurement of liver enzyme activities in the serum samples, which are crucial indicators of liver function and health in experimental animals.[13]

After 4 weeks, the rats were dissected, and liver samples were taken. Initially, liver samples were fixed in 10% formalin, followed by sectioning into 5 µm slices using a microtome. These sections were then mounted on glass slides. Subsequently, the slides were subjected to examination under a light microscope and stained using the Hematoxylin-Eosin technique. Images of the stained ss were captured with a camera for detailed analysis. This process ensured that the tissue samples were adequately prepared and visualised for histopathological assessment.[13]

Images were shifted to Image J software, and qualitative analysis of sinusoidal dilatation and congestion (blood-filled dilated sinusoids) and hepatocyte vacuolation was done.[13]

Sinusoidal dilatation and congestion and hepatocyte vacuolation were categorized based on the extent of involvement in the evaluated fields: none (0) indicated no observable damage, mild (1) represented damage affecting 0–25% of the evaluated area, moderate (2) indicated damage affecting 25–50% of the evaluated area, and severe (3) indicated damage affecting 50–100% of the evaluated area across various experimental groups.[14,15]

Data analysis was performed using SPSS version 27, employing Chi-square tests for qualitative data and analysis of variance (ANOVA) for quantitative data. A significance level of $p \leq 0.05$ was used to determine statistical significance. This analysis aimed to identify and quantify pathological changes in liver tissue, providing an understanding of the severity and distribution of hepatocellular damage within the hepatic lobule.[10]

Results

When comparing the control group to experimental groups B and C, the serum levels of ALT were 35.5 ± 4.7, 42.4 ± 3.9, and 49.8 ± 4.5 in groups A, B, and C, respectively. The serum levels of AST were 140.6 ± 4.3, 263.5 ± 5.1, and 295.3 ± 4.6 in groups A, B, and C, respectively. On comparing the mean value of serum ALT and AST between all three groups, ANOVA showed significant results (*p* value<0.001). The post-hoc multiple comparisons revealed a significant difference in the serum levels of ALT and AST between experimental Group B and Group C, with a p-value less than 0.001. (Table 1)

In group A, 10% of the rats showed mild sinusoidal congestion and dilatation. In group B, only 10% of the rats had normal liver architecture, while 50% showed mild and 40% showed moderate sinusoidal congestion and dilatation. In group C, 60% of the rats exhibited moderate while 20% showed severe sinusoidal congestion and dilatation in hepatic lobules. The Chi-square analysis indicated a highly significant difference among the experimental groups, with a *p*-value of < 0.001.(Figure2,4)

In group A, all the rats showed normal liver architecture. In group B, 50% of rats showed moderate hepatocyte vacuolation, while 40% showed mild hepatocyte vacuolation. In group C, 10% of the rats exhibited severe while 40% showed moderate hepatocyte vacuolation. The Chi-square analysis indicated a highly significant difference among the experimental groups, with a *p*-value of < 0.001. (Figure 3,4).

Table 1: Table showing levels of liver enzymes (ALT and AST)

Liver Enzyme Levels			
Quantitative Parameter	**Group A (IU/L)**	**Group B (IU/L)**	**Group C (IU/L)**
ALT	**35.5±4.7**	**42.4±3.9**	**49.8±4.5**
AST	**140.6±4.3**	**263.5±5.1**	**295.3±4.6**

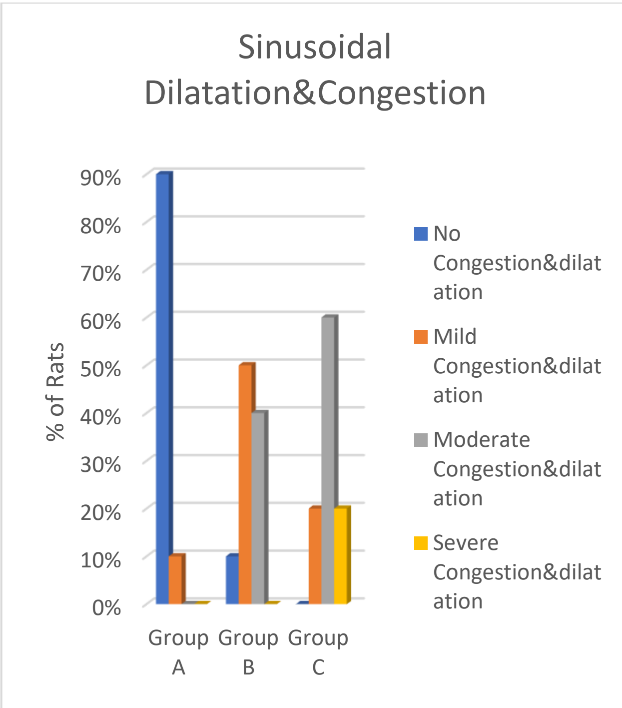

Figure 2: Bar chart showing different grades of sinusoidal dilatation and congestion

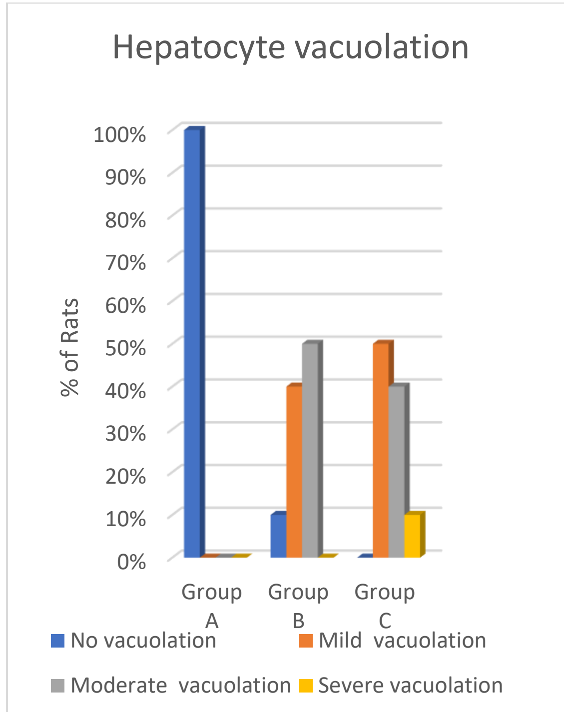

Figure 3: Bar chart depicting severity of hepatocyte vacuolation in group A,B and C

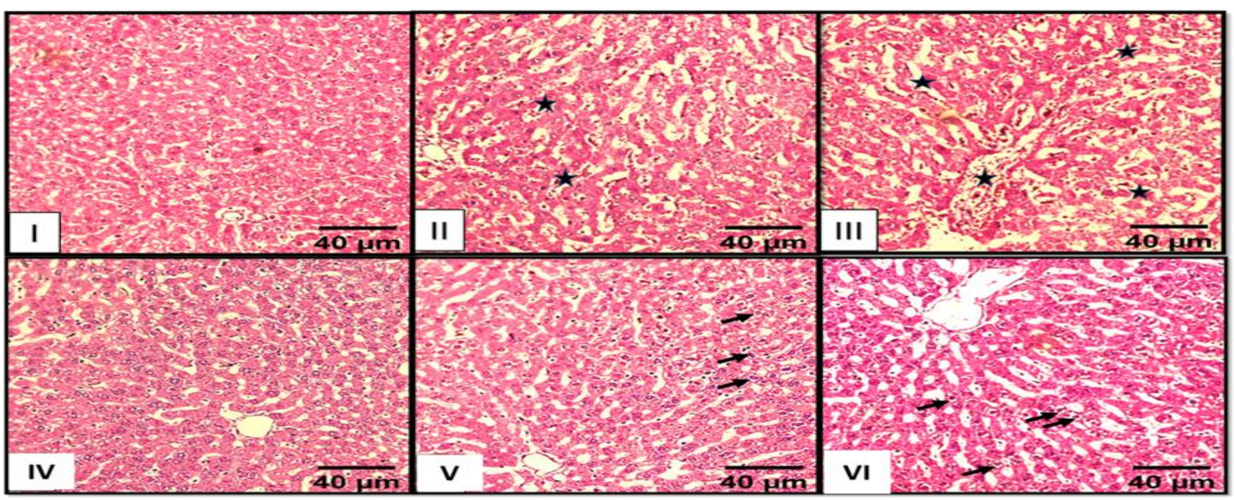

Figure 4: Photomicrograph showing normal hepatic architecture in control group A(I&IV). II & III showing sinusoidal dilatation and congestion in group B& C, respectively. V showing hepatocyte vacuolation in group B, whereas VI shows hepatocytes vacuolation in group C.H& E stained.20x magnification.

Discussion

The study investigates the effects of mobile phone radiation on liver health, specifically examining histological changes in hepatic sinusoids and liver functions in a frequency-dependent manner. Serum transaminases, specifically AST and ALT, are key liver enzymes that are typically confined within liver cells, resulting in low and stable levels in peripheral blood under normal conditions. However, when cell membranes are damaged or mitochondria are lysed, these enzymes are released into the bloodstream, increasing serum levels. This increase serves as an indicator of liver cell injury.[10]

Our study shows a significant rise in serum ALT and AST levels in animals exposed to different frequencies of EMF, which suggests potential hepatic damage. These findings align with previous research and indicate that long-term exposure to pulsed electromagnetic fields (PEMF) can harm the liver and disrupt its function.[13] Anjali Sharma's work also supports these findings, confirming elevated liver function tests (LFTS) in rats exposed to EMR in a frequency-dependent manner.[10]

An imbalance between free radicals and antioxidants plays a crucial role in causing histopathological damage in the liver. Disruptions in lipid inclusions and fat metabolism have been linked to increased cytoplasmic vacuolation. Additionally, damage and fragmentation of the rough endoplasmic reticulum around the mitochondria may contribute to the accumulation of fat droplets, resulting in intracellular vacuolation of hepatocytes, which is more pronounced in rats exposed to 5G EMF.[9,16] Sinusoidal congestion and dilatation may be associated with increased vascular permeability, as seen by the buildup of blood and fluid in sinusoids, causing sinusoidal dilatation. Given that the liver's health depends on appropriate blood flow and drainage, such alterations may result in hepatic dysfunction.[16]

These findings align with some previous research suggesting that EMF may have adverse effects on liver structure and function by inducing oxidative stress. Muradpour observed oxidative stress leading to severe damage to the liver tissue structure, including sinusoidal dilatation, Kupffer cell proliferation, congestion, and inflammatory cell infiltration around the portal region after exposure of healthy mice to mobile phone radiation. Additionally, other research has linked EMR exposure to severe liver damage.

Azam Vafaei demonstrated that whole-body exposure to electromagnetic radiation at frequencies of 1800 and 2100 MHz, emitted by cell phones, can induce oxidative stress. This exposure alters oxidant-antioxidant balance and results in DNA strand breaks and oxidative DNA damage in the liver of rats.[17]

Conclusions

In conclusion, this study highlights significant alterations in liver health associated with electromagnetic radiation exposure, such as sinusoidal congestion, dilatation and hepatocyte vacuolation. These findings contribute to the growing body of evidence suggesting possible adverse effects of EMF on liver structure and function. Nevertheless, further research, particularly with human participants, is essential to validate these results and understand the underlying mechanisms. This will be important for developing public health recommendations and safety guidelines regarding mobile phone use.

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