Glutathione Effects on Liver Enzymes and Serum Electrolyte in Mice Exposed to 850–1900 MHz Mobile Phone Radiation

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Abstract

The widespread presence of electromagnetic fields (EMF) produced by modern technologies may pose a significant threat to animal health. Ionizing radiation, a byproduct of EMF exposure, has the potential to contribute to serious diseases, including cancer. This study examines the impact of chronic exposure to 900-1800 MHz GSM-EMF-induced electromagnetic radiation on liver enzymes and serum electrolytes in mice, and the potential mitigating effects of exogenous glutathione (GSH) administration. Thirty-five adult male mice were randomly divided into seven groups and exposed to various modes of mobile phone radiation for five weeks, with or without GSH administration. Liver enzymes, Alkaline phosphates (ALP), Alanine transaminase (ALT) , Aspartate aminotransferase (AST) and, serum electrolytes, sodium, potassium and bicarbonate were analyzed. Results showed significant increases in ALP levels in the Silent, Ringtone + GSH, and Silent + GSH groups compared to control group, while ALT and AST levels remained largely unchanged with the exception of some groups across the experimental cohorts. Serum electrolyte concentrations did not significantly differ across the control group. Our study found that chronic mobile phone radiation exposure in mice generally does not significantly affect liver enzyme levels or serum electrolyte concentrations. However, certain combinations with GSH administration notably increased some biochemical markers like ALP, ALT, and AST levels. Our study concluded that chronic mobile phone radiation had no significant impact on liver enzymes or serum electrolytes in mice. However, glutathione administration in combination with radiation increased liver enzyme levels, suggesting a potential role in oxidative stress mitigation. Further research is needed to explore these findings.

Keywords: Mobile phone radiation, Glutathione, Liver enzymes, Serum electrolytes, Physiology

Background

The widespread presence of electromagnetic fields (EMF) generated by contemporary technologies, encompassing common household appliances and mobile devices, presents a palpable threat to animal health. Research indicates that ionizing radiation (IR), a byproduct of EMF, may serve as a precursor to future cancer development, as evidenced by studies linking elevated rates of chromosome abnormalities and micronuclei occurrence in populations exposed to radiation from various sources such as nuclear incidents, environmental contaminants, and medical diagnostic procedures like computed topography (CT) scans and x-rays (Imai *et al*. 2019). Such exposure to radiation triggers oxidative stress (OS) within bodily tissues (Salameh et al. 2023), emphasize the potential health consequences associated with prolonged EMF exposure. This phenomenon arises from an imbalance between the levels of reactive oxygen species (ROS) and the capacity of the antioxidant system to mitigate their effects (Kıvrak et al. 2017). Moreover, OS levels can escalate during different stages of development (Jauniaux et al. 2000). Various physiopathological and physiological conditions, nutritional factors (Newsholme et al. 2016), and exposure to external pollutants like electromagnetic radiation (EMR) (Syaza et al. 2017) can contribute to heightened OS status. Moreover, Non-ionizing Radiation (NIR) spans from extremely low frequency to visible light and IR encompasses higher frequencies (Syaza et al. 2017).These radiations primarily impact the liver, kidneys, and brain due to their proximity to mobile phones during daily use (Salameh et al. 2023). The increasing use of multi-slice CTs also further amplifies concerns, especially for children, who are at a higher risk of radiation-induced malignancies due to their longer lifespan (Kutanzi et al. 2016). Additionally, research reveals the potential adverse effects of electromagnetic frequencies emitted by mobile phones, including DNA damage, increased cancer susceptibility, OS, chromosomal abnormalities (Akdag et al

2016; Cam and Seyhan 2012; Chauhan et al. 2017; Dasdag et al. 2015; Deshmukh et al. 2013; Megha et al. 2015), and hormonal and physiological imbalances.

Numerous studies indicate that EMR increases the production of ROS, leading to various physiological effects, such as DNA damage. IR, due to its ability to rapidly ionize cellular structures, poses a significant risk to animal health, and capable of causing immediate harm. On the other hand, NIR, like that emitted from devices such as mobile phones, lacks ionizing power but can still present risks with long-term exposure, which is particularly important to consider regarding its interaction with the body (IARC 2000) tissues. Many researchers agree that NIR carries substantial health risks (Omer 2021). The potential health consequences of electromagnetic pollution and the growing awareness of EMF exposure underscore the need for increased vigilance (Bortkiewicz 2019) to animal exposure.

The liver, the body's largest internal organ, plays a pivotal role in detoxification, ridding the body of harmful toxins (Casas-Grajales and Muriel 2017). Yet, the detrimental effects of free radicals generated during hepatic metabolism can largely be counteracted by various antioxidant enzymes and non-enzymatic mechanisms (Kıvrak et al 2023; Martínez-Sámano et al 2010). Nevertheless, a decline in the antioxidant system's response, coupled with an overproduction of ROS from radiation, may escalate hepatic OS, leading to liver injury. EMR emitted by 900 MHz cell phones has been shown to impact the expression of Nrf2 protein, trigger oxidative damage, and alter the morphology of liver cells (Luo et al. 2014).

Electrolytes are electrically charged molecules that play pivotal roles in regulating acid-base balance, blood clotting, and the contraction of muscles and body fluids. Within the body, several common electrolytes serve specific and vital functions, collectively maintaining the balance of fluids between intracellular and extracellular environments. This equilibrium is crucial for

hydration, nerve impulses, muscle function, and pH levels. Among the major electrolytes are Potassium, Sodium, Magnesium, Calcium, and Chloride. Notably, Serum Sodium, Potassium, and Chloride are fundamental determinants of the electrophysiological properties of myocardial membranes (Kughapriya and Evangeline 2016; Onyiriuka andOyenusi 2018). In the body, serum electrolytes (SE) perform essential roles in numerous physiological and metabolic processes. Particularly among acutely ill individuals, including children, adolescents, and animals, fluctuations in serum sodium and potassium concentrations are prevalent, often leading to heightened morbidity and mortality (Ibinda et al. 2016). SE concentrations constitute key tests for evaluating a patient's clinical condition and are closely linked to morbidity and mortality. Any deviations from the normal range of electrolyte levels in the body are termed electrolyte disorders (Timerga et al. 2020). Despite their critical importance, electrolyte disorders in the context of EMF exposure, encompassing both IR and NIR, have received scant attention. However, these disorders hold significant potential in influencing the initiation, diagnosis, and prognosis of diseases (Liamis et al. 2024).

Glutathione, a potent antioxidant, plays a crucial role in reducing oxidative stress by neutralizing free radicals and regenerating other antioxidants. It supports the immune system, detoxifies the body, and maintains cellular health (Pizzorno 2014). By mitigating oxidative damage, glutathione helps protect against stress-related cellular dysfunction and enhances overall resilience to stressors (Wu et al. 2004) like that of daily mobile radiation exposure.

As technology becomes increasingly integrated into our daily lives, there's a notable gap in research concerning the effects of radiation on liver enzymes and electrolyte balance, both of which are closely linked to elevated mortality and morbidity rates. Therefore, this study aims into investigate the repercussions of EMR exposure on liver enzymes and serum electrolytes.

The research focuses on exploring the impact of daily exposure to GSM-EMFs and 900-1800 MHz-induced EMR on OS levels in animal model (mice) and the impact of GSH to such exposure and to shed light on the potential physiological consequences and implications for mice which might also serve as preliminary study for human's health as well.

Methodology

Animals and grouping

The animals involved in the study were maintained and used in accordance with the Animal Welfare Act and the Guide for the Care and Use of Laboratory animals prepared by the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC), and ethical approval was obtained from the ABUCAUC with the approval number of ABUCAUC/2021/005.

At the start of the experiment, thirty five (35) apparently healthy adult male mice between the ages of 8-12 weeks were obtained from the animal house facility of the Department of Human Physiology, College of Medical Sciences, Ahmadu Bello University, Zaria, Nigeria.

Animal welfare and Anesthetic protocol

The animals were housed in standard polypropylene cages and were maintained and allowed free access to feed (standard mice feed) and water 24/7 and 12h alternate day light and darkness. The animals were allowed to acclimatize to the environment of the behavioral laboratory for two weeks before the commencement of the experiment. During the cause of the experiment, they were handled under anesthetic protocol of Ketamine (100mg/kg) and Diazepam (5 mg/kg) and were administered through intraperitoneal route to minimize pain and distress and at the end of the experiment, they were humanly euthanized by cervical dislocation, also under the same anesthetic protocol to minimize pain and distress in accordance to animal welfare act.

As shown in Table 1, the animals were randomly selected and divided into seven (7) groups of five (n=5) mice per group as shown below

Table 1: Showing different groups of mice used during the experiment

GROUP	CATEGORY
I	Normal control group
II	Group exposed to mobile phone radiation in ringtone mode
III	Group exposed to mobile phone radiation in vibration mode
IV	Group exposed to mobile phone radiation in silent mode
V	Group exposed to mobile phone radiation in ringtone mode plus glutathione (GSH)
VI	Group exposed to mobile phone radiation in vibration mode plus glutathione and the last group
VII	Group VII were exposed to mobile phone radiation in silent mode plus glutathione

Radiation exposure and Glutathione administration

Radiation exposure was done based on the method used by Kesari *et.al.* (Kesari e al. 2010) with little modification. In this study we used Itel mobile phone (it2160) with specific absorption rate (SAR) of 2W/kg and 2G Network Bands, of frequency 850-1900 MHz, the animals in groups II to VII were all exposed to 4 hours mobile phone radiation of an average of 300 missed calls per day for six (6) weeks. Animals in groups V, VI and VII were administered glutathione (250 mg/kg) thirty minutes prior to mobile phone radiation daily. Glutathione was administered orally with cannula at a dosage doubled that of intraperitoneal administration. Higher oral doses were used to achieve comparable systemic levels of the drug throughout the experiment. Therefore, a 250 mg/kg oral dose compensates for the reduced bioavailability and ensures an adequate amount of glutathione reaches systemic circulation during the course of the experiment. The oral route was chosen to overcome differences in bioavailability, first-pass metabolism, and gastrointestinal degradation, while also ensuring the welfare of the study animals. This approach ensures that therapeutic levels of glutathione are achieved, maintaining the efficacy of the treatment while minimizing potential stress and harm to the animals throughout the experiment. .

Although, this route diverges from the common approach used by Zhang *et al. (2008)*, who used intraperitoneal administration (100mg/kg) with a faster absorption rate.

Sample collection and storage

The animals were humanely euthanized by cervical dislocation under anesthesia by an expert (AHU). Blood was collected via cardiac puncture. Immediately after collection, the blood was transferred to micro centrifuge tubes and allowed to clot at room temperature for 30 minutes. The tubes were then centrifuged at 2,000 revolutions per minute for 10 minutes to separate the serum. The serum was carefully transferred using a pipette into aliquot tubes without disturbing the cell pellet. The serum samples were stored at -80°C until they were analyzed. Each sample was labeled with its unique ID and the date of collection. Liver samples were collected by same expert (AHU) who performed a midline laparotomy to expose the liver. The liver was then excised using sterile scissors and forceps, followed by rinsing in cold phosphate-buffered saline (PBS) to remove any blood. All the liver tissue was then snap-frozen in liquid nitrogen immediately after collection to preserve enzyme activity and prevent degradation. They were then transferred to a -80°C freezer until analysis.

Determination of Liver enzymes

ALP was analyzed using the method described by Bassey *et al.* (1946) and modified by Wright *et al.* (1972), with Randox kits. In this procedure, 10 µl of the sample was mixed with 500 µl of the ALP reagent in a cuvette, and absorbance at 405 nm was recorded over 3 minutes. ALP (IU/l) was calculated using the formula 2742 × ΔA 405 nm/min. ALT was measured using the method by Shaw *et al.* (1978) with Randox kits. Here, 50 µl of the sample and 500 µl of the ALT reagent were mixed, and absorbance at 340 nm was recorded over 3 minutes. ALT (IU/l) was calculated using the formula 1746 × ΔA 340 nm/min. AST, also measured using Shaw *et al.*

(1978) with Randox kits, followed a similar procedure, and AST ((IU/l)) was calculated with the same formula 1746 × ΔA 340 nm/min.

Determination of Serum electrolytes

Serum sodium and potassium concentrations were measured using the flame emission photometry method described by Magoshes and Vallee (1956). This involves desolvating a solution by a flame, which leaves solid salts that dissociate into neutral ground state atoms. These atoms become excited and emit light at characteristic wavelengths (590 nm for sodium and 770 nm for potassium) as they return to the ground state. The emitted light passes through a filter onto a photosensitive element, producing a measurable current. Bicarbonate ion concentration was determined using the titration method by Van Slyke and Neil (1924), where carbon dioxide is released from bicarbonate ions by adding dilute hydrochloric acid, and the remaining acid is titrated with sodium hydroxide, using phenol red as an indicator.

Statistical analysis

All data obtained from the study were expressed as mean ± standard error of mean (SEM). Analysis was carried out using the statistical package for social sciences (SPSS) version 27. *P-value* was gotten by using a two-sample t-test to compare the control group to each experimental group, followed by Tukey's post-hoc test for multiple comparisons. Values of p □ 0.05 were considered statistically significant.

Results

Liver enzymes

Alkaline Phosphate (ALP)

The impact of chronic exposure to mobile phone radiation, coupled with GSH administration, on ALP levels in mice was analyzed. Statistical analysis revealed no significant differences between the control group (25.97 ±3.09 (IU/L)) and other experimental groups, except for the Silent (141.13±12.87 (IU/L)), Ringtone + GSH (71.09±6.38 (IU/L)), and Silent + GSH (80.73±17.21 (IU/L)) groups with mean difference of 115.16, 45.12 and 54.76 for the significant groups respectively with *p*-values < 0.0001as shown in (Fig 1a).

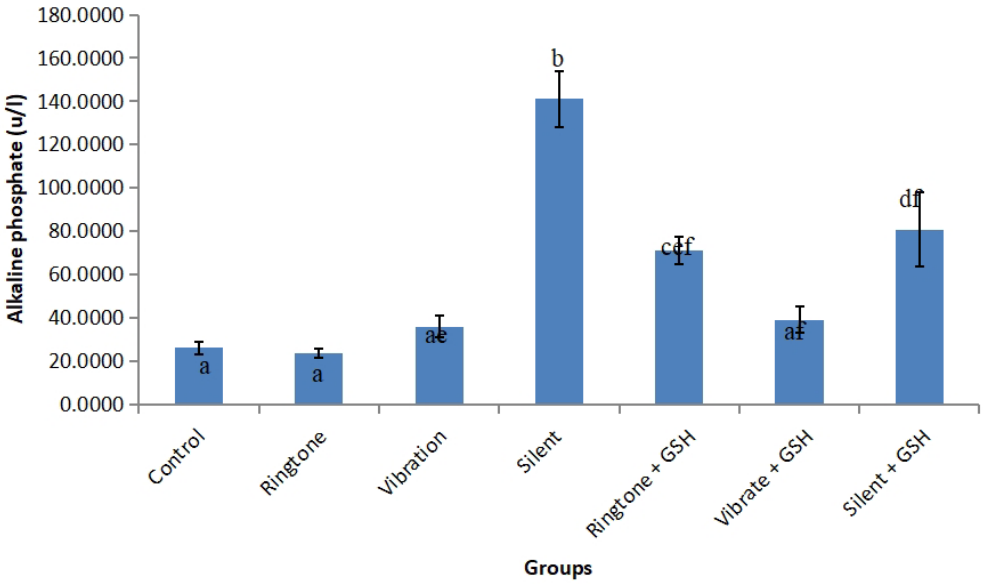

Figure 1a: Effect of chronic exposure to mobile phone radiation and GSH administration on serum ALP in mice in comparison with control group and with *p*-values of < 0.0001

Alanine Aminotransferase (ALT)

The impact of chronic exposure to mobile phone radiation, alongside GSH administration, on ALT in mice was analyzed. Statistical analysis revealed no significant differences (p>0.05) between the control group Control (111.0±10.47 (IU/L)) and the other experimental groups, except for the Ringtone + GSH (145.5±12.10 (IU/L)) and Vibration + GSH (148.75±23.57

(IU/L)) groups with mean difference of 34.5 and 37.75 for the significant groups respectively with *p*-values < 0.01 and < 0.05 as shown in (Fig 1b).

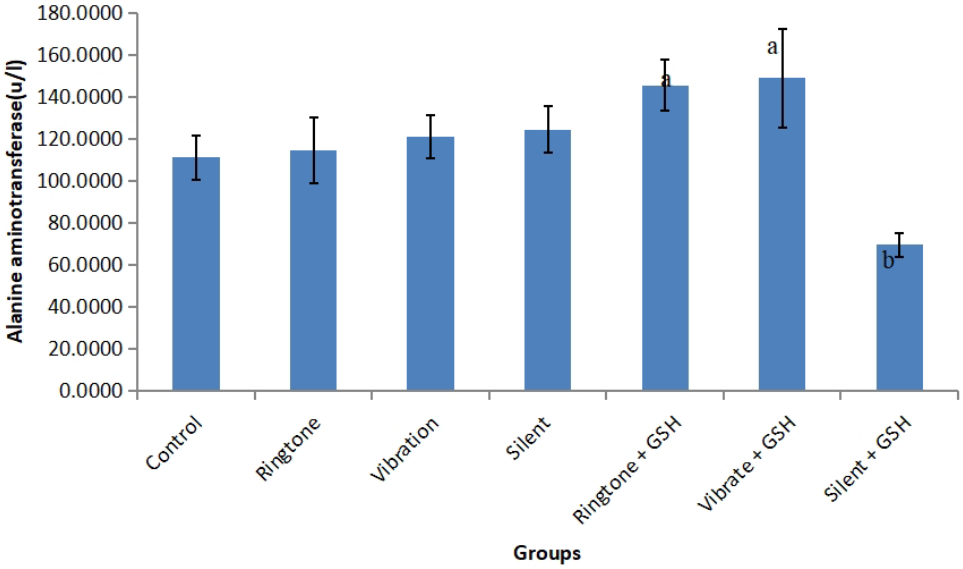

Figure 1b: Effect of chronic exposure to mobile phone radiation and GSH administration on serum ALT in mice in comparison with control group and with *p*-values of < 0.01 and 0.05

Aspartate Aminotransferase (AST)

The impact of chronic exposure to mobile phone radiation, coupled with GSH administration, on AST in mice was analyzed. Statistical analysis revealed no significant differences ($p>0.05$) when comparing the control group (262.25±17.79(IU/L)) to the other experimental groups except the vibration+GSH group (392.25±58.41(IU/L)) with mean difference of 130.0 for the significant groups respectively with *p*-value < 0.01as shown in (Fig 1c).

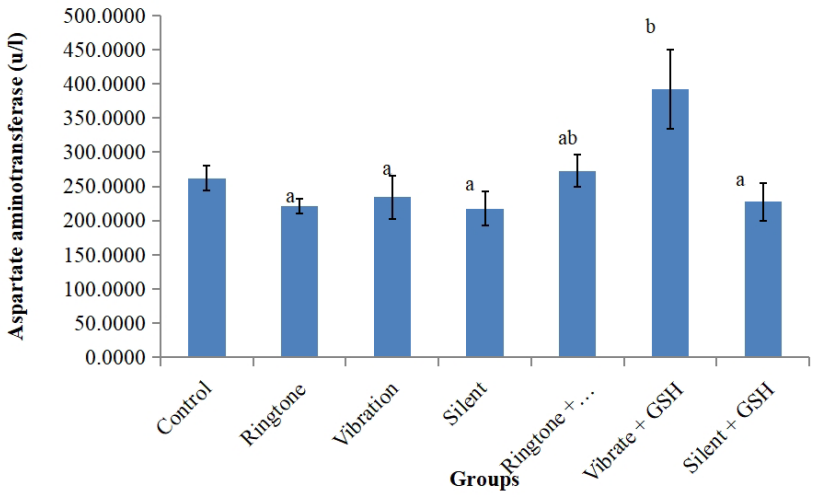

Figure 1c: Effect of chronic exposure to mobile phone radiation and GSH administration on serum AST in mice in comparison with control group and with *p*-values of < 0.01

Serum electrolytes

Sodium ion Concentration

The impact of chronic exposure to mobile phone radiation, alongside GSH administration, on serum sodium ion concentration was analyzed. No significant differences were observed ($p > 0.05$) when comparing the control group with the entire experimental groups as shown in (Fig 2a).

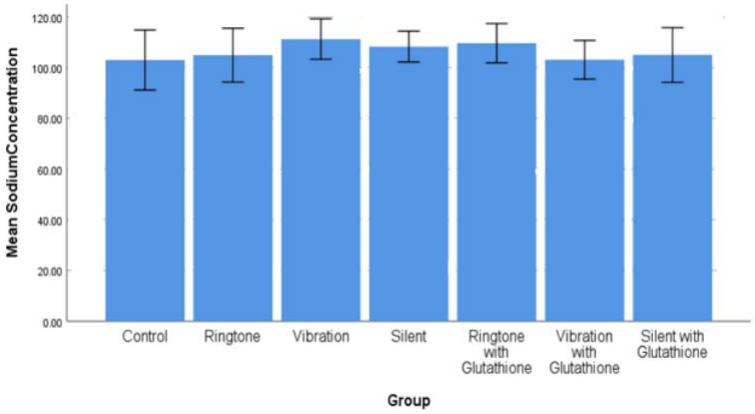

Figure 2a: Effect of chronic exposure to mobile radiation and GSH administration on serum sodium ion concentration in mice

Potassium ion Concentration

The impact of chronic exposure to mobile phone radiation, coupled with GSH administration, on serum potassium ion concentration was analyzed. Result revealed no significant differences statistically ($p > 0.05$) when comparing the control group with the various experimental groups as shown in (Fig 2b).

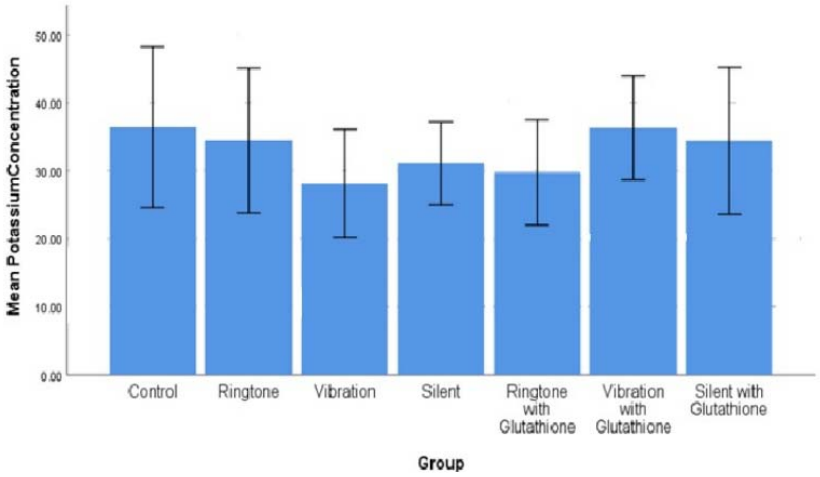

Figure 2b: Effect of chronic exposure to mobile radiation and GSH administration on serum potassium ion concentration in mice

Bicarbonate Concentration

The impact of chronic exposure to mobile phone radiation, alongside GSH administration, on serum bicarbonate concentration was analyzed. No significant differences ($p > 0.05$) when the control group was compared with the various experimental groups as shown in (Fig 2c).

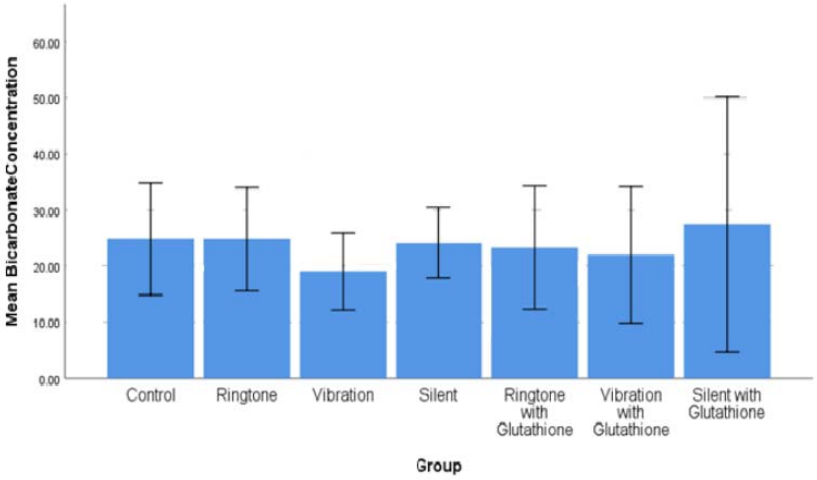

Figure 2c: Effect of chronic exposure to mobile radiation and GSH administration on serum bicarbonate ion concentration in mice

Discussion

The utilization of mobile phones stands as one of the most rapidly advancing technological fronts, prompting concerns regarding the interplay between EMR and liver function (Kesari et al. 2010). Amidst the different applications of low-intensity, extremely low-frequency EMF in combating ailments like malaria and cancer (Lai and Singh 2010), the utilization of radiofrequency radiation (RFR) is emerging as a promising noninvasive strategy for tumor therapy, capitalizing on its ability to induce shock responses and apoptosis in human cancer cells (Maeda et al. 2004). Nonetheless, findings by Costa *et al*. (2011) suggest that even minute levels of EMF, modulated at specific frequencies, might disrupt the growth of Hep G2 cells. However, their study delves into the in vitro mechanisms elucidating the antiproliferative impact of such low-intensity EMF on Hep G2 cells (Costa *et al*. 201; Zimmerman et al. 2012). Moreover, various studies have explored the repercussions of EMF exposure on the delicate balance of

antioxidants and oxidants in vital organs and tissues such as the liver, heart, and lens (Irmak et al. 2002; Esmekaya et al. 2011; Balci et al. 2007). Despite this wealth of research, scant literature exists on the influence of EMF radiation on physiological parameters exposure such as serum electrolytes and liver enzymes, particularly regarding the interplay with GSH.

A previous study conducted by Kosanic *et al.* (2001) reported the effects of vitamin C, a potent antioxidant, on individuals exposed to Wi-Fi radiation for one day. Their findings unveiled a significant rise in blood glucose levels and a concurrent decrease in triglyceride levels compared to the control group. However, no notable impact was observed on cholesterol levels, low-density lipoprotein (LDL), high-density lipoprotein (HDL), or hepatic enzyme activities such as ALP, AST, and ALT relative to the control group. This finding suggests that the observed effects could potentially stem from the antioxidant properties of vitamin C. In our study, where we investigated the influence of GSH, a compound closely related to vitamin C in its role as an antioxidant, on radiation exposure from different modes of phone activity, distinct patterns were noted. Notably, a significant increase in ALP was only noted in the Silent, Ringtone+GSH, and Silent+GSH modes, while other modes exhibited no significant increase.

Despite GSH's known physiological and metabolic roles and its capacity to shield against radiation-induced damage by scavenging free radicals and converting them into non-radical forms (Kosanic et al. 2011; Hasan et al. 2009), our findings revealed a non-significance difference in most of the group aside the one mentioned earlier. This could arise from an upsurge in free radical production in those groups, and a decline in antioxidant defense systems, or a combination of both factors. The lack of significant effects observed in ALP, ALT, and AST across most phone modes may be attributed to prolonged exposure to mobile phone radiation, which has been linked to increased serum ALT and AST levels, as reported by Li et al. (2015)

and Rahman et al.(2022) . However, insights from Fatima *et al*. (2017) and colleagues shed further light, revealing that rats exposed to radiation over a two-month period, three days per week, exhibited heightened liver enzyme activity, elevated levels of MDA and H202, and decreased antioxidant levels such as GSH in liver tissue. While this aligns partially with our findings, it's worth noting differences in exposure duration between our study and theirs.

Our findings is also similar to the conclusions drawn by Nersesova *et al.* (2014), who elucidated that despite observing changes in enzyme activity levels of liver-sensitive enzymes in both liver tissue and blood serum upon radiation exposure, no discernible effects on hepatocytes cell membrane permeability were evident. Instead, they noted alterations in energetic metabolism. However, despite these observations, correlation analysis of post-radiation enzyme activity level changes failed to reveal a clear relationship among them. Our study aligns with the findings by Ma *et al*. (2014), who reported no significant changes in levels of ALT, AST, and AST/ALT in the radiated group. Notably, they observed hepatocytes nuclear atrophy and cytoplasmic vacuolar degeneration in histological sections, which was not explored in our study.

Additionally, in Cetin *et al.*'s (2014) investigation on brain and liver GSH peroxidase activities, along with liver vitamin A and β-carotene concentrations, they noted a decrease in GSH peroxidase levels in the EMF radiated groups. Conversely, brain iron, vitamin A, and β-carotene concentrations increased in the EMR groups, a result consistent with some outcomes from our phone model assessments on glutathione's role in mitigating EMF radiation effects on liver enzymes.

Furthermore, our results share similarities with the findings of Nwokocha *et al*. (2012). In their study, they observed that AST liver enzymes elevated with increasing radiation, which is also observed in some certain phone models in our study, especially vibration+GSH, where AST

liver enzyme levels elevated with increasing radiation, while others within the same category did not exhibit statistical significance in our study. Similarly, they found that ALT levels increased with radiation, which is also similar with our ringtone+GSH and vibration+GSH models, while others in this same category showed no significant changes. Notably, our study also identified an increase in AST liver enzyme levels, particularly in the vibration+GSH model, an aspect not explored in their research. However, the remaining phone models in our study, including ringtone, vibration, silent, ringtone+GSH, and silent+GSH, did not show any significant increase. Vibration+GSH mode shows similar findings in both ALT and AST, and this might be that more radiation were emitted while the phone vibrate compare to when the phone is on silent or ringtone, although, vibration+GSH in ALP did not show similar trend.

Almost similar to our findings, Nwokocha *et al.* (2012) reported that serum electrolyte concentrations remained within normal ranges following total body irradiation, this findings aligned with our results as presented earlier in our results. Specifically, sodium, potassium, and bicarbonate levels were found to be statistically not significant than the control group after irradiation.

Conclusion

In conclusion, our study found that chronic mobile phone radiation does not significantly affect liver enzyme levels or serum electrolyte concentrations in mice across all groups. However, certain combinations with glutathione administration notably increased ALP, ALT, and AST levels which help in mitigating OS level. Also, our study found no significant differences in all serum electrolytes between the control group and the experimental groups exposed to chronic mobile phone radiation with or without glutathione administration. These findings indicate that

such radiation exposure, even when combined with glutathione supplementation, does not substantially influence these serum electrolyte levels. Overall, our findings suggest minimal immediate impact on specific biochemical markers but highlight the need for further research to understand the underlying mechanisms and potential health implications of chronic mobile phone exposure.

Availability of data and materials

All datasets related to this manuscript have been provided and uploaded as supporting information files.

Ethical statement

This study was conducted at the Department of Human Physiology, Faculty of Basic Medical Sciences of Ahmadu Bello University, Zaria. Approval for the project was granted by the Ahmadu Bello University Ethical Committee on Animal Use and Care, with the assigned approval number ABUCAUC/2021/005. All ethical protocols concerning the handling, care, and welfare of the animals were meticulously observed throughout the study, from its inception to its conclusion.

Consent for publication

All the listed authors approved the final version of this manuscript and consent to its submission for publication. We affirm that the manuscript is original, has not been published elsewhere, and is not under consideration by any other publication. We also declare that all data, images, or other materials included in the manuscript, where applicable, have been obtained with proper

authorization and that necessary permissions have been secured for their publication. By granting this consent, we accept the journal's policies regarding copyright, licensing, and publication ethics.

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