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Effect of Different Intensities of Radiofrequency-Electromagnetic Radiation (RF-EMR) from Mobile Phone on Biological Stress Markers in Lactating Wistar Rats

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Abstract

Background: The increase in global exposure to intensities of RF-EMR from mobile phone has been linked to rise in body temperature (BT) and alterations in biological stress markers (BSM), particularly during pregnancy and lactation, but with paucity of scientific validation.

Objectives: This study therefore assessed the effect of different intensities of mobile phone radiation on BT and BSM in lactating Wistar rats.

Methods: Twenty lactating Wistar rats (LWR) were assigned into five groups (n=5). From the first to the twenty-first day of lactation, the animals were exposed to cell phone radiation frequencies. There was no radiation exposure for Group A (Control). Groups B-E were given access to 900MHz talk mode (TM), 1800MHz standby mode (SBM), 1800MHz TM and multiparous at 1800 MHz TM respectively. Thereafter, the levels of cortisol, superoxide dismutase (SOD), and body temperature were measured.

Results and Conclusion: The exposure of animals to all intensities of RF-EMR significantly ($p<0.05$) elevated serum cortisol level when matched with the control (13.84±0.83). While the 900 MHz TM and 1800MHz SBM primiparous rats did not significantly ($p>0.05$) alter superoxide dismutase (SOD) activity and body temperature, the primiparous and multiparous rats at 1800 MHz TM significantly ($p<0.05$) elevated serum SOD activity and BT when compared with the control value. The study revealed that the exposure of LWR to different intensities of RF-EMR elevated BT and BSM. This increase may be due to the ability of RF-EMR to generate reactive oxygen species and temperature spike, which may predispose the animals to oxidative stress.

Keywords: Radiofrequency electromagnetic radiation (RF-EMR), Body temperature, Mobile phone, Lactation, Superoxide dismutase.

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INTRODUCTION

These days, people are continuously exposed to radiofrequency electromagnetic radiation (RF-EMR) because of advancements in technology and the growing usage of electronic devices. Natural sources such as the sun and earth also emit radiation on a regular basis, in addition to radiation from man-made sources (1,2). Numerous studies have demonstrated that, depending on their energy, electromagnetic radiations have a range of significant effects on various bodily parts. Concerns regarding the effects of radio waves are growing as concerns about society's modernity rise. Electrical equipment, transmission lines, and generating plants can all be powered by these non-ionizing electromagnetic radiation waves, which are also released by industrial producing sources (3).

The relevance of RF-EMR to mobile phone cannot be overemphasized. Mobile phones utilize RF-EMR to transmit and receive data. Exposure to certain intensities of RF-EMR from mobile phones has raised concerns about potential health effects, including thermal damage, cellular stress, and even possible links to certain health problems (4). Strategies to minimize exposure to RF-EMR include the use hands-free devices, increasing distance, limiting call time and carrying phones away from the body (5,6). Exposure to RF-EMR from mobile phone can cause oxidative stress by increasing the production of free radicals and reactive oxygen species (ROS) within cells, which can damage cellular components like DNA and proteins (7). The imbalance between ROS production and the body's antioxidant defenses can lead to oxidative stress and cellular damage (8).

While biological stress refers to the general reaction of the body to changes that disrupts its normal state, especially during lactation, the biological system responds to the harmful effect of oxidative stress through the activities of antioxidants to maintain and restore normalcy. Antioxidants can play a protective role to mammary cells and allied tissues against the potential oxidative stress caused by exposure to RF-EMR by neutralizing free radicals and reducing oxidative damage to these cells. Antioxidant and oxidative stress markers that can be examined following RF-EMR exposure during lactation are superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), cortisol, glutathione peroxidase (GPx) among others. The assessment of stress markers during lactation is beneficial as it indicates the imbalance between exposure to RF-EMR and antioxidant level (9).

Documented reports on the effect of RF-EMR exposure to oxidative and antioxidative stress include the study by Salameh et al. (11) who investigated the effect of GSM-EMR exposure to Sprague–Dawley pregnant rat liver for oxidative stress indices at 900MHz. Kıvrak et al. 2017 (10) examined the EMR on antioxidant defense system. Salameh et al. (12) observed increased oxidative stress status following exposure to 900MHz GSM-modulated during prenatal and postnatal periods. Koohestanidehaghi et al. (13) studied the harmful effects of radiofrequency electromagnetic waves on oxidative stress and other related indices in mouse preimplantation embryos. EL-baeth et al. (14) observed an increase in cortisol level and a decrease in behavioural and other performance markers upon exposure to 900 MHz from third generation Huawei mobile phones in quail chicks. Zheng et al. (15) studied the biological effects of exposure to 2650 MHz electromagnetic radiation in mice. The above reports have emphasized on the effect of exposure to RF-EMR on oxidative stress indices without focusing on the effect of these radiations during lactation creating a knowledge gap. This study therefore evaluated the effect of different intensities of RF-EMR from mobile phone on biological stress markers in lactating Wistar rats.

METHODOLOGY

Experimental Animals

Twenty-five Wistar rats weighing between 190 and 260g were used in the study. The Wistar rats were purchased from the Animal House of the Department of Physiology, University of Jos, Plateau State, Nigeria and raised in the animal house of College of Health Sciences, University of Abuja, Nigeria. They were housed in well-ventilated metallic cages with saw dust beddings maintaining normal room temperature of 28-32 °C, relative humidity of 50-70%. The animals also had access to food (normal rat feed pellets manufactured by Vital feeds Plc, Nigeria) and water ad libitium. All housing and care were consistent with the guide for the care and use of Laboratory animals of 2011 (8th edition).

Ethical Approval

Ethical approval was obtained from the University of Abuja Ethics Committee on Animal Use with reference number (UAECAU/2020/0004).

RF-EMR Exposure

The method previously used by Onaadepo et al. (15) was adopted for exposing the animals to RF-EMR from mobile phones. Briefly, freely moving rats were exposed to RF-EMR from mobile phone in GSM quad band: 800/900/1800/1900 MHz. Itel 2060 and Itel P13 (Transsion Holdings, Honk Kong) were used as the sources of RF-EMR (Figures 1a and b). The batteries statuses were frequently checked and the phones were equipped with power bank to ensure power supply.

RF-EMR exposure from mobile phones was performed for 21 consecutive days (lactation period) at random. A mobile handset was placed in each cage with the farthest distance of 30 cm and exposure was for 10 hours a day between 7:00 am and 5:00 pm. Animals were exposed to RF-EMR from the mobile phones in standby and active talk mode at varying frequencies of 900MHz and 1800MHz. Calls were made 15 times with two minutes duration daily and text messages 10 times a day for animals in active talk mode and phones were left on without any calls or messages for the standby mode (Table 1). The control group of animals were not exposed to any phone radiation and were in a separate part of the room away from all effective radiations.

Measurement of RF-EMR

Radio frequency electromagnetic meter was used in the measurement of RF-EMR emitted from the phones. The detector (Model: GM 3120, BENETECH Company, China) measurement range was calibrated according to the electronic company. It is a highly sensitive device capable of measuring frequency ranging from 5Hz -3500 MHz, has a memory function, easy and safe to use. The meter measures the value of electric field and magnetic field. The Smart RF-EMR meter was used to measure the radiation level emitted from the phones and the radiation level in the room and lack of effective radiation from external interference was confirmed. The radiation was measured three times at three different distances from the mobile phone with the RF meter to verify an adequate RF-EMR output. The meter measured the value of electric field and magnetic field and the power density was calculated using the Poynting theorem (17-19) as shown below:

Pd = E- ×H-

Where:

Pd = the power flux density expressed in watt per meter squared (W/m2);

E = the electric field strength in volt per meter (V/m);

H = magnetic field strength in ampere per meter (A/m).

Measurement of Temperature

The temperature of the rats was taken twice daily (morning and evening) before exposure to RF-EMR and after 12 hours of exposure to RF-EMR. The temperature was taken with a body infrared thermometer (Model No: DT 8861, Cocare Company, Sussex, England) with accuracy of ±0.2 0C (0.36 0F).

Laboratory Analysis

Cortisol and SOD assays were done at the Labcrest Laboratories, Gwagwalada, Abuja by adopting standard protocols.



Figure 1a: RF-EMR Device for 900MHz



Figure 1b: Smart RF-EMR Device for 1800MHz

Animal Grouping

Using the information obtained from the measurement of body weight, twenty-five lactating Wistar rats were divided into five groups (n=5) (Table 1). From the first to the twenty first day of lactation, the rats were exposed to different intensities of RF-EMR (Table 1). Thereafter, selected serum oxidative stress indices and body temperature were measured using standard protocols.

Blood Collection and Preparation of Serum Supernatant

Blood samples were collected through the retro orbital vein with plain sample bottles and were thereafter separated using a Centrifuge (Farfly Energy Technology Co., Ltd, Model: ZJY-3, ShangHai, China) at 2000 g for 10 minutes to obtain a solution. The upper portion of the solution was carefully decanted to obtain serum. The serum was them refrigerated for 12 hours until it is used for the determination of SOD activity and cortisol concentration.

Table 1: Group of animals and their treatment for 21 days.

Group (n=5)	Treatment in Hz
A: PLR	No. RF-EMR exposure
B: PLR	RF-EMR 900MHz talk mode (GSM 900MHz +15 phone calls + 10 text messages)
C: PLR	RF-EMR 1800MHz standby mode (10 text messages+ internet connection)
D: PLR	RF-EMR 1800MHz talk mode (15 phone calls +Text 10 text messages + internet connection)
E: MLR	RF-EMR 1800MHz talk mode (15 phone calls + 10 text messages + internet connection)

PLR = primiparous lactating rats: MLR = multiparous lactating rats

Procedure for Estimation of Serum Cortisol Concentration

Blood samples were also collected on the postnatal day 21 for the determination of serum cortisol. Cortisol level determination was with Enzyme-linked immunosorbent assay (ELISA) and performed with serum from control and RF-EMR–exposed animals using the Cortisol rat ELISA kit (Calbiotech Inc CA, USA, CO 103S) according to the manufacturers manual. Principle was based on what was described by Turpeinen and Hamalainen, (20). The microplate was pre-coated with cortisol and placed in the holder. 25µl of cortisol standards, control and experimental sera and 200 µl of cortisol enzyme conjugate was added to the wells and thoroughly mixed for 10 seconds. This was then incubated for 60 minutes at 25 0C. Liquids were removed from the wells and washed thrice with 300 µl of 1X wash buffer and then blotted on absorbent paper towels and 100 µl of tetra-methyl benzidine (TMB) substrate added to all the wells. The absorbance was then read at 450 nm within 20 minutes after adding the stop solution.

Procedure for Estimation of Serum Superoxide Dismutase Activity

Blood samples were also collected on the 21st day of parturition for the determination of SOD. SOD activity was assayed using the North West Life Science Specialties (NWLSS) SOD assays kit (Product: NWK-SOD02, Specificity: Cu/Zn, Mn and Fe superoxide dismutase, Sensitivity: 5 U/mL). The method was based on the principle of superoxide inhibition of auto-oxidation rate of hematoxylin as originally described by Kazlou, (21) with modifications to increase robustness and reliability. In brief, 920 µL of assay buffer was added to each cuvette. This was followed by addition of 40 µL of assay buffer (for blank) and 40 µL of sample. The mixture was incubated for two minutes after which 40 µL hematoxylin reagents was added and mixed quickly to start the auto-oxidation reaction. An absorbance was measured at 560 nm for every 10 seconds for 5 minutes.

Statistical Analysis

Values are given as mean ± Standard Deviation (SD) of five replicates per group. All data obtained from the study were analyzed using the Statistical Product and Service Solutions (SPSS) software version 20.0. One way analysis of variance (ANOVA) was used to analyze the means and significant difference. Comparisons between the groups were made using least significant difference (LSD) Post Hoc tool. Differences at $p<0.05$ (95% confidence interval) were taken to be statistically significant.

Results

RF-EMR Energy Emitted from Mobile Phones

The calculated power density emitted from the mobile GSM phones used in Group B (primiparous rats + RF-EMR 900 MHz talk mode) was significantly ($p<0.05$) lower (1.9725 mw/m2) than that from the smart phones in Group C (primiparous rats + RF-EMR 1800MHz, standby mode, 2.0572 mw/m2), and Group D (primiparous rats + RF-EMR 1800 MHZ talk mode, 3.2591 mw/m2) and Group E (multiparous rats +RF EMF 1800MHz, talk mode, 3.25 mw/m2). The calculated power density from the smart phone that was on standby mode in Group C was significantly ($p<0.05$) lower than that calculated from the talk mode. The electric field waves were effective at a maximum distance of 40 cm for all mobile phones (Table 2).

Effect of RF-EMR on SOD Activity in Lactating Wistar Rats

The exposure of primiparous rats to RF-EMR at 900MHz talk mode and 1800 MHz standby mode did not significantly ($p>0.05$) alter the activity of serum SOD activity when compared with the control value (12.47±1.31µ/dl). However, the exposure of both primiparous rats at 1800 MHz talk mode and multiparous rats at 1800MHz talk mode significantly ($p<0.05$) elevated serum SOD activity when matched-up with the normal control group (12.47±1.31µ/dl) (Table 3).

Effect of RF-EMR on Cortisol Level

There was statistically significant ($p<0.05$) increase in the level of cortisol in Group B (primiparous rats + RF-EMF 900MHz talk mode, 16.72±0.79µ/dl), group C (primiparous rats + RF-EMF 1800MHz, standby mode, 16.45±0.65µ/dl), group D (primiparous rats + RF-EMF 1800MHz talk mode, 15.35±1.96) and group E (multiparous rats + RF EMF 1800MHz talk mode, 15.42±1.99µ/dl) when compared to the control group (13.84±0.83µ/dl) (Table 3).

Table 2: Component values of electromagnetic radiation and calculated power densities from the mobile phones used

Groups of animals and RF-EMF exposed to	Mean ± SD Electric field strength (mV/m)	Mean ± SD Magnetic field strength (mA/m)	Mean ± SD Computed power density (mW/m²)
A (Control) No RF-EMF exposure	0	0	0
B (primiparous rats + RF-EMF 900MHz talk mode)	0.45±0.01[a]	1.47±0.01[a]	1.97±0.01[a]
C (primiparous rats + RF-EMF 1800MHz, standby mode)	0.84±0.01[b]	1.55±0.01[b]	2.05±0.00[b]
D (primiparous rats + RF-EMF 1800MHz talk mode)	2.03±0.01[c]	2.12±0.01[c]	3.25±0.00[c]
E (multiparous rats + RF-EMF 1800MHz, talk mode)	2.03±0.01[c]	2.12±0.01[c]	3.25±0.01[c]

Data presented as mean ± SD, (n=5)
Different superscripts a, b, c indicates statistical significance at $p<0.05$ compared to control

Table 3: Effect of RF-EMR exposure on cortisol and SOD level in lactating rats on the 22nd day

Group	CORTISOL (µ/dl)	SOD (µ/dl)
	Mean±SD	Mean±SD
Group A (Control) No RF-EMF exposure	13.84±0.83[a]	12.47±1.31[a]
B (primiparous rats +RF EMF 900MHz talk mode)	16.72±0.79[b]	13.85±0.48[a]
C (primiparous rats + RF EMF 1800MHz, standby mode)	16.45±0.65[b]	14.23±0.47[a]
D (primiparous rats +RF EMF 1800MHz, talk mode)	15.35±1.96[b]	16.03±1.05[b]
E (multiparous rats + RF EMF 1800MHz, talk mode)	15.42±1.99[b]	17.70±1.52[b]

Data presented as mean ± SD, (n=5); SOD = Superoxide dismutase
Different superscripts from 'a' indicate statistical significance at $p<0.05$ compared to control

Effect of RF-EMR on Body Temperature of the Lactating Rats

The exposure of both primiparous rats to RF-EMR at 900MHz talk mode and 1800 MHz standby mode did not significantly ($p>0.05$) affect the body temperature when liken with the control group. However, the exposure of both primiparous rats at 1800 MHz talk mode and the multiparous rats at 1800MHz talk mode significantly ($p<0.05$) increased body temperature when compared with the normal control group (Figure 1).

Effect of RF-EMF on Basal Body Temperature of Lactating Rats

There was statistically ($p<0.05$) significant increase in the daily basal body temperature all through the twenty-one days in Group D (primiparous rats + RF-EMF 1800MHz, talk mode) and group E (multiparous rats + RF-EMF 900MHz, talk mode) when compared with the control group. However, there was no significant ($p>0.05$) difference in basal body temperature of animals in Group B (primiparous rats + RF-EMF 900MHz talk mode) and Group C (primiparous rats + RF-EMF 1800MHz, standby mode) (Figure 2).

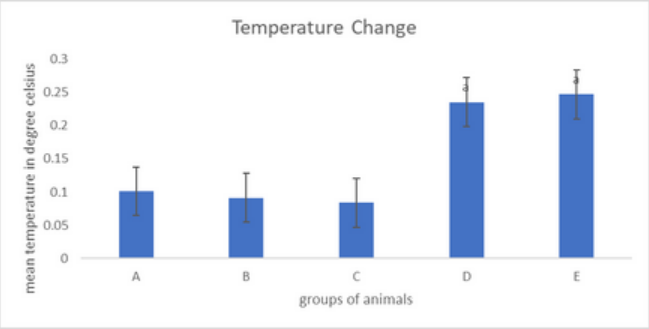


Figure 1: Effect of RF-EMR on body temperature

Results are presented as mean ± SD; N= five animals per group; a= ($p<0.05$) when compared with the control group.

Group A: control rats with no RF-EMR exposure; Group B: primiparous rats + RF-EMR 900MHz talk mode; Group C: primiparous rats + RF-EMR 1800MHz, standby mode; Group D: primiparous rats + RF-EMR 1800MHz, talk mode; Group E: Multiparous rats + RF-EMR 1800MHz, talk mode

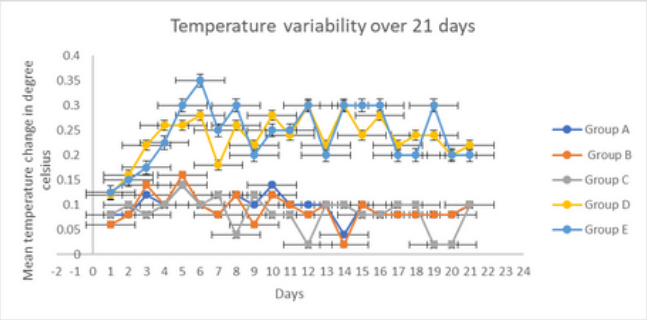


Figure 2: Effect of RF-EMR on basal body temperature of all the groups of animals over a period of the 21-day

Group D and E are statistically significant at $p< 0.05$ when compared with the control group.

Group A: control rats with no RF-EMR exposure; Group B: primiparous rats + RF-EMR 900MHz talk mode; Group C: primiparous rats + RF-EMR 1800MHz, standby mode; Group D: primiparous rats + RF-EMR 1800MHz, talk mode; Group E: Multiparous rats + RF-EMR 1800MHz, talk mode.

Discussion

SOD is an enzyme that acts as antioxidant, protecting cells from damage caused by superoxide radicals. It converts harmful radicals into less dangerous molecules like oxygen and hydrogen peroxide (22,23). SOD is found in virtually all aerobic organisms. SOD plays a crucial role in mitigating the oxidative stress caused by RF-EMR by acting as an antioxidant. It primarily functions by scavenging superoxide radicals, converting them into less harmful hydrogen peroxide, which can then be further neutralized by other antioxidant enzymes like catalase (24). The elevation of serum SOD activity following exposure to RF-EMR at 1800 MHz TM (for primiparous rats) and 1800MHz TM (for multiparous rats) may be attributed to the ability of RF-EMR to generate more reactive oxygen species (ROS) that exceeds the natural capacity of the cells to act as antioxidants, which may predispose to oxidative stress and tissue damage in the animals. The high SOD activity could lead to interference with normal cellular processes/functions and the onset of neurodegenerative diseases in the animals (25). The non-significant effect of primiparous rats to RF-EMR at 900MHz TM and 1800 MHz SBM may indicate ability of the radiation to ROS that equal that of the natural ability of the cell to generate antioxidants, which might not be detrimental to the animals.

Cortisol is a steroid and stress hormone produced by the adrenal glands. It plays a crucial role in regulating various bodily functions, including metabolism, blood pressure, and the sleep-wake cycle (26). While essential for managing stress, consistently high cortisol levels following RF-EMR exposure can lead to health issues as they contribute to increased production of ROS, which are unstable molecules that can damage cells and tissues (27,28). The elevated serum cortisol concentration observed in the study following exposure to all intensities and frequencies of RF-EMR from mobile phone could have arisen due to the potential of RF-EMR to act as potential biological stressor, triggering the release of cortisol, a stress hormone. The observed increase could also have occurred due to stimulation of the adrenal glands, leading to higher cortisol production; effect of RF-EMR on Hypothalamic-Pituitary-Adrenal (HPA) axis, leading to a shutdown of the stress response, thereby increasing cortisol levels; indirect effect of RF-EMR on cortisol level through thermoregulation, as increased body temperature which might influence cortisol concentration in the animals (29). The increased cortisol level by exposure to RF-EMR could lead to high cortisol which may alter normal metabolism, and potentially lead to weight gain, especially around the abdomen of the animals. The elevated cortisol level can contribute to high blood pressure and other cardiovascular problems via a compromise on the normal functioning/obstruction of the coronary artery as well as to predisposition to anxiety, depression, and other mood disorders in the animals (18). The high cortisol level can also impair normal cognitive abilities and predisposition to Cushing syndrome (which occur due to prolonged high cortisol level) (30).

Temperature plays an important role in the biological system, influencing oxidative stress/antioxidant balance, enzyme activity, metabolic rates, and the physical state of water, which in turn affects the distribution and survival of organisms (31,32). Exposure to RF-EMR leads to tissue heating (thermal effects) and non-thermal effects including impact on oxidative stress (33). The exposure of animals to RF-EMR triggers the interaction of body tissues, causing molecules to vibrate and rotate. This molecular motion generates heat. If the rate of heating is high and the cooling capacity of the body mechanism are overwhelmed, it can lead to hyperthermia, a dangerous condition where body temperature rises excessively (34). The specific absorption rate (SAR) of the tissue is a key factor for determining the potential for temperature increase (35). Exposure limits are set to ensure that SAR levels remain below those that could cause significant thermal damage. RF-EMR radiation also affects the temperature regulatory capacity of the body. The increase in temperature could also be due to RF-EMR heat generating power which exceed its cooling effect causing hyperthermia (1). Therefore, the elevation of body temperature by exposure of the animals to RF-EMR at 1800 MHz TM (for primiparous rats) and 1800MHz TM (for multiparous rats) may be adduced to the ability of RF-EMR to initiate interaction among body tissues which makes it internal molecules to vibrate and rotate causing body movement and heat generation, affecting the homeostasis of the animals. This could mean that the increase in temperature is a sign of stress to the animals. Acute stress causes sympathetically-mediated vasoconstriction, which quickly lowers skin temperature. This peripheral blood supply and stress-induced thermogenesis both boost core temperature at the same time. The increase in core temperature, known as "stress-induced hyperthermia" (SIH), is dependent on the severity of the stressor (36). Furthermore, the small body size and head circumference of rats may have contributed to the elevated body temperature increase since their SAR was higher than that of human, making them more susceptible to RF-EMR. Brief temperature elevations can occur when there is not enough time for temperature to dissipate, which can lead to larger temperature elevations in small regions given the same absorbed radiofrequency power. This is in contrast to steady-state temperature elevations, which occur when temperature rises gradually and gives time for temperature to dissipate over a larger tissue mass and for thermoregulatory processes to counteract temperature rise (37). The non-significant effect in body temperature observed by the primiparous rats to RF-EMR at 900MHz TM and 1800 MHz SBM may imply the ability of RF-EMR to maintain its heat emitting ability (38). The findings from this study are of public and environmental health importance, as it creates awareness to the general population, especially to the pregnant and breastfeeding mothers to take caution in contact and exposure to RF-EMR for their health, safety and that of their babies.

Conclusion

The study revealed that the exposure of lactating animals to RF-EMR could trigger a rise in oxidative stress markers and body temperature. The exposure which occurs by alterations in the normal oxidative stress/antioxidant balance as well as in the thermoregulatory framework could have harmful effect on the lactating animals. These findings would help the general population, especially the lactating mothers to exercise caution with exposure to RF-EMR. These cautions include: holding mobile phone at some distance away (at least 10cm) from the body of lactating mothers to avoid the effects of thermal heat. Antioxidants supplements could be taken regularly to combat the oxidative effect of RF-EMR. Mobile phone calls should be kept short or used with hand free kit whenever they are switched on.
We hereby recommend the assessment of more oxidative stress biomarkers that are relevant to RF-EMF exposure for breastfeeding mothers as well as the introduction of a positive control treatment group. Also, further research is required to discover other effects and possible mechanism(s) of action or pathway involved in RF-EMR during lactation.

Conflict of Interest

The authors declare no conflict of interest in this study.

Authors Declaration

The authors hereby affirm that the findings contained in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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