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Acute and Sub-acute Effects of Exposure to Radiofrequency-Electromagnetic Radiation (RF-EMR) from Mobile Phone on Some Lactogenic Hormones in Female Wistar Rats

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Abstract

Background: The radiofrequency-electromagnetic radiation (RF-EMR) produced by mobile phones and their base station antennae for the Global System for Mobile Communications (GSM) ranges from 900 to 1800 MHz. The increased use of mobile phones has been accompanied by epidemiologic studies and public concerns over possible harm to human reproductive health, especially to pregnant and lactating women.

Objectives: This study investigated the potential effect of exposure to EMRs at 900 and 1800MHz on lactogenic hormones at day 3 and 12 of lactation.

Methods: Twenty animals were divided into four groups (n=5). There was no radiation exposure for Group A (Control). Groups B-D were given access to 1800MHz standby mode, 1800MHz talk mode, and 900MHz speak mode, respectively. From the first to the twenty first day of lactation, the rats were exposed to several cell phone radiation frequencies. Selected lactogenic hormone concentrations were measured using standard protocols.

Results and Conclusion: On day 3 of lactation, the exposure of RF-EMR to animals produced oxytocin, prolactin and progesterone values that are not significantly ($p>0.05$) different from those of their respective control values (16.50±0.06, 25.68±2.68, 23.64±1.64). The same pattern of non-significant ($p>0.05$) effect was observed on day 12 of lactation when liken with their respective control (15.94±1.32, 23.54±2.64, 23.64±2.84). The study indicated that the frequency and duration of RF-EMR exposure to animals are not potent enough to cause any profound hormonal alteration in the animals. These findings are relevant for public and environmental health concern about RF-EMR exposure, particularly among the pregnant or nursing populations.

Keywords: Lactogenic hormones, Mobile phone, Lactation, Radiofrequency electromagnetic radiation (RF-EMR), Prolactin, Global System for Mobile Communications (GSM).

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INTRODUCTION

Radiofrequency electromagnetic radiation (RF-EMR), also known as RF radiation, is a type of non-ionizing radiation with frequencies ranging from 3 kHz and 300 GHz. It is a form of electromagnetic radiation that occurs within the wide electromagnetic spectrum, comprising basically radio waves and microwaves (1). RF-EMF radiation is emitted by a broad range of sources, with varying frequencies, including natural sources like the sun, outer space and the earth magnetic field itself, as well as human-made sources like communications devices, microwave ovens, computers, electric power lines, and appliances (2). Radiofrequency radiation (RF) is generally considered low-energy compared to other types of radiation in the electromagnetic spectrum. RF-EMR is extensively used in various applications among which are radar, microwave ovens, and medical treatments as well as in communications (radio, television, mobile phones) (3). Despite its beneficial role, it is considered harmful as it could pose potential health effects from prolonged exposure, especially as it relates to mobile phones and wireless networks, which is of interest in the present study.

The emission of radiofrequency electromagnetic radiation from mobile phone occurs in order to transmit and receive signals for calls, data use and text messaging. This radiation is considered non-ionizing and falls within the radio frequency portion of the electromagnetic spectrum (4). Long-term exposure to mobile devices during transmission and receipt of signals poses potential long-term health effects of RF-EMR on reproductive organs of the biological system of the general population, and has

become a matter of public and environmental health, particularly to the pregnant and nursing population as the life of mother and their baby is at risk (5). The probable health effects of RF-EMR can be ascertained through assessment of serum concentration of lactogenic hormones including prolactin, oxytocin, progesterone among others.

Lactogenic hormones are hormones that stimulate lactation and milk synthesis from the mammary gland. Major lactogenic hormones are prolactin, human placental lactogen (hPL), progesterone/estrogen, oxytocin, which are all involved in preparing and initiating the mammary glands for milk production (6,7). Exposure to RF-EMR from mobile phones may have varying effects on lactogenic hormones in women, with potential detrimental effect on milk composition and alteration in levels of lactogenic/steroidogenic hormones (8).

Previously documented studies on RF-EMR include those by Singh et al. (9) who reported the adverse effect of RF-radiation from mobile phone base stations on the reproductive health status of laboratory animals. Olarinoye et al. (10) observed alterations in the levels of follicle-Stimulating Hormone (FSH), progesterone, estrogen, LH as well as oestrous cycle interference of rats upon exposure. Koohestanidehaghi et al. (11) observed that radiation released from mobile phone at a frequency of 900-1800 MHz via induction of oxidative stress could alter the pre-implantation of embryo in mice. Aldad et al. (12) opined that mice exposed to emission from cellular phones exhibited altered cognitive and reproductive performances. Alekperov et al. (13) showed that the exposure of female rats to electromagnetic field at low frequency mode (30 Hz) displayed no significant defect in the levels of progesterone, luteinizing Hormone (LH), FSH, and estradiol E2 as well as in the structure of the rat ovaries at estrous cycle. Sundaram et al. (14) demonstrated that a two-month exposure of female Sprague Dawley rats to electromagnetic radiation (EMR) at a frequency of 150 KHz triggered changes in the gross, histochemical, hematological and histopathological profiles of the liver and lungs but were not potent enough to predispose the animals to other clinical and functional alterations. Onaadepo et al. (15) showed that RF-EMR from mobile phone had a significant effect on the milk content and not on the volume of milk produced in lactating Wistar rats. Yuksel et al. (16) reported that Wi-Fi and cell phone-induced EMR could be among the causes of the rise in oxidative uterine injury and lowered hormone levels in maternal rats.

Given these studies, to the best of our knowledge, no one has specifically focused on the effect of RF-EMR from mobile phone on lactating Wistar rats at 900 and 1800 MHz (acute and sub-acute frequencies) in relation to standby mode, talk mode and speak mode, on selected hormones of lactogenic importance, creating a research vacuum that needs to be filled. Therefore, in the present study, we assessed the acute and sub-acute effects of exposure to RF-EMR from mobile phone on some lactogenic hormones in female Wistar rats.

METHODOLOGY

Ethical Approval

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

Experimental Drugs

Oxytocin injections used were manufactured by Pitons Pharmaceuticals, China (Batch No: 191032) and vitamin C from Emzor Pharmaceuticals Industries Ltd, Nigeria (Batch No: 2033X) and were procured from the Skylark Pharmaceuticals Limited, Abuja, Nigeria.

Laboratory Analyses

Progesterone, oxytocin, and prolactin assays were done at the Labcrest Laboratories, Gwagwalada, Abuja.

Animal Grouping

Using the record of the body weight, twenty female Wistar rats were divided into four groups (n=5) (Table 1). From the first to the twenty first day of lactation, the rats were exposed to several cell phone radiation frequencies. Thereafter, selected serum lactogenic hormone concentrations were measured using standard protocols.

Table 1: Groups of Animals and their Treatment for 21 Days

Groups (n=5)	Treatment in Hz
A: primiparous lactating rats	No exposure to RF-EMF
B: primiparous lactating rats	GSM 900MHz +15 phone calls + 10 text messages
C: primiparous lactating rats	1800MHz standby mode. 10 text messages + internet connections
D: primiparous lactating rats	1800MHz in Talk mode +15 phone calls + Text 10 text messages + internet connections

RF-EMR Exposure

The procedure previously described by Onaadepo et al. (15) was adopted for exposing experimental animals to RF-EMR from mobile phone. 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 1b). 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 (Figure 1c). 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 (13-15) 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).



Figure 1a: RF-EMR Device for 900MHz



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



Figure 1c: Rat around a ringing phone

Blood Collection

Blood samples were collected through the retroorbital vein with heparinized capillary tubes with open ends and the samples were transferred into universal sample bottles for determination of lactogenic hormone analysis.

Estimation of Serum Progesterone

Enzyme-linked immunosorbent assay (ELISA) was performed with serum from control and RF-EMF exposed animals using a rat ELISA kit according to the manufacturer's protocols (Enzo Life Sciences, Inc. Farmingdale, NY, United States).

The desired number of coated wells in the holder were secured. A known volume (25 µl) of standards, specimens and controls were dispensed into appropriate wells and 100 µl of working Progesterone-HRP Conjugate Reagent was introduced into each well. A known volume (50 µl) of rabbit anti-progesterone reagent was dispensed to each well;

this was thoroughly mixed for 30 seconds. It was then incubated at room temperature (18-25 °C) for 90 minutes. The microwells were rinsed and flicked 5 times with distilled water. A known volume (100 µl) of TMB reagent was dispensed into each well and gently mixed for 10 seconds and incubated again at room temperature (18-25 °C) for 20 minutes. The reaction was stopped by the addition of 100 µl of Stop Solution to each well and was gently mixed for 30 seconds and absorbance was read at 405 nm with a Microtiter Well Reader within 15 minutes (17).

Estimation of Serum Prolactin

ELISA was performed with serum from control and RF-EMF exposed animals using a Rat ELISA Kit (Chemux Bioscience, Inc, Hayward, Canada). Prolactin assay was carried out using the specie-specific kit which was designed for the quantitative evaluation of rat prolactin according to the manufacturers manual. The microplate was first coated with monoclonal antibody prolactin. The sera sample containing the antigen was pipetted into an antibody coated microplate and allowed to stand for two hours during which the prolactin antigen in the sample bound to the antibodies fixed on the inner surface of the wells. Non-reactive components were removed by washing steps. Afterwards, a second polyclonal horseradish peroxidase-labelled antibody was added, a sandwich complex which consisted of two antibodies and the rat prolactin was formed during one hour of incubation. The excess enzyme conjugate was washed out and a chromogenic substrate, tetra-methyl benzidine (TMB) was added to the wells and was allowed to incubate for 30 min during which the substrate was converted to a coloured end product (blue) by the fixed enzyme.

The enzyme reaction was inhibited by addition of hydrochloric acid as the stop solution. Absorbance was measured using a Microplate Reader at 450 nm. The standard curve was obtained by plotting the concentration of standard against the absorbance from which the prolactin concentration was gotten (18).

Estimation of Serum Oxytocin

Oxytocin assay was carried out using the oxytocin rat ELISA kit according to the manufacturers manual. The oxytocin specie-specific ELISA kit (Melsin Medical Company, China) in microplate was designed for the quantitative evaluation of rat oxytocin. Before the commencement of the experiment, the microplate was pre-coated with oxytocin. The sera sample containing the antigen was pipetted into an antibody coated microplate, and allowed for two hours. During the reaction, the oxytocin component of the sample and standard competed with a fixed amount of oxytocin on the solid phase support for sites on the Biotinylated medium. Excess conjugate and unbound sample were washed from the plate, and HRP-Streptavidin (SABC) was properly added to each microplate well and incubated. Then, TMB substrate solution was added to each well. The enzyme-substrate reaction was terminated by the addition of a sulphuric acid solution and color change was measured spectrophotometrically at a wavelength of 450 nm. A standard curve was obtained by plotting the concentration of standard against the absorbance from which the oxytocin concentration was gotten (Olarinoye et al.., 2021) (10).

Statistical Analysis

Values are given as means ± 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

Lactogenic Hormones on Day 3 of Lactation

RF-EMF did not significantly ($p>0.05$) alter the level of the lactogenic hormones (oxytocin, prolactin and progesterone) measured in the rats. This study did not observe any significant ($p>0.05$) change in the level of lactogenic hormones measured on the third day of lactation when compared to the control group. Thus, there was no acute effect of RF-EMR as all groups of animal-initiated lactation in like manner even under the influence of RF-EMR (Table 2).

Lactogenic Hormones on Day 12 of Lactation

Measurements of the same lactogenic hormones taken on the twelfth (12th) day of lactation (peak phase 12-14 days) were not statistically ($p>0.05$) significant as well, thus no sub-acute effect observed on the hormones evaluated. Since prolactin and oxytocin are major determinant in the control of synthesis and let down of breast milk are not affected, therefore, the study observed no effect of RF-EMR on neither the initiation nor maintenance of lactation, thus within safety limits RF-EMR had no effect. However, the exposure of animals to RF-EMR at 900MHz talk mode significantly ($p>0.05$) lowered progesterone level when compared with the group control (Table 3).

Table 2: Effect of RF-EMR on Some Lactogenic Hormones on Day 3 of Lactation

Group (n=5)	Oxytocin(ng/ml)	Prolactin (ng/ml)	Progesterone (ng/ml)
A. (Control) No RF-EMR exposure	16.50±0.06[a]	25.68±2.68[a]	23.64±1.64[a]
B. (primiparous rats + RF-EMR 900MHz talk mode),	16.84±0.48[a]	25.72±2.31[a]	24.94±1.44[a]
C. (primiparous rats + RF-EMR 1800MHz, standby mode)	16.49±0.19[a]	25.67±2.15[a]	23.77±1.23[a]
D. (primiparous rats + RF-EMR 1800MHz talk mode)	16.45±0.25[a]	25.53±2.43[a]	23.59±1.51[a]

a =Values are not statistically significant (p>0.05) when compared with the control group

Table 3: Effect of RF-EMR on some Lactogenic Hormones on Day 12 of Lactation

Group (n=5)	Oxytocin (ng/ml)	Prolactin (ng/ml)	Progesterone (ng/ml)
A (Control) No RF-EMR exposure	15.94±1.32[a]	23.54±2.64[a]	23.64±2.84[a]
B (primiparous rats + RF-EMR 900 MHz talk mode)	15.92±1.51[a]	24.94±2.82[a]	19.74±1.30[b]
C (primiparous rats + RF-EMR 1800 MHz, standby mode)	15.98±1.72[a]	23.77±2.47[a]	23.76±2.14[a]
D (primiparous rats + RF-EMR 1800 MHz talk mode)	15.85±1.83[a]	23.59±2.96[a]	23.59±2.36[a]

a =Values are not statistically significant (p>0.05) when compared to control group

Discussion

The measurement of the degree of exposure to varying frequencies of RF-EMRs emitted from mobile phones and other allied devices by experimental animals is a useful strategy for providing vital information on levels of lactogenic/steroidogenic hormones.

Oxytocin, mostly referred to as love hormone plays a crucial role in lactation, facilitating milk ejection reflex and enhancing mother-infant bond (19,20). It triggers the myoepithelial cells on the breast alveoli, to contract. These contractions squeeze the milk into the milk ducts, making it available for the baby to feed. Oxytocin stimulates mother-infant bonding via stimulation of feelings of love and affection between mother and child, thereby creating positive relationship between the mother and her baby (21,22). Also, oxytocin triggers a n emotional and psychological effects on the lactating mother, thus reducing stress and promoting relaxation. Furthermore, it stimulates uterine contraction during child birth via contraction of uterus after delivery, aiding in the delivery of the placenta and reducing bleeding (23,24). Therefore, the non-significant effect of exposure of rats to RF-EMR on oxytocin concentration in the lactating rats on day 3 and 12 in the present study, may indicate no alteration in oxytocin level or milk production. This suggests that RF-EMR exposure to animals, at 900 and 1800 MHz in the varying studied modes, did not interfere with the normal hormonal and physiological processes involved in breastfeeding (25,26).

Prolactin is a hormone produced by the pituitary gland and it plays a major role in lactating mothers via stimulation of breast milk production and development of mammary glands (27). It also helps in the regulation of menstrual cycle and leads to amenorrhea (cessation of menstruation) during breastfeeding. More so, prolactin participates in postpartum milk production through stimulation of decrease in progesterone and estrogen level and increased level of prolactin to stimulate milk secretion after delivery (28). Therefore, the non-significant effect in the level of prolactin day 3 and 12 of lactation following exposure of the animals to RF-EMR, at 900 and 1800 MHz, from mobile phone, may signify that the exposure level was not strong enough or the duration was too short to trigger a noticeable alteration in prolactin level (29). The non-significant effect in prolactin level by RF-EMR could also be adduced to different levels of sensitivity of the animals to RF-EMR. In addition, it may also imply that the emitted RF-EMR is not within the range of normal fluctuations limit to allow a measurable change in prolactin level. This indicates that the frequency of the RF exposure was not sufficient enough to reveal statistically different value in the treatment groups when compared with the normal control group (30).

Progesterone is a lactogenic hormone that prepares the breasts for milk production during pregnancy and supports ongoing lactation after childbirth. This occurs via development of the glandular tissue within the breasts, stimulates growth of mammary glands and initiates lactation by inhibiting further differentiation of the alveolar cells (31). Also, progesterone helps maintain the uterine lining during pregnancy and prevents uterine contractions prematurely. Furthermore, the high level of progesterone during pregnancy inhibits lactation by blocking the release of prolactin and inhibiting milk production from the breast. It plays a significant postpartum role in lactation (after childbirth) where the declined progesterone level triggers the release of prolactin and allow for full initiation of milk production from the breast tissues, leading to onset of lactation (32). The non-significant effect in progesterone concentration on day 3 and 12 of lactation, except for 900 MHz talk mode on day 12 of lactation, following exposure of rats to RF-EMR at 900 and 1800 MHz may imply that the frequency and intensity of exposure may not significantly trigger changes in progesterone production, a hormone crucial for lactation (33,34). The low progesterone concentration by exposure to 900 MHz talk mode on day 12 of lactation could be adduced to lower progesterone levels leading to decreased milk supply,

which could impact the nutrition of the baby and potentially necessitate supplementing with formula. The low progesterone levels could also contribute to mood swings, fatigue, and other symptoms that affect the total well-being of the mother (35). This reduction in progesterone level agrees with the findings of Yuksel et al. (16) who did his study with pregnant animals.

Conclusion

The study indicated that the mobile phone mediated RF-EMR exposure to animals at 900 and 1800 MHz could not reveal profound alteration in the examined lactogenic hormones. The non- significant effect might arise because the frequency and duration of RF-EMR exposure to animals might not be sufficient enough to reveal profound hormonal alteration in the animals. These observations would be relevant for public and environmental health concern about RF-EMR exposure, particularly among the pregnant or nursing populations.

We hereby recommend that further studies with consideration on varied frequency, grouping randomization increased sample size, use of multiple mobile phones, single timepoint measurement, and increased duration of exposure of lactating experimental models to RF-EMF from mobile phones are encouraged.

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Conflict of Interest

Authors declare no conflict of interest on this work.

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