Original Article

Evaluation of Haematological Parameters and Oxidative Stress-Induced in Rats Exposed to Radio-Frequency Radiation from Mobile Phones

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Abstract

Introduction: The use of new wireless technologies emitting radio-frequency electromagnetic field (RF-EMF) radiation has been introduced worldwide, raising concerns about their biosafety. So far, there have been contradictory scientific reports which have led to active debates over the bio-effects of EMF on the ecosystem. **Aim:** This study, therefore, aims to evaluate the bio-effect of exposure to RF-EMF from a mobile phone simulator. **Materials and Methods:** The experimental study used 16 healthy albino rats (8 females/8 males) randomly selected and divided equally into two groups: Group A (8 rats exposed to mobile phone simulators) and Group C (8 rats as control); the study procedure was carried out for 6 weeks. The rats were examined for physical changes, hematological profiles, and serum oxidative stress (OS) biomarkers. **Results:** The outcome of the study showed that exposure to RF-EMF affected the weight of the animals; this is illustrated when comparing the baseline weight/final weight of the exposed as compared to the control. This trend was also replicated when compared across gender, though further analysis showed no significance across the two groups ($P > 0.05$). The result of the hematological analysis showed that only granulocytes (neutrophils, basophils, and eosinophils) showed significance ($P = 0.04$), while for OS biomarkers, the result showed that superoxide dismutase and catalase showed significant difference ($P = 0.02$), respectively, across the two groups. **Conclusion:** This study concludes that exposure to RF-EMF has an associated effect on hematology and OS induction and therefore recommends the adherence to the precautionary principle while further research has been carried out on their specific mechanism and site of action.

Keywords: *Albino rats, bio-effect, mobile phone simulator, radiation*

Introduction

The use of mobile phones and wireless technology has gradually increased throughout the world with increasing concern about their potential biological effect on the ecosystem; this has also raised the need for scientific research into these effects. This situation became even more complicated with the rapid development of the new generation of wireless networks, designed for communication purposes, and specifically for mobile phones along with associated infrastructure.[1]

The effects of radio-frequency electromagnetic field (RF-EMF) radiation on biological systems are suggested to be via (a) thermal and (b) nonthermal mechanisms. The former could increase body temperature caused by mobile phone radiation, resulting in damage to tissues and cells. On the other hand, the

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nonthermal mechanism causes cell injury via oxidative stress (OS) due to free radical formation, which may lead to alterations in membrane structure and function.[2]

OS occurs if there is a disequilibrium between the formation of reactive oxygen species (ROS) and the capacity of the antioxidant system to neutralize them. This OS has been implicated in most health symptoms associated with RF-EMF, especially through the induction of biomarkers of OS and inflammation in the brain,[3] as demonstrated in both human and animal studies,[4,5] though the intensity of RF-EMF radiation emitted by cell phones is much below the limiting specific absorption rate value of 0.08 W/kg for whole-body exposure[6,7] to cause any thermal effect.

Of course, there are several gaps in the existing knowledge which do not permit one to reach a concrete conclusion on the possible harmful effects. This may be why exposure to these types of radiation is being considered an environmental pollutant, therefore posing an environmental risk that needs further investigations, as well as re-evaluation of established limits and standards.

Materials and Methods

Animal care

The experimental rats were obtained at the Nigerian Institute of Medical Research (NIMR) Animal House, Yaba, Lagos, Nigeria, following the NIMR Institutional Research Board for the Care and Use of Laboratory Animals, and ethical approval (IRB/20/107) was obtained from the NIMR Institutional Review Board (IRB).

A total of 16 (8 females/8 males) healthy rats about 2 months old, weighing between 150 and 230 g, were randomly selected and divided equally into two groups: Group A (8 rats [4 males and 4 females housed in separate cages] exposed to mobile phone simulators) and Group C (8 rats [4 males and 4 females housed in separate cages] nonexposed as control). All the animals were maintained in clean plastic cages with wired tops to allow for ventilation food and water. Animals were acclimatized in an ambient environment at 25°C room temperature and to the pathogen-free laboratory conditions 3 days before experiments at 12:12-h day/night cycle. Food and water were provided *ad libitum*. Cages had wood shavings to protect them from their urine and stools; this was cleaned and changed every 2 days. Animals of all the groups are habituated to exposure cages for 1 week before the start of exposure to avoid procedure-related stress; body weight of animals was then taken at baseline and the end of the study period.

Exposure setup

After the short adaptation period, samples of the experimental groups were continuously exposed to a 3G (UTMS) mobile phone simulator with properties (frequency: 1800–2100 MHz, bandwidth – 2.0 GHz, transient burst – 10 mW/m^2, variability – 0.667–3 mW/m^2, and max field strength – 2 V/m), using that described in another study[8] as a guide, and built-in the Department of Physics, Faculty of Science, University of Lagos, Nigeria. The antenna was 25 cm above the center of the rat cages. The exposure systems were placed in different rooms with similar conditions as the control; this procedure is similar to but slightly modified to a previous study.[9] There was no movement restriction for rats in the cages during the study period.

At the end of the 6 weeks, blood samples were drawn from the retro-ocular sinus of all the animals. The samples were collected in ethylenediaminetetraacetic acid for hematological analysis while another portion was collected in a plain sample bottle, which was centrifuged, and serum decanted for biochemical analysis for OS biomarkers.

Hematological analysis

Whole blood was used to evaluate the effect of exposure on the level of hemoglobin (Hb), red blood cell (RBC), mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), mean corpuscular Hb (MCH), white blood cells (WBCs), neutrophils, lymphocytes, and platelets. This was carried out using the Sysmex XS-1000i hematology analyzer at the Center for Human Virology and Genomics Laboratory, NIMR, Yaba, Lagos. Medical laboratory personnel were blinded to the group of the samples before analysis to guard against bias.

Biochemical analysis

For the biochemical study, the following antioxidant enzymes activities were used to evaluate the level of OS-superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), malondialdehyde (MDA), GSH peroxidase, and GSH S-transferase (GST), which were determined spectrometrically. This was carried out by a biochemist who was blinded to the group of each sample before analysis at the Department of Biochemistry, College of Medicine, University of Lagos.

Determination of superoxide dismutase activity

SOD activity was determined by its ability to inhibit the auto-oxidation of epinephrine determined by the increase in absorbance at 480 nm. The Superoxide Dismutase (SOD) activity was determined by its ability to inhibit the auto-oxidation of epinephrine, determined by the increase in absorbance at 480nm, as described by Sun and Zigma (1978).[10]

Catalase activity determination

CAT activity was determined according to Sinha.[11] It was assayed calorimetrically at 620 nm and expressed as μmoles of H2O2 consumed/min/mg protein at 25°C. The reaction mixture (1.5 ml) contained 1.0 ml of 0.01M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate, and 0.4 ml of 2M H2O2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). $\sum$ =40M − 1 cm^{-1}.

Lipid peroxidation

MDA, an index of lipid peroxidation, was determined using the method of Buege and Aust.[12] 1.0 ml of the supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA-HCl reagent (thiobarbituric acid 0.37%, 0.24 N HCl, and 15% TCA) tricarboxylic acid-thiobarbituric acid-hydrochloric acid reagent boiled at 100°C for 15 min and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed, and the absorbance read at 532 nm against a blank

Reduced glutathione determination

The reduced GSH content of liver tissue as nonprotein sulfhydryl was estimated according to the method described by Sedlak and Lindsay.[13] To the homogenate, 10% TCA was added and centrifuged. 1.0 ml of supernatant was treated with 0.5 ml of Ellman's reagent (19.8 mg of 5,5-dithiobisnitrobenzoic acid in 100 ml of 0.1% sodium nitrate) and 3.0 ml of phosphate buffer (0.2M, pH 8.0). The absorbance was read at 412 nm.

Determination of glutathione-S-transferase activity

GST activity was determined by the method according to Habig *et al.*[14] This is since all known GST demonstrate a relatively high activity with 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate. Consequently, the conventional assay for GST activity utilizes CDNB as substrate. When this substrate is conjugated with reduced GSH, its absorption maximum shifts to a longer wavelength. The absorption increases at the new wavelength of 340 nm which provides a direct measurement of the enzymatic reaction.

Data analysis

Data were entered and analyzed with the IBM Statistical Package for Social Sciences (IBM, SPSS version 25.0) (IBM SPSS version 25 – Licensed material of IBM corporation and its licensors 1989, 2017 in Chicago, USA), Licensed Materials – Property of IBM Corporation and its Licensors 1989, 2017. Descriptive analysis of the baseline and final weight (FW) was done, while a bivariate analysis comparing the exposed and control groups was carried out using the independent *t*-test. Power density was measured in μW/m^2.

Results

The study used 16 albino rats randomly grouped into exposed and control, with each group consisting of four males and four females. The descriptive analysis is shown in Table 1. A closer look at the table also shows that the FW of control was higher than that of the baseline weight (BW) as expected, but this was the reverse for the exposed, as illustrated in Figure 1. This trend was replicated when compared across gender [Figure 2]. Further analysis with an independent *t*-test showed no statistical significance across gender [Table 2] or the groups [Table 3].

Analysis of hematological parameters showed a general increase in the mean level of these parameters in the exposed as compared to the control except for lymphocytes [Table 4]. Further analysis using an independent *t*-test showed that only granulocytes had statistical significance at $P < 0.05$. The analysis of biochemical OS markers showed that the mean level of these markers was reduced in the exposed when compared to that of the control, except for GST [Table 5]. Further analysis using an independent *t*-test showed that only SOD and CAT showed statistical significance across the two groups.

Discussion

This experimental study used 16 albino rats randomly grouped into exposed and control, with each group consisting of four males and four females. The outcome of the study shows that exposure to RF-EMF affected the weight of the animals; this is illustrated when comparing the BW/FW of the exposed as compared to the control. This trend was also replicated when compared across gender, though further analysis showed no significance across the two groups. The hematological analysis result showed that only granulocytes (neutrophils, basophils, and eosinophils) showed significance, while for OS, the result showed that SOD and CAT showed significant differences across the two groups.

This study has shown that RF-EMF has some effects on the body weight of animals as illustrated by the

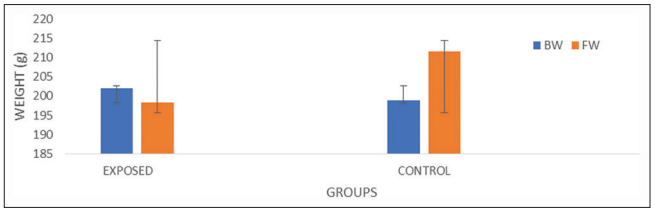

Figure 1: Comparing the baseline weight and final weight across the two groups

Table 1: Effect of radio-frequency electromagnetic field from mobile phones on weight

Groups	Rat	Mean±SD		BW		FW	
		BW	FW	Maximum	Minimum	Maximum	Minimum
Total	Male	200.04±30.83	201.35±34.81	249.21	150.41	242.91	160.34
	Female	203.44±22.33	199.05±22.33	245.01	181.51	241.23	195.42
Exposed	Male	204.39±23.09	196.94±19.49	249.21	150.41	242.91	172.01
	Female	204.44±23.98	184.34±30.07	225.32	181.52	205.46	198.32
Control	Male	197.25±27.70	212.00±37.00	223.65	158.81	239.45	160.34
	Female	200.36±30.08	211.19±20.49	245.01	181.51	241.23	195.42

BW: Baseline weight (g), FW: Final weight (g), SD: Standard deviation

Table 2: Comparison of effect of radio-frequency electromagnetic field from mobile phones on weight across gender

Groups	Weight	Gender	Mean±SD	*P*	95% CI	
					Lower	Upper
Exposed	BW	Male	198.49±46.32	0.79	-76.80	62.75
		Female	205.52±18.11			
	FW	Male	195.12±49.66	0.81	-85.29	72.31
		Female	201.61±3.41			
Control	BW	Male	197.25±27.70	0.88	-53.22	47.00
		Female	200.36±30.08			
	FW	Male	212.00±37.00	0.88	-53.22	47.00
		Female	211.19±20.49			

BW: Baseline weight, FW: Final weight, SD: Standard deviation, CI: Confidence interval

Table 3: Comparison of effect of radio-frequency electromagnetic field from mobile phones on weight across the groups

Group	Weight	Mean±SD	*P*	95% CI	
				Lower	Upper
Exposed	BW	202.01±32.78	0.83	-28.92	35.31
	FW	198.37±32.77			
Control	BW	198.81±26.82	0.40	-45.76	19.31
	FW	211.59±27.69			

BW: Baseline weight (g), FW: Final weight (g), SD: Standard deviation, CI: Confidence interval

difference between the BW and FW of both the control and the exposed though not significant. This is supported by a previous study,[15] while another study showed that there was a statistically significant weight difference between the exposed and the control, with significant weight loss in the exposed.[16,17]

The outcome of the analysis of hematological parameters showed an increase in the mean level of all the blood parameters for the exposed as compared with the control except for lymphocytes, though only granulocytes (neutrophils, basophils, and eosinophils) showed statistical significance. This agrees with a previous study that indicated that long-term exposure of mice to RF-EMF would lead to an increased number of neutrophils. This increase in the level of neutrophils has

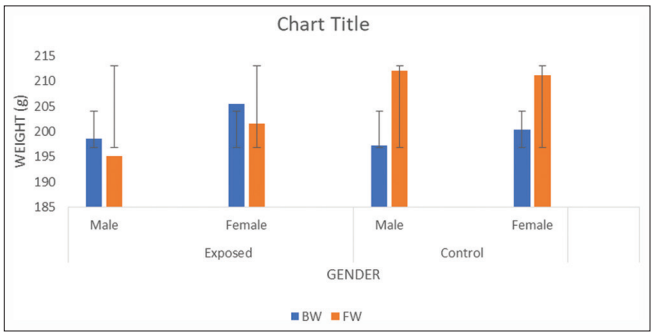

Figure 2: Comparing the baseline weight and final weight across gender

been attributed to the compensation for the impairment of neutrophils caused by cell phone radiation.[9,18] Various experimental studies have shown different outcomes on exposure to RF-EMF; for instance, a study which used Sprague Dawley rats exposed to 2450MHz of EMF for 1 year showed significant increases in RBC count, Hb content, and HCT value.[19] Similarly, authors like Bonhomme-Faivre *et al.* reported a decline in lymphocytes in mice depending on the duration of exposure to the magnetic field,[20] while another reported that chronic exposure to a 0.2–6.6-μT magnetic field can lead to decreased total lymphocytes in humans and mice.[21] Furthermore, previous studies reported that exposure of experimental animals to RF EMFs resulted in a significant increase in blood platelet count, which is confirmed by the proliferation of megakaryocytes, the precursor cells of blood platelets.[22-24] In contrast, another study showed that there were decreases in RBC count, Hb, and HCT, RBCs indices (MCV, MCH, and MCHC) upon exposure of rats to 900MHz for 2 months. The depletion in the values of hematological parameters following EMF radiation exposure may be attributed to OS due to overproduction of ROS by microwave radiation interaction,[22] while another study on rats showed no significant difference in total leukocyte, neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts, or in erythrocyte, HCT, MCH, MCHC, RDW, PLT, and PDW levels between the exposed and sham-exposed groups.[25] Some other studies showed an increase in RBC count and a decrease in WBC count

Table 4: Comparison of effect on hematological parameters across groups

Parameters	Exposed	Control	*P*	95% CI	
				Lower	Upper
WBC	3.25±0.57	3.00±0.08	0.42	-0.46	0.96
Lymphocytes	0.56±0.17	0.71±0.15	0.23	-0.42	0.12
Granulocyte	1.58±0.32	1.14±0.11	0.04	0.02	0.85*
Hb	10.75±0.38	10.15±1.05	0.32	-0.76	1.96
RBC	6.32±0.38	5.74±0.53	0.12	-0.21	1.38
MCV	51.24±0.62	49.50±4.62	0.48	-3.96	7.44
MCH	16.65±0.87	16.35±1.86	0.78	-2.21	2.81
MCHC	33.25±1.34	33.18±0.98	0.93	-1.95	2.10
PLT	332.75±113.69	221.50±11.56	0.10	-28.56	251.06

*P<0.05 (independent t-test). WBC: White blood cell, RBC: Red blood cell, Hb: Hemoglobin, MCV: Mean corpuscular volume, MCH: Mean corpuscular Hb, MCHC: MCH concentration, PLT: Platelet, CI: Confidence interval

Table 5: Comparison of effect on oxidative stress markers across groups

Biomarkers	Exposed	Control	*P*	95% CI	
				Lower	Upper
Serum GSH	32.94±1.62	33.31±3.32	0.85	-4.89	4.15
Serum SOD	2.44±0.53	3.38±0.21	0.02	-1.62	-0.24
Serum CAT	10.89±2.91	16.84±2.05	0.02	-10.30	-1.60
Serum MDA	0.55±0.18	1.02±0.38	0.07	-0.99	0.05
Serum GST	40.55±1.76	39.44±3.81	0.62	-4.03	6.25
Serum GPX	38.70±2.35	41.23±6.69	0.50	-11.20	6.15

*P<0.05 (independent t-test). CI: Confidence interval, SOD: Superoxide dismutase, GSH: Glutathione, CAT: Catalase, MDA: Malondialdehyde, GPX: Glutathione-peroxidase, GST: Glutathione S-transferase

and lymphocyte count after prolonged exposure to microwave radiation.[26] Exposure to RF-EMF has been shown to result in deterioration of RBC function and metabolic activity, and it was expected that the increase of toxicity in specific organs was a result of the RBC functional failure which has been attributed to OS.[19] Reasons for the difference in the outcome of the different studies may not be unconnected with the methodology used and the type of exposure, duration, and pattern of exposure. To support this, an overview concluded that exposure of human and experimental animals to EMFs may cause disturbance in hematological parameters depending on species, the sources of EMFs, frequencies, intensities, and duration of exposure, etc.[27]

Literature has also shown that increased RF-EMF exposure can modify the cellular balance by generating ROS and some antioxidant mechanisms such as SOD, CAT, and GSH GST protect tissues. SOD is the first step of defense mechanism against ROS and catalyzes the dismutation of the superoxide anion into hydrogen peroxide which is now converted into H_2O and O_2 by CAT.[28] The outcome of the analysis on serum OS in this study showed that the mean level of serum OS biomarkers was reduced in the exposed when compared with the control except for GST. Although only SOD and CAT showed statistical significance across the two groups. This is in concordance with a previous experimental study which showed a significant decrease in the activity of SOD, CAT, and GSH peroxidase in rats exposed to EMR compared to the control group.[29] Similarly, Sowa *et al.* found some associations between EMF exposure and OS biomarkers, though not consistent across the different biomarkers.[30] The decreased SOD activity may occur either via direct oxidative damage of the SOD or by the altered pattern of SOD gene expression by OS or both.[31]

In a study using Wistar albino rats, the result showed an increased level of MDA but a reduced level of GSH, SOD, and CAT between control and exposed for 2 weeks,[32] while another study, in which continuous RF-EMF was applied at 2.45 GHz for 20 weeks, showed no significant difference in lipid peroxidation, whereas a significant decrement was reported in the CAT and GSH-Px activities of rats' testes.[33] The differences in the OS biomarker in this study may be because RF-EMF effects are dependent on some physicals such as frequency, modulation, polarization, duration, the water content of tissues, and humidity and biological variables (species, size, weight, the geometry of the body, and nutritional and health status).[34]

Experimental studies have also associated the generation of ROS due to exposure to RF-EMF to induction of OS which may also lead to DNA fragmentation. This is shown in a study by Campisi *et al*.[35] which investigated acute effects of 900 MHz RF for different exposure periods (5, 10, or 20 min) on primary rat neocortical astroglial cell cultures and indicated the importance of the amplitude modulation and observed that only 20 min exposure of RF-EMF increased ROS and DNA fragmentation. Literature has shown that if this unfavorable state (OS) persists over a long period or occurs repeatedly, it can lead to changes in the biological material, as well as the genetic and epigenetic information, and can lead to health-related malfunctions, which have been observed in many diseases, such as diabetes and congenital malformations,[36] and they have a potential role in the initiation, promotion, and malignant conversion stages of carcinogenesis[37] and protein oxidation may play a significant role damaging biomolecules other than DNA in the pathogenesis of neurodegenerative diseases including Alzheimer's disease, multiple sclerosis, or amyotrophic lateral sclerosis and Parkinson's disease.[38]

Conclusion

The biological effect of exposure to RF-EMF will continue to generate controversies as well as concerns, as different studies have shown a different level of outcomes. This study has particularly shown its associated effect on OS biomarkers and blood parameters. Therefore, it is advised to limit the use of EMF emitting devices for household and occupational activities, where possible, while further studies are being carried out on their potential adverse effect. In addition, it is recommended that policymakers should push for the use of the precautionary principle in the use of this technology generally and encourage further research areas to enable understanding the exact mechanism and site of action upon continuous exposure to such radiations.

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Conflicts of interest

There are no conflicts of interest.

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