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# Cell Phone Alters Oxidative Status and Impairs Testicular Function of Male Wistar Rats

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## Abstract

Mobile phone has become a necessity rather than a communication tool. In fact, it has been integrated into everyday life. However, exposure of humans to radiofrequency electromagnetic radiation (RF-EMR) from mobile phone has raised public concerns because of the adverse effects of RF-EMR emitted from this communication device. The current study was designed to investigate the effects of RF-EMR from mobile phone on the testes. Adult male Wistar rats weighing 180 g – 200 g were randomly allotted to control (switched off mode exposure), 1 h exposure group (1H-exposure), 2 h exposure group (2H-exposure) and 3 h exposure group (3H-exposure). The Animals were exposed to RF-EMR of Nokia 105 for a period of 28 days. Histomorphometry and biochemical investigations were carried out. The microscopic examination of spermatozoa showed low sperm count, altered sperm morphology and progressivity in the experimental groups. Also, histomorphometric parameters showed altered cross section, lumen and germinal epithelia diameter in all the experimental groups, and sera levels of malondialdehyde and superoxide dismutase, which are markers of reactive oxygen species significantly increased and decreased respectively in all the experimental groups compared to control group. In addition, sera levels of male sex hormones (follicle stimulating hormone, luteinizing hormone and testosterone) significantly decreased ( $p < 0.05$ ) in 1H- and 2H- exposure groups compared to the control group. The study demonstrates that chronic exposure to RF-EMR from cell phone causes oxidative stress and impairs testicular function that is accompanied by decreased sex hormone profile.

### Keywords:

Cell phone; Histomorphometry; Malondialdehyde; Radiofrequency; Electromagnetic radiation; Superoxide dismutase

## Introduction

Cell phone has gained a global acceptance as an integral part of life not minding its impact on our health. Early studies have documented a possible link between the use of cell phone and male infertility [1]. In addition, recent studies have shown that electromagnetic fields (EMF) generated by mobile phones affect reproductive functions which is cortisol dependent [2]. Cellular phones operate more commonly about at a frequency of 850 to 1800 MHz; and the radiant energy is incorporated by human body tissues and organs. Thus, the habit of keeping a mobile phone in the trouser pocket or during the time of its use may have an impact on possible generation of hyperthermia and oxidative stress as well [3]. However, concerns have been raised from carrying cellular phones near the reproductive organs most especially the testes, which might cause spermatogenic dysfunction, thus changing the sperm cell cycle due to deformation of leydig and sertoli cells that is associated with cell proliferation and decrease fertility in men [2,4].

Cellular phones are defined as devices emitting radiofrequency electromagnetic waves (RF-EMW), which transmit signals from the cellular phone to the base stations and antennas. The frequency of such waves ranges from 800-2200 MHz. However, there is still risk to the human health, because our bodies can act as antennas that absorb these waves and alter them into eddy currents [5]. The mechanisms of cell phones function in such a way that the sound wave created from the speaker goes through a transmitter that converts the sound into a sine wave. This sine wave then travels to the antenna, which then projects the wave out into space. Average power usage of the transmitter is about 0.75 to 1 watt, with a maximum of 2 W [5]. The force of the electric sine wave running through the transmitter circuit also yields an electromagnetic field. As the electric current oscillates back and forth, these electro-magnetic fields continue to build up and collapse, resulting in electromagnetic radiation. The preliminary cell phone system, Analogue NMT (Nordic Mobile Telephone) system, was introduced in the 1980's, and operated at an

electromagnetic resonance of 902.5 MHz. A decade later, the GSM (global system of mobile communications) succeeded it, operating at a radiofrequency of 902.4 MHz, pulsing at 217 Hz. The most recent DCS (digital cellular system) operates at a radiofrequency of 1800 MHz [6]. Therefore, the advancement in cellular phone telecommunication systems are clearly connected with an increasing signals, and studies have shown that cell phone exposure on male Wistar rats result in decreased sperm production, function and increased oxidative stress [1,7]. However, there is paucity of information regarding the quantitative and qualitative studies of the effect of cell phone on the testis of adult male Wistar rats. The present study was designed to investigate the histomorphometry and biochemical studies of cell phone on the testis of male Wistar Rats.

## Materials and Methods

### Animals and grouping

Twenty matured male Wistar rats weighing 180-200 g were obtained from Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The rats were housed in wire mesh cages and maintained in a well-ventilated room at  $25 \pm 2^\circ\text{C}$ , on a 12 h light/12 h dark cycle. Rats had unrestricted access to standard rat chow and tap water. After acclimatized for two weeks, the animals were randomly allotted to: Control (n=5), 1 h exposure group (1H-exposure; n=5), 2 hours exposure group (2H-exposure; n=5) and 3 hours exposure group (3H-exposure; n=5). The investigation was conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and was approved by the Institutional Review Board of University of Ilorin, and every effort was made to minimize both the number of animals used and their suffering.

### Protocol

Nokia 105 which has a body of  $107 \times 44.8 \times 14.3$  mm, 64 cc ( $4.21 \times 1.76 \times 0.56$  in) and weighs 70 g (2.47 oz), Dual-band EGSM 900/1800 MHZ was used and attached to each cage containing 5 rats per cage. The screen size is  $128 \times 128$  pixels and alert types are vibration and polyphonic. Control group was exposed to cell phone in switch off mode in a separate room similar to the room used for the experimental groups, the experimental groups were exposed to cell phone frequency for 1 h/day (1 H-exposure group), 2 h/day (2H-exposure group) and 3h/day (3H-exposure group) respectively. All the cages were brought back to the home room following phone exposure. The exposure of the cell phone was done in the morning between 7.00 am and 10.00 am every day for a period of 28 days.

### Sample preparation

At the end of the exposure, the rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.). Blood was collected by cardiac puncture into sample bottle and was centrifuged at 5000 rpm for 15 min. Serum was stored frozen until it was needed for

biochemical assay. Testes were excised, blotted and weighed immediately.

### Testicular histology

The testes of all the rats were fixed in 10% buffered formal saline for histology to estimate histomorphometric parameters (cross section diameter, lumen diameter, germinal epithelia height), dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5  $\mu\text{m}$  thick paraffin section of each testicular tissue was stained with hematoxylin and eosin, followed by examination under a light microscope at 200 magnification and micrographs taken [8].

### Sperm motility

One drop of caudal epididymis sperm was dropped into a slide and diluted with few drop of normal saline at room temperature. The slide was examined under compound microscope. The number of motile and non-motile sperm was counted in ten random fields. The number of motile sperm was then expressed as a percentage of the total number of sperm [8].

### Sperm counts

Sperm count was performed as described in Chees bough laboratory manual [8] with modifications. The caudal epididymis was carefully separated from the testis and homogenized in 2 ml of normal saline and the suspension was obtained. The suspension was diluted with sodium bicarbonate-formalin in ratio 1 to 20. The improved Neubauer hemocytometer chamber was filled with well diluted sperm then the sperm were counted in 2 sq mm of Neubauer hemocytometer chamber. The sperm counts were calculated in 1 ml of fluid multiplied the number counted by 100,000.

### Sperm viability

The caudal epididymis sperm was dropped on the slide and mixed with a drop of 0.5% eosin solution. After 2 min, the slide was examined under compound microscope with  $40 \times$  objective lens to count the percentage of viable (unstained) and non-viable sperm (stain red) [8].

### Sperm morphology

A drop of sperm suspension was smeared on a glass slide, fixed with 95% ethanol for 10 minutes and was allowed to air-dry. The smear was washed with sodium bicarbonate formalin solution to remove any mucus and then rinsed with several changed of water. The smear was covered with diluted (1 in 20) carbon fuchsin and allowed to stain for 3 min. The stained was washed off with water and counter stained with covered smear with diluted (1 in 20) Loeffler's methylene blue for 2 min. The normal sperm which were stained (nucleus of head –dark blue; cytoplasm of head-pale blue; middle piece and tail- pink red) were counted and expressed in percentage [8].

## Sperm progressivity

The progression is determined by subjecting grading system (grade): A or 4; excellent forward directional movement, B or 3; good forward directional movement, C or 2; fair forward directional movement, D or 1; poor forward directional movement as described by world health organization (2005).

## Biochemical assays

Serum levels of oxidative stress markers (Malondialdehyde; MDA and Superoxide dismutase; SOD) were measured by standardized enzymatic colorimetric methods using assay kit obtained from Randox Laboratory Ltd. (Co. Antrim, UK).

## Histomorphometric analysis

The testicular tissues prepared with Haematoxylin and Eosin (H&E) staining was also used for the histomorphometry. A computer software Image-J-win 32 (National Institute of Health, USA) was used.

## Statistical analysis

All data were expressed as means  $\pm$  SEM. Statistical group analysis was performed with SPSS version 22.0 statistical software. One-way analysis of variance (ANOVA) was used to compare the mean values of variables among the groups. Bonferroni's test was used to identify the significance of pair wise comparison of mean values among the groups. Statistically significant differences were accepted at  $p < 0.05$ .

## Results

### Effect of RF-EMR emitted from cell phone on the sperm count, motility, morphology, progressivity and life and death ratio of spermatozoa of the male Wistar rats

**Table 1** shows the assessment of sperm count, morphology, progressivity and viability, which are the criteria for assessing

**Table 1:** Effects of RF-EMR emitted from cell phone on the sperm count, motility, morphology, progressivity and life and death ratio of spermatozoa; Data are expressed as means  $\pm$  S.E.M.  $n=5$ ; Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test (\* $p < 0.05$  vs. CONTROL; # $p < 0.05$  vs. 1H-EXPOSURE) For sperm progressivity (grade) A; excellent forward directional movement B; good forward directional movement C; fair forward directional movement D; poor forward directional movement.

Groups	Control	1H-Exposure	2H-Exposure	3H-Exposure
Sperm count ( $\times 10^6/\text{mL}$ )	61.27 $\pm$ 8.90	50.93 $\pm$ 7.12	37.87 $\pm$ 7.18*	24.20 $\pm$ 5.00#
Sperm motility (%)	86.10 $\pm$ 10.00	78.43 $\pm$ 9.70	76.60 $\pm$ 72.40	72.46 $\pm$ 15.00
Sperm morphology (%NORMAL)	84.45 $\pm$ 14.00	80.47 $\pm$ 18.50	72.65 $\pm$ 19.20	61.05 $\pm$ 25.00*#
Sperm progressivity (grade)	A	B	B	C
Viability ratio (%)	91.06 $\pm$ 25.00	82.74 $\pm$ 15.00	82.04 $\pm$ 9.50	76.77 $\pm$ 20.30

sperm fertility potential. There was a significant decrease in sperm count of the rats exposed to RF-EMR emitted from cell phone for 2 h and 3 h exposure while the rats exposed for 1 h remain unchanged when compared with control rats respectively. Also, sperm count of the rats exposed for 3 h was significantly different from the rats exposed for 1 h. The normal sperm morphology was significantly decreased in group with 3 h exposure to RF-EMR emitted from cell phone when compared with control and 1 h exposure rats. The sperm progressivity remarkably declined in all the experimental groups with more declination in group that was exposed for 3 h compared with control group. The sperm motility and viability remained unchanged in all the experimental groups compared with control group.

### Effects of RF-EMF emitted from cell phone on the testicular morphometry parameters; cross section diameter, lumen diameter, germinal epithelia Height of the male Wistar rats

The testicular morphometry parameters showed no significant change in all the experimental groups compared to control group. However, lumen diameter significantly decreases in 3 hrs exposure rats compared to 1 h exposure rats **Table 2**.

### Effect of RF- EMF emitted from cell phone on MDA, SOD and circulating sex hormones of the male Wistar rats

**Table 3** depicts markers of ROS and circulating sex hormones. Two and three hours exposure groups showed comparable significant increase in MDA level compared to the control and one hour exposure groups. In addition, **Table 3** showed SOD level. Comparable significant decreases were observed in two and three hours exposure groups compared to the control and one hour exposure groups. Also, Sera levels of sex hormones (FSH, LH and testosterone) significantly decreased in 2 and 3 h exposure groups compared to the control group. In addition, 3H-exposure group is significantly lower when compared with 1H-exposure group.

Non-motile sperm (%)	13.9 ± 5.10	21.57 ± 8.25	23.40 ± 6.02	27.54 ± 5.05
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**Table 2:** Effects of RF-EMR emitted from cell phone on the testicular morphometric parameters (cross section diameter, lumen diameter, germinal epithelia height) of male Wistar rats; Data are expressed as means ± S.E.M. n=5; Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. (#p<0.05 vs. 1H-EXPOSURE); CS/D (cross section diameter); L/D (lumen diameter); GE/H (Germinal epithelia height).

Groups	Control	1H-Exposure	2H-Exposure	3H-Exposure
CS/D (µm)	3.27 ± 1.18	4.26 ± 0.29	4.23 ± 0.42	4.52 ± 0.19
L/D(µm)	0.17 ± 0.05	0.22 ± 0.05	0.18 ± 0.03	0.14 ± 0.02 <sup>#</sup>
GE/H (µm)	0.28 ± 0.04	0.26 ± 0.02	0.25 ± 0.04	0.24 ± 0.02

**Table 3:** Effects of RF-EMR emitted from cell phone on oxidative stress markers and circulating sex hormones of male Wistar rats; Data are expressed as means ± S.E.M. n=5; Data were analyzed by one-way ANOVA followed by Bonferroni post hoc test. (\*p<0.05 vs. CONTROL; # p<0.05 vs. 1H-EXPOSURE); MDA (Malondialdehyde); SOD (Super oxide dismutase); FSH (Follicle stimulating hormone); LH (Luteinizing hormone) and TT (Testosterone).

Groups	Control	1H-Exposure	2H-Exposure	3H-Exposure
MDA (µmol/L)	3.75 ± 0.25	4.35 ± 0.80	6.10 ± 1.00 <sup>#</sup>	7.25 ± 1.30 <sup>#</sup>
SOD (µmol/L)	6.05 ± 1.20	5.05 ± 0.70	3.35 ± 0.50 <sup>*</sup>	2.50 ± 0.50 <sup>#</sup>
FSH (mIU/mL)	1.35 ± 0.05	1.10 ± 0.10	0.75 ± 0.04 <sup>*</sup>	0.65 ± 0.04 <sup>#</sup>
LH (mIU/mL)	3.25 ± 0.25	1.05 ± 0.15	0.35 ± 0.05 <sup>*</sup>	0.15 ± 0.04 <sup>#</sup>
TT (mIU/mL)	9.30 ± 1.30	8.30 ± 0.70	6.80 ± 1.00 <sup>*</sup>	3.75 ± 0.50 <sup>#</sup>

## Discussion

The present study demonstrates that exposure to RF-EMF emitted from cell phone for 1 h, 2 h and 3 h per day for a period of 28 days altered sperm count and sperm progressivity but none of the current exposure to RF-EMR emitted from cell phone altered sperm motility and sperm viability. However, 3 h exposures led to a significant decrease in the percentage of normal sperm morphology. In addition the exposures did not significantly affect the histomorphometric parameters except lumen diameter that was significantly lower in 3 h exposure compared to 1 h exposure. Our results further demonstrate increase in circulating MDA level and decrease in circulating SOD level for 2 h and 3 h exposure to RF-EMR emitted from cell phone. A significant decrease in FSH, LH and testosterone hormone was observed in 2 h and 3 h exposure but not in 1 h exposure.

The present findings that 2 h and 3 h exposure to RF-EMR emitted from cell phone decrease the sperm count and alter the sperm progressivity even without significant change in sperm motility, sperm viability and histomorphometric parameters suggest a defective sperm function. These findings were in consonance with earlier study that exposure of male reproductive organs to cellular phones might cause spermatogenic dysfunction, thus changing the sperm cell cycle due to deformation of leydig and sertoli cells that is associated with cellular proliferation and decrease fertility in men [1]. Since the 1 h exposure to RF-EMR emitted from cell phone did not significantly alter the spermatogenic function, whereas even 3 h exposure significantly alter the normal sperm morphology, suggesting that the severity of the effect of RF-EMR emitted from cell phone to testes is duration dependent.

In addition, our present study show increased circulating MDA level (oxidative stress marker) in 2 and 3 h exposures but not in 1 h exposure to RF-EMR emitted from cell phone, suggesting that the defective spermatogenic function in the present study is associated with increased oxidative stress. This finding provides further evidence to previous studies that increased MDA concentration induces lipid peroxidation of sperm membrane which damages the cell plasma membrane and results in cell death, thus high lipid peroxidation inhibits sperm motility and viability which may reduce the capacity of sperm to undergo acrosomal reaction and fertilization [9]. The observed significant decrease in the level of circulating antioxidant enzyme SOD in 2 and 3 h exposures to RF-EMF emitted from cell phone suggest a decline in sperm antioxidant capacity, which in turns leads to a surge in oxidative stress. ROS are continuously neutralized by antioxidants; antioxidants protect spermatozoa against oxidative stress which act as a defense mechanism. This result of biochemical analysis is in consonance with that lipid peroxidation is associated with poor sperm function which causes infertility and oxidative stress, impairs sperm motility and will result in less sperm reaching the oocyte for fertilization [9,10]. The present observation is associated with a decline in circulating sex hormone.

## Conclusion

The study suggests that chronic exposure to radiofrequency electromagnetic radiation of cell phone causes oxidative stress and impairs testicular function that is accompanied by decreased sex hormone profile.

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