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EFFECT OF CHRONIC EXPOSURE TO GSM 900/1800 MHZ RADIOFREQUENCY RADIATION ON GENERAL BLOOD PHYSIOLOGY AND REPRODUCTIVE FUNCTION IN MALE RATS

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ABSTRACT

Introduction: The long term interaction of mobile phone radio-frequency radiation (RFR) on general health, especially on male fertility and repercussion of above is becoming a major concern for current researchers for obvious reasons. Objective of the current study was aimed for evaluating the effect of long term exposure of RFR from mobile phones on blood pressure, hematological parameter, testosterone level, sperm count and their morphology. To perform the current study the RFR generator was used and a uniform radio frequency radiation with frequency range of 900 MHz and 1800 MHz were exposed to the rats placed individually in hexagonal plastic cage which is divided into six compartments. After the experimental period, blood pressure, hematological parameter, testosterone level, sperm count and their morphology were studied. Total sperm count, their morphology, total platelet count were significantly affected at both RFR. BP, neutrophil, lymphocyte percentage were significantly affected at 900 MHz however no significant changes were observed in hemoglobin content, total RBC and WBC count, eosinophil percentage and serum testosterone level. Hence it can be concluded that a long term (12week) mobile phone RFR exposure significantly affected hematological parameter, and had perilous effect on male fertility in rats.

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INTRODUCTION

In today's society, our fast pace lives have been the driving forces behind vast technological innovations such as the Internet, email, and most recently, the "Smartphone". Cell phones have become a vital part of our lives. We seldom give importance to the aspect associated with the impact of these devices on human health, more specifically male fertility. Cell phones emit radiofrequency electromagnetic waves (RF-EMW) to nearby relay base stations or antennas. The human body acts as antennas that absorb the radiation and convert it into alternating eddy currents. [1] The frequencies of these radio waves fall in the low frequency microwave range (800- 2200 MHz), therefore, non- ionizing radiation type as the energy emitted is too low to break chemical bonds in biological system. On the other hand, the energy carried in extremely high frequencies (1,000,000 MHz) electromagnetic waves such as x-rays is so intense that the electromagnetic particles have sufficient power to break chemical bonds and cause serious damage to human tissue; this type of radiation is known as ionizing radiation. Given the large number of mobile phone users, investigating, understanding, and monitoring of any potential public health impacts of mobile phone use are important [2].

Studies to assess the potential long-term effects of mobile phone use are ongoing. There is currently no consensus on whether mobile phones might have health or even biological effects, and in particular, plausible mechanism for the effects of mobile phone radiation is not well understood, although a few studies have suggested the involvement of lipid peroxidation and free radical formation including biochemically induced oxidative stress [3]. Although the radio waves of cellular phones do not have enough energy to cause the ionization of atoms and molecules, the recent concerns over long-term exposure to the electromagnetic radiation emitted by mobile phones should be taken more seriously given the fact of growing trend toward deterioration of the male germ line (spermatogenesis and sperm maturation) [4]. A common finding is that these effects were associated with the production of reactive oxygen species (ROS) which can damage many cellular components, including cell membranes and DNA [5, 6]. More recently, Agarwal *et al.* (2009) found in controlled experiments that ejaculated sperm from healthy donors showed reduced viability and motility and an increase in ROS after one hour's exposure to a cell phone in talk mode [7]. Mailankot et al (2009) found that exposure to GSM signals from a mobile phone for 1 hour per day for month significantly reduced sperm motility in rats. No SAR estimation or other measures of power output of the phone were provided. Again, nothing of value can be deduced from this study due to the complete lack of dosimetry [8]. In the present study, we tried to focus on evaluating the effect of really long-term (12 week) exposure to RFR emitted from a mobile phone (900/1800 MHz) on male fertility in terms of total sperm count and their morphology. Besides the above mentioned, probable impact of long term exposure on general hematology and blood pressure were also evaluated.

MATERIALS AND METHODS

Animals:

Inbred healthy Wistar Male rats weighing 150-220g were used as experimental animals. They were obtained from Al-Ameen College of Pharmacy, central animal facility. The rats were housed in plastic cages of size 36 cm - 23 cm - 21 cm (three rats in each cage) inside a temperature- and humidity-controlled environment with free access to food and water ad libitum, with a 12 h light and 12 h dark cycle. All the experiments were carried out with prior approval from the institutional animal ethics committee. Care was taken to handle the rats in a humane manner, and all precautions were taken to use the minimum number of animals required to generate significant data. The use of animals in these experiments was authorized by IAEC. The experimental rats were handled and monitored throughout the experiment as per the CPCSEA guidelines.

Experimental design:

The radio frequency electromagnetic radiation generator (manufactured by AND Solutions, India) was used to emit 900MHz and 1800MHz frequencies, SAR set at 1.6 W/kg and power density was 0.9 watt/m² (which was fixed statically by manufacturer as per IEEE guidelines) via monopole antenna that directed the signal to the cages. The antenna generated uniform RF radiations as a genuine GSM mobile phone. The length of the monopole antenna was set so that the antenna resonant at the operating frequency. The antenna had an Omni-directional pattern in the azimuth plane through which the rats were to be uniformly distributed. Male and female Wistar rats were placed individually in an hexagonal acrylic plastic cage (40 cm in diameter) which was divided into six compartments and exposed to RF-EMR daily 2 h for 3 months. The cage was designed in such a way that the animals were not restrained and ventilated by drilling holes. The animals were left for 3 days for acclimatization. Sham exposed animals underwent the same transportation, habituation and handling procedure without switching on the signal generator i.e. no RFR exposure. The antenna was placed 30 cm above the ground. [Figure 1]

Groupings:

Animals were divided in to 3 groups, each containing 6 male Wistar rats: Group I (n = 06), negative control, i.e. sham exposure to radiation for 2 hours per day for 3 smonths, Group II (n=06) were exposed to RFR 1800 MHz 2 hours per day for 3 months, Group III (n=6) were exposed to RFR 900 MHz 2 hours per day for 3 month. After duration of 3 months at the end of the study, blood pressure was measured by non-invasive method. Blood was withdrawn from retro orbital sinus under light anesthesia for the measurement of hematological parameters. Rats were sacrificed by overdose of anesthesia and epididymis was isolated for the estimation of sperm count and sperm morphology.

Measurement of blood pressure:

Non-invasive blood pressure was performed by tail cuff using MLT125 NIBP Controller (AD Instruments, Australia). Animals were trained for 2 to 3 sessions to acclimatize the condition and placed in the Perspex cylinder restraint cage comfortably. Rats were kept under constant temperature between 28 to 30°C to improve the blood circulation in the tail and the signal to noise ratio in the recording. The tail cuff was positioned at the proximal end of the tail which was used to occlude blood flow in the tail and thereby interrupt the pulse that is measurable in the caudal artery. The active site of the pulse transducer was placed on the ventral surface of the tail, directly below the caudal artery. The transducer was positioned directly following the tail cuff. Maximum sensitivity was achieved when the artery was positioned above the most sensitive position on the transducer. Movement from this position can reduce the amplitude of the measured pulses. The transducer which is used to make the pulse measurements is very sensitive and subject to vibrations. So it was ensured that mechanical vibrations from other laboratory devices do not affect the transducer. The basic signals recorded by the MLT125 NIBP system are the cuff pressure and the caudal artery. Systolic blood pressure (SBP) occurs when the cuff pressure corresponds to the restoration of the first caudal artery pulse. Each measurement was repeated five to six times to ensure reproducible results. Although direct observation of the pulse is usually sufficient to determine the systolic blood pressure point, alternative methods of detecting is the onset of the pulse. [9, 10]

Collection of serum:

The blood sample collected in the Eppendorf tube was allowed to clot for half an hour. The sample was then centrifuged using cold centrifuge at 8000 rpm for 10 minutes. The clear supernatant was carefully separated for experiment.

Estimation of Blood parameters**Hemoglobin estimation:**

Hemoglobin content was estimated by using Sahli's haemoglobinometer. Hemoglobin value was expressed as % Hb. [11, 12]

RBC count:

RBC count was done by using Neubauer's Haemocytometer. The number of cells in undiluted blood are calculated and reported as the total number of cells per cubic mm of whole blood [13].

Total WBC estimation

Total WBC count was done by using Neubauer counting chamber. Crystal violet was used to stain the nucleolus of the leucocytes. The number of leucocytes in a diluted fluid can be counted by using a Neubauer counting chamber and represented as cells/cumm of blood. [14]

Platelet count

Platelet count was done with the help of hemocytometer chamber using appropriate diluents (1% ammonium oxalate) and reagent. Platelets appear greenish, not refractive. [15]

Differential leukocyte count

Differential leukocyte count was done by using polychromic staining solutions (Wright, Leishman's, and Giemsa) methylene blue and eosin. These basic and acidic dyes induce multiple colors when applied to cells, methanol acts as fixative and also as solvent. Differential leukocyte count was expressed in percentage term. [16]

Evaluation of serum testosterone level in male rats using testosterone assay

The testosterone levels of blood serum were measured by immunoassay method. The testosterone analysis was conducted by an automated mini VIDAS[®] instruments "Bio-Merieux, France" (Medical Avicenna Laboratory, Algiers), for the quantitative determination of total testosterone in serum or plasma using the ELFA technique (Enzyme Linked Fluorescent Assay).

Evaluation of sperm parameters in male rats:**Total sperm count**

Epididymis was separated carefully from testis and divided into 3 segments: head, body and tail. The epididymal tail was trimmed with scissors and placed in petri dishes containing 1.0 ml of 0.1 M phosphate buffer of pH 7.4. It was swirled for homogeneity and then was allowed for dispersion of sperm cells in the solution for 10 min under 37°C. Sperm count was done using an improved Neubauer's chamber. Concentration is usually expressed in terms of millions/ml. [17, 18]

Sperm morphology [17, 18]

The sperm morphology was also determined using eosin staining method. For this purpose 10 µl of 1% eosin Y was added to a test tube containing 40 µl of sperm suspension and were mixed by gentle agitation. Then, sperm were incubated at room temperature for 45 to 60 min for staining and then re-suspended with a Pasteur pipette. Two hundred sperm per animal were examined microscopically at ×40-100 magnifications and the number of morphologically abnormal sperm was recorded to give the percent abnormal sperm.

Statistical analysis

All the values are expressed as Mean \pm SEM (n =6). The data were analyzed with Graph Pad Prism version 5 (San Diego, CA). Statistical analysis of data was done by one-way ANOVA, followed by followed by tukeys multiple comparison test. A level of $p < 0.05$ was accepted as statistically significant.

RESULTS

Effect of RFR on blood pressure in male rats

There was no significant changes in BP was observed in rats exposed to 1800MHz compared to sham, but groups exposed to 900MHz have shown significantly lower BP when compared with sham as well as 1800MHz exposed. [Figure 2] [Figure 3]

Effect of RFR on blood parameters

Effect of RFR on blood hematological parameters in male rats

There was no significant change was observed in terms of %hemoglobin content, total RBC and WBC count when RFR exposed rats were compared with sham exposed. [Figure 4.1, 4.2, 4.3]. Male rats exposed to RFR at 1800 MHz and 900 MHz frequency have shown significantly higher platelets count compared to sham exposed rats. [Figure 4.4].

Effect of RFR on blood on differential leukocyte content in male rats

Male rats exposed to RFR at 900 MHz frequency had shown significantly decrease in lymphocyte percentage ,but increase in neutrophil percentage as compared to sham as well as 1800MHz exposed rats, where as no significant change was observed in case of eosinophil percentage. [Figure 4.5.1, 4.5.2, 4.5.3] [Table I]

Effect of RFR on serum testosterone level in male rats

There was no significant change was observed in terms of serum testosterone level when RFR at 900 and 1800MHZ exposed rats respectively were compared with sham exposed. [Figure 5]

Effect of RFR on sperm parameters in male rats

Effect of RFR on sperm count

Male rats exposed to 900MHz and 1800MHz had shown significant decrease in sperm count when compared with that of sham exposed. But no significant changes were observed between the two frequencies. [Figure 6.1][Table II].

Effect of RFR on sperm morphology

Male rats exposed to 900MHz and 1800MHz had shown significant percentage increase of defective sperms when compare with that of sham exposed rats. However no significant changes were observed between the two frequencies. [Figure 6.2] Table II].

Table I: Effect of exposure to RFR on differential leukocytes count in male rats.

Sl.No	Group	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)
1	Group 1 (Sham exposure)	69.67 \pm 2.17	19.00 \pm 2.70	11.33 \pm 1.38
2	Group 2 (1800 MHz)	66.00 \pm 3.12	21.50 \pm 2.91	12.50 \pm 0.50
3	Group 3 (900 MHz)	55.50 \pm 2.41**	31.50 \pm 2.15*	13.00 \pm 0.85

Values are expressed as Mean \pm SEM; n= 6. ** $p < 0.01$, * $P < 0.05$ when compared with sham exposed by using one way ANOVA followed by Tukey's multiple comparison test.

Table II: Effect of exposure to RFR on sperm count and sperm morphology in male rats.

Sl.No	Group	Total Sperm count	% of Defective sperms
1	Group 1 (Sham exposure)	84.00 \pm 6.10	11.17 \pm 1.22
2	Group 2 (1800MHz)	14.77 \pm 3.08***	28.50 \pm 1.83***
3	Group 3 (900MHz)	14.06 \pm 4.86***	30.33 \pm 3.818***

Values are expressed as Mean \pm SEM; n= 6. *** $p < 0.001$, when compared with sham exposed by using one way ANOVA followed by Tukey's multiple comparison test.

DISCUSSION

Mobile phones create an EMF around them when in use, thus increasing the electromagnetic contamination, also known as “electrosmog” in the vicinity. In response to public and governmental concern, the WHO established the International Electromagnetic Fields (EMF) Project in 1996 to assess the scientific evidence of the possible adverse health effects of the electromagnetic fields and to conduct a formal health risk assessment of radiofrequency field exposure by 2012 to fill these knowledge gaps [19]. In current study the results were elaborately analysed and it revealed that, chronic exposure to RFR from mobile phone could have adverse effect on the well being of general health and more importantly on reproductive function. As such there is no published data regarding the chronic exposure of RFR at 900MHz and 1800MHz on vital sign of male rats. Though Alhousseiny et al (2012) in a study showed that the radiofrequency of cell phone interferes with the cardiac conduction [20]. Blood pressure (BP) is the pressure exerted by circulating blood upon the walls of blood vessels and is one of the principal vital signs. Blood pressure varies depending on situation, activity, and disease states, and is regulated by the nervous and endocrine systems. Effect on blood pressure due to RFR did not show significant differences between sham exposed (normal rats) and radiation exposed 1800MHz rats, but there was a slight decrease in blood pressure among male rats exposed to 900 MHz when compared to normal rats but the decrease in blood pressure was found to be in the normal range. This decrease in blood pressure might be due to depression, hormonal changes, abnormal breathing. Measurements of blood parameters are most important means to determine the health status of experimental animals, as it is the diagnostic for certain diseases such as anemia, leukemia and detect the possibility of any infection or inflammation. Study performed by Usman et al on mice suggested that long term exposure to 1800 MHz and 900 MHz RF fields showed that hematological parameters of the exposed mice were in within the normal range [21].

Absorption of radiation have direct relation with body mass hence to get the possible impact on human, in present study, analyses of hematological parameters on rats was performed which have higher body mass then the mice and has shown miscellaneous effects of exposure to RF radiations. There was not significant change was observed in case of hemoglobin (%), RBC count, total W.B.C count, eosinophils (%) when compared to normal rats. Though the study was for 12 week and no significant change in above mentioned parameter denies any hazardous effect, but given the fact in case of humans who uses cell phones for lifelong, moreover deranged pattern in observed data does not completely negate the possible impact. Further platelet count found to be significantly increased in male rats exposed to 1800 MHz and 900 MHz, compared to normal. Radiation could have some inflammatory effect, though not any plausible mechanism can be deduced in present situation, but the apparent result from present study definitely needs to be taken in to consideration for further study. At the same time increase in platelets may leads to blood clotting, bleeding or stroke. High platelets are also accompanied by a persistent headache, difficulty breathing, dizziness, and seizures, changes in speech, or confusion or loss of consciousness for even a brief moment. Interestingly, rats exposed to RFR at 900 MHz frequency had shown significantly decrease in lymphocyte percentage, but increase in neutrophil percentage as compared to sham exposed rats. However significant difference between 900 and 1800 MHz was also observed which shows the possibility of differential impact of frequencies on WBCs. 900 MHz had shown more impact as compared to 1800 MHz. Though the present study finding is miscellaneous, and mechanism behind this could not be explained but it draws the attention for further elaborate study. Till that probable pathological effect of this deranged hematological parameter couldn't be neglected. In this study, a significant change in testosterone levels was not seen on chronic exposure of RFR at 900MHz and 1800MHz. In contrast with our finding, rats exposed to 30 min per day, 5 days a week for 4 weeks to 900 MHz EMF causes significant decrease in serum total testosterone level [22]. Whereas exposure to electromagnetic field 1800 and 900 MHz for 2 hr continuously per day for 90 days caused an increase in testosterone. This might be due to long term exposure might have induced some compensatory mechanism. To understand the reason behind this is possible only if elaborate experiment regarding interrelationship of testosterone with other hormones on long term basis is performed. As men usually carry mobile phones in their pockets or in holders close to their reproductive organs, it is important to evaluate the effects of mobile phone use on male fertility. Mobile phones might have undesirable impact on the reproductive system via a RFR specific effect, a thermal effect, or a combination of these effects. As per Agarwal A et al. in 2008, the use of cell phones by men is associated with a decrease in their semen quality. According to published data the decrease in sperm count, motility, viability, and normal morphology was related to the duration of exposure to cell phones [23]. In a very recently published study by Kuzay D et al has shown exposure of rats to both RFR caused an increase in oxidative stress and decrease in antioxidant level in testis tissue. The effect is more severe if the subject is already suffering from metabolic disorder like diabetes. [24]. As per Falzone N, et al, 2011 a significant reduction in sperm head area and acrosome percentage of the head area was reported among exposed sperm compared with unexposed controls. It was concluded that although radiofrequency electromagnetic fields exposure did not adversely affect the acrosome reaction, it had a significant effect on sperm morphometry [25]. In present study effect of RFR on sperm count was carried out and rats exposed to both 900 and 1800MHz has shown significant decrease in sperm count besides showing significant increase in percentage of defective sperms as compared to control rats. This finding is with accord to earlier published results. Based on earlier published result and present study data, it signifies that exposures to RFR not only adversely affect the male fertility, but there is also a negative impact on general health in terms of hematological parameter, blood pressure. In order to get the more insight on effect of RFR of 900 and 1800MHz on extent of damage and mechanism, further studies are warranted.

CONCLUSION

The radiofrequency radiations (RFR) exposures from mobile phone do have the negative effect on general well being of rat. Meanwhile differential response at two frequencies seeks more elaborate research for upcoming days. However the evidences from present study definitely draws the attention towards the quite precarious effect of RFR at both the frequencies from the mobile phone on general health of possibly humans too in terms of hematological parameter, blood pressure and very importantly on male fertility.

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Authors' Statements

Competing interests

The authors have no conflict of interest.

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