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# Mobile phone usage and male infertility in Wistar rats

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A significant decrease in protein kinase C and total sperm count along with increased apoptosis were observed in male Wistar rats exposed to mobile phone frequencies (2 h/day × 35 days at 0.9 W/kg specific absorption rate). The results suggest that a reduction in protein kinase activity may be related to overproduction of reactive oxygen species (ROS) under microwave field exposure. Decrease in sperm count and an increase in apoptosis may be causative factor due to mobile radiation exposure leading to infertility.

**Keywords:** Apoptosis, Mobile phone radiation, Protein Kinase C, Sperm count

In recent decades, concern has aroused about decreasing fecundity and fertility in man[1,2]. The reasons for this are often linked to various types of environmental and occupational exposure, leading to possible causes for the reduced sperm quality and impact on neurological dysfunction[3]. The occupational exposure emitted from mobile phone and several other gadgets may affect biological systems due to an increase in temperature i.e. thermal[4], though non-thermal effects have also been established[5].

A hazardous effect of mobile phone exposure on male infertility is linked to a decrease in sperm count, disorders in their motility and structure. Several studies on mobile phone emissions indicate that these radiations may alter hormone secretion due to deformation of leydig and sertoli cells, which may lead to cell proliferation (activated by follicle-stimulating hormone)[6]. Such type of alteration may also decrease the sperm count and may also cause DNA strand break[7,8]. Wang *et al.*[9] in their study on mice, pointed out that leydig cells are among the most susceptible cells to electromagnetic wave (EMW) and injury to these cells may affect spermatogenesis. Decrease in sperm count, weight of testicular organs (i.e. caput, cauda)[10] and destruction in leydig cells due to these radiations are indication of male infertility. Agarwal *et al.*[11] experimenting with 361 men attending an infertility clinic, concluded that the use of cell phones adversely affects the quality of semen by decreasing the sperm counts, motility, viability and morphology, which might contribute to male infertility. It is suggestive that these are induced by oxygen and oxygen derived free radicals commonly known as reactive oxygen species (ROS). These can contribute to hormonal imbalance, gonadal dysfunctions, poor sperm motility, decrease or increase in level of antioxidant enzyme and protein kinase C (PKC) activity, thereby leading to infertility [11-13,7].

PKC is a key regulatory enzyme in signal transduction mechanism governing cellular responses[14], which may be present in seminiferous tubules and leydig cells[15,16] in male. A decrease in level of PKC indicates a decrease in level of $G_2$/M phase and an increase in apoptotic phase. Use of mobile phones has negative affects on sperm motility[17], antioxidant enzymes[18] and sperm concentration[19]. In addition PKC has also been implicated in tumor promotion[20].

Acute and chronic, continuous or pulsed wave irradiation of animals can produce morphological alterations in biological cells and tissues[21]. Present investigation is aimed at providing additional data confirming above findings in mobile phone exposure in wistar rats.

## Materials and Methods

*Material*— Propedium iodide (PI) for fluorescence-activated cell sorting (FACS) was purchased from Sigma Chemicals, USA. $P^{32}$ radio active labeled ATP was purchased from BRIT, Hyderabad, India. Rests of the chemicals were purchased from Thomas Baker Chemicals Limited, Marine Drive, Mumbai.

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*Animals exposure*—Male wistar rats (70 days old weighing 200±20 g body weight) were obtained from animal facility of the University. The animals were divided into two groups of 6 each: control and exposed group. All the experiments were repeated and done in blind pattern. All animals were housed in an air conditioned room (25°-27°C) with 40-50% RH and kept on 12/12 h light/dark cycle throughout the experiment. Animals were provided with standard food pallets (Tetragon Cheime Private Limited, Bangalore) and water *ad libitum*.

The protocols for animal experimentation were approved by the Institutional Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All subsequent animal experiments adhered to the 'Guidelines for Animal Experimentation' of the University.

*Exposure chamber*—Rats were placed in Plexiglas cages, fixed with anechoic material which was ventilated with holes of 1 cm diameter. Exposure with mobile phone radiations, specific absorption rate (SAR) was 0.9 W/kg. Each animal cage (6) was attached with separate mobile phone. Variation of SAR within each animal cage is minimal as discussed earlier[22].

*Power calibration*—For the purpose of calibration, the emitted power of mobile phone was measured by using a specially designed monopole antenna. Mobile was kept on exposure box and was excited by an external source, whereby the emitted power was measured by a power meter (RF power sensors 6900 series and IFR 6960 B sensors RF power meter) (at a distance of about 2 cm), attached to the antenna by SMA connector simulating an exposure scenario. The maximum and minimum emitted power turned out to be 2mW and 100 μW respectively. A diagrammatic presentation is shown in Fig. 1.

*Protein kinase C (PKC) assay*—After 35 days of exposure, the animals were sacrificed with rat cocktail anesthesia (Ketamine, Xylazine; 0.1-0.2 ml/250g, ip). Sperms were collected in 40 volumes of ice-cold 1 m*M* sodium bicarbonate (*p*H 7.5) and centrifuged at 600 g for 10 min at 4°C. The supernatant were centrifuged at 20,000 *g* for 30 min at 4°C. The pellet was pipetted with ice cold 1 m*M* sodium bicarbonate and centrifuged at 20,000 *g* for 30 min. at 4°C. The pellet was re-suspended in incubation buffer (100 m*M* HEPES, 120 m*M* NaCl, 1.2 m*M* $MgSO_4$, 2.5 m*M* KCl, 15 m*M* $NaHCO_3$, 10 m*M* glucose, 1 m*M* EDTA, *p*H 7.4) and protein concentration was measured by Lowry's method[23]. Protein kinase activity was assayed in a total volume of 0.5 ml incubation medium (50 m*M* HEPES, 10 m*M* $MgCl_2$, 0.5 m*M* $CaCl_2$, and 0.2 m*M* EGTA (free calcium level of 0.1 m*M*), *p*H 7) with a total protein concentration of 100 μg. $P^{32}$ labeled ATP (specific activity 3000Ci/mmole ATP) was added to initiate the reaction and then incubated at 25°C. Samples (50 μl) were taken out and pipetted upon 3 mm filter discs (pretreated with 10% trichloroacetic acid, 20 m*M* sodium pyrophosphate, and 10 m*M* EDTA). These filter discs were dropped into 500 ml of the TCA mixture (10% trichloroacetic acid, 20 m*M* sodium pyrophosphate, and 10 m*M* EDTA) and left overnight at 4°C. Filters were washed once in 5% TCA, heated to 90°C for 15min in 10% TCA. Further 5% TCA wash were extracted in hot ethanol/ether (3:1 v/v) before drying. Radioactivity was measured in a Hewlett Packard scintillation counter.

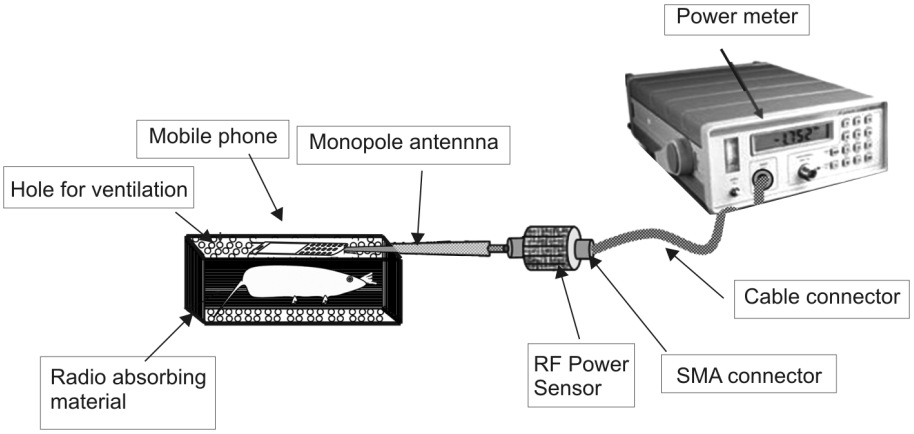


Fig. 1— Calibration methodology of power emitted from mobile phone during exposure.

*Flowcytometer detection of apoptosis and total sperm count*—Sperm samples (100 µl, $2\times10^6$ cells/ml) were taken in falcon 12×75 mm polypropylene tube with snap cap and 1 ml ice-cold 70% ethanol was added. Sample was incubated overnight at 4°C. After the completion of an incubation period, sample was centrifuged at 600 g for 10 min at 4°C and finally supernatant was decanted. 100 µl RNAase (100 unit) was added to the pellet and pipetted thereafter. Samples were incubated at room temperature for 20 min. Finally these were stained with 50 µl (25 µg/ml) propidium iodide (PI) just prior to the analysis.

## Results

*Protein kinase C Activity*—The important role of PKC is the transduction for the activation of many cellular functions and control of cell proliferation. Cells, which are subjected to prolonged exposure, may cause tumor promotion (phorbol esters), showed depletion in PKC level. In the present investigations PKC activity in sperm cells showed significant decline ($P < 0.05$) in mobile phone exposed group (2876 ± 617.9/mg protein) as compared to the sham exposed ones (3013± 520.67/mg protein).

*Flowcytometer detection of total sperm count and apoptosis*—Fluorescence activated cell sorting (FACS) analysis was carried out to confirm the occurrence of apoptosis and total sperm count in the epididymis after mobile phone exposure followed by propidium iodide (PI) staining (Fig. 2). A significant increase in peak height (M2) due to an increased apoptosis was observed in exposed group as compared to the sham exposed (2b). Peak (M1) showed a significant decrease in sperm count of exposed group as compared to the sham exposed group. In exposed rats a significant decrease in the mean value of total sperm count (31.14 ± 13.6) was observed as compared to the sham exposed ones (61.33 ± 3.68). Also the number of apoptotic cells in exposed group (13.15 ± 1.26) was significantly higher as compared to sham exposed (5.93±1.64 % mean) animals.

## Discussion

The first report that these radiations could have harmful effects on sperm was published over 64 years ago[24] and it is now generally accepted that reactive oxygen species (ROS) production in sperm suspensions, lipid peroxidation and DNA oxidation are associated with poor sperm function causing infertility[25-27]. ROS are able to damage many biomolecules, including DNA, enzyme, lipids and protein. Lai and Singh[28] reported that free radical generation in microwave exposed body may alter biological damage like DNA strand breaks in rat brain cells when exposed to 2.45 GHz continuous and pulsed RF radiation for 2 h per day. These findings also support the results that mobile phone radiation may lead to oxidative stress due to overproduction of ROS in human semen[29].

The outcome of oxidative damage induced by electromagnetic fields will therefore depend on various factors, including the oxidative status of the cell, capability of endogenous antioxidation enzymes and processes to counteract free radical buildup,

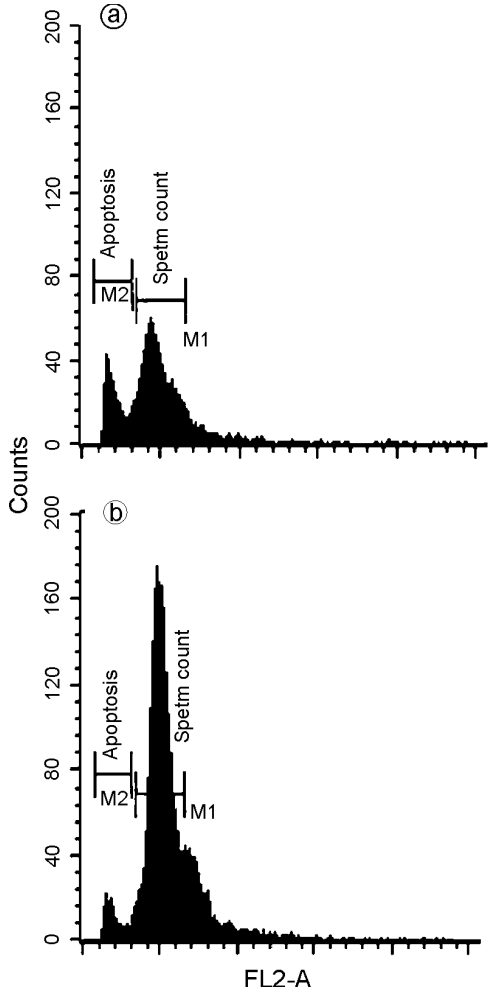


Fig. 2— Flow cytometry analysis of mobile phone exposed (a) and sham exposed (b) sperm. Sample shows comparative analysis of apoptotic and total sperm cell count. Histogram of fluorosphere blue-fluorescence (FL2-A) used to count the total apoptotic cells (M2) and total sperm cell count (M1).

availability of exogenous antioxidants, the parameters of exposure (e.g., generated power, duration of exposure and wave shape), and whether the oxidative damage is cumulative.

The present findings viz protein kinase C (PKC), apoptosis and sperm count are suggestive that their levels are affected due to mobile phone radiation exposures. Such type of alterations may have implications leading to infertility. It is well known that PKC plays a key role in a variety of pathologic states including oncogenesis[30,31] and in mediating cellular responses to extracellular stimuli involved in proliferation, differentiation, apoptosis, and exocytotic release in a number of non-neuronal and sperm cells[32]. It was found to be localized mainly in the equatorial segment of the human sperm[33,34]. Several lines of evidence suggest that PKC modulates ion conductance by phosphorylating membrane proteins such as channels, pumps, and ion exchange proteins, besides its role in extrusion of $Ca^{2+}$ immediately after its mobilization into the cytosol. Moreover the sperm plasma membrane contains a $Ca^{2+}$ channel that is activated by PKC[35]. Protein phosphorylation and $Ca^{2+}$ elevation will activate actin-severing proteins, leading to dispersion of F- actin, which enables the plasma and the outer acrosomal membranes to come into contact and fuse, release the acrosomal exocytosis.

The activation of this enzyme is thought to be biochemically dependent on $Ca^{2+}$. Infact, calcium and cAMP are thought to be the two pivotal regulators of sperm flagellar motility. PKC activate $Na^{+}/H^{+}$ exchange[36], which may represent one possible mechanism of action in sperm. PKC is highly localized in equatorial segment suggesting a specific compartmentalized role for PKC in human sperm physiology. Thus, the PKC enzyme is important in sperm motility, and mobile phone has been reported to decrease PKC activity. Presently we suggest that mobile phone can cause declines in sperm motility by affecting PKC. Mobile phone-altered PKC activity has linked with various types of malignancies[37,38]. Changes in intracellular calcium levels and activities of PKC are interrelated as well as can be secondary to mobile phone exposure and infertility. Many studies reported decline in sperm motility with decreases in PKC activity[39,40]. Paulraj and Behari[41] and Kesari and Behari[42] reported a decrease in the activity of PKC in rats exposed to 147 MHz amplitude modulated and 50 GHz microwave exposure respectively in developing rat brain. This has been earlier corroborated in our study[43].

Yan *et al.*[44] reported with 6 hours of daily cellular phone exposure for 18 weeks exhibited a significant higher incidence of sperm cell death than control group rats. De Iuliis *et al.*[45] have also reported that the radio frequency-electromagnetic radiations emitted from mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells and stimulating DNA fragmentation. These authors[44,45] suggest that carrying cell phones near reproductive organs could negatively affect male fertility and health. Kilgallon and Simmons[46] also concluded that a decrease in sperm concentration has been found due to keeping cell phones close to waist.

Lee *et al.*[47] reported that EMF may induce cell death (apoptosis) in several *in vivo* studies mostly on mice and rats. The present findings, at much lower power show a significant decrease in sperm count and an increase in apoptosis are also in support of the findings of Agrawal *et al*[11]. Fejes *et al.*[48] observed negative effects on sperm motility due to prolonged use of cell phones. Kesari and Behari[49] also observed an increase in apoptotic cells (by TUNEL assay) and decrease in sperm count at 2.45GHz exposed animals. The changes in histone kinase and cells cycle has also been observed in 50GHz exposed animals[50]. Otieno and Kensler[51] reported ROS-mediated changes in the activities of PKC and sperm quality. Recently Kumar *et al.*[13] also reported significant changes in different phases of sperm cell cycle and histone kinase in rats following microwave exposure.

## Conclusions

Study concludes that the changes observed may have been due to an overproduction of ROS by an induced field of microwave radiations. This can trigger cell differentiation by its action on PKC which may have adverse affects on spermatogenesis. These findings also indicate that decrease in sperm count and increase in apoptosis is caused due to this mobile phone frequency exposure leading to infertility.

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