

## Studies on Genotoxic Effects of Mobile Phone Radiation on A375 Cells

Debashri Manna<sup>1</sup>, Sagar Sanyal<sup>2</sup>, Rita Ghosh<sup>1\*</sup>

1. Department of Biochemistry and Biophysics, University of Kalyani, Kalyani, Nadia, West Bengal, India

2. Department of Physiology, West Bengal University of Animal and Fishery Sciences, 37 K B Sarani, Kolkata, West Bengal, India

### ARTICLE INFO

**Article type:**  
Original Article

**Article history:**  
Received: Mar06, 2018  
Accepted: Jul15, 2018

**Keywords:**  
Cell Cycle Arrest  
DNA damage  
Electromagnetic Radiation  
Reduced Glutathione  
Reactive Oxygen Species  
Viability

### ABSTRACT

**Introduction:** Radiation from cell phones has been associated with an increased risk of cancer. The literature has reported evidence of certain biological effects resulting from exposure to various wavelengths, doses, and intensities of radiofrequency radiation. The present study aimed to evaluate the possible adverse effects of radiation from a GSM mobile phone operating at 900 MHz on human melanoma A375 cells.

**Material and Methods:** Cellular morphology was observed under an inverted phase contrast microscope. Cell viability was determined through trypan blue dye exclusion and clonogenic assay. Moreover, flow cytometry was applied to detect DNA damage, cell cycle arrest, and reactive oxygen species (ROS) production. Cellular reduced glutathione (GSH) content was estimated by measuring the total soluble thiol. In addition, the physico-chemical changes were assessed using spectrophotometer and viscometer.

**Results:** This study revealed that there was no change in cellular morphology and necrotic cell killing; although a small effect was observed on delayed cell death. Depletion in GSH content was noted, but ROS generation was not significantly different from that of the control group. No DNA damage was found during such exposure and there was no alteration in cell cycle distribution. *In vitro* evaluation of radiation effect on calf thymus DNA showed a slight perturbation in absorption spectra that was completely reversible with time. On the other hand, viscometric analysis showed no changes.

**Conclusion:** From the findings, it can be concluded that this range of mobile phone radiation for 60 min of continuous exposure has no genotoxic impact on A375 cells.

► Please cite this article as:

Manna D, Sanyal S, Ghosh R. Studies on Genotoxic Effects of Mobile Phone Radiation on A375 Cells. Iran J Med Phys 2019; 16: 75-84.10.22038/ijmp.2018.29992.1329.

### Introduction

Radiofrequency radiations (RFRs) are a part of electromagnetic spectrum with wavelength ranging from 1mm-1m and corresponding frequencies of 0.3-300 GHz. The widespread applications of RFR in communication systems and daily appliances have led to interest in its biological consequences. Moreover, with introduction of the Global System for Mobile (GSM) communications in 1992, use of these radiations has increased tremendously.

Today about one third of the world population relies on mobile phones for daily communication. The widespread use of mobile phones raises concerns about the associated health hazards. The International Agency for Research on Cancer as a part of the World Health Organization has already declared RFR as a possible carcinogen (Group 2B) [1]. Apart from cancer, cell phone usage has been linked to other health-related problems, such as cognitive disorders [2], sleep disorders [3], behavioral disorders [4], male infertility [5], and hematological changes [6-8].

Using RFRs of different frequency ranges several investigators have found deleterious consequences in cells. Damage to proteins has been detected that includes denaturation and misfolding leading to

dysfunctional enzymes or receptors [9,10]. DNA damage was also observed [11], which may be responsible for the altered cell proliferation rate [12]. In addition, changes in the cell cycle progression [13] and induction of apoptosis were detected [14].

Most studies focused on the influence on various parameters of brain and neuronal cells, as well as the cells of reproductive organs during whole body irradiation of animals. In these studies, generation of reactive oxygen species (ROS) was believed to be responsible for disruption of the hormonal communications between brain, pituitary gland, and ovary [15]. However, findings in these areas were often contradictory and have since been a topic of interest and controversy [16,17].

Some studies have raised serious concern, while others have failed to detect any damaging effects. Therefore, the effect of RFR on various endpoints in the cultured cells is highly important. Cellular endpoints, including viability, DNA damage, micronuclei formation, apoptosis, and cell cycle arrest were observed. The literature indicated that the frequency of irradiation, dose rate, wave form, its modulation, exposure condition, exposure time, and

\*Corresponding Author: Tel: +91 033 2582 8750 ext. 294; Email: rghosh\_bcbp@klyuniv.ac.in

above all, cell type play a significant role in imposing biological effects [18–21].

It was found that the frequencies in the GHz range has an important impact as it affected most endpoints in different cells [22–25]. However, at 800–1800 MHz range, the power density of radiation was important as well. The impacts were detected only at low power densities, while such effects were not evident at moderate to high intensities [26–29].

Studies using various cell lines have often exhibited varied responses. Using two cell lines- a parental and its derived one, the set of genes expressed upon RFR exposure was found to be distinct, which could account for the variability in responses [30]. Therefore, it is necessary to study the effect in separate cell types.

Skin is the largest organ of body and is maximally exposed to such radiations. The influence of RFR on different normal cells in relation to gene expression, DNA damage, and apoptosis has been evaluated, which we have reviewed earlier [21]. However, there was only one report regarding skin cancer cell line; in which was shown that RFR alone had no effect on stress-related protein, but could be influenced when combined with other stress factors [31].

We, therefore, investigated whether RFR from mobile phone could have any genotoxic action in cancer cells. In this report, we present our findings on the impact of RFR coming directly from mobile phone (900 MHz) on A375, a human melanoma cell line. We assessed the genotoxic potential of this radiation through evaluating cellular DNA damage, cell cycle distribution, ROS generation, reduced glutathione (GSH) content, and cell viability. Furthermore, the effects of such irradiation on calf thymus DNA (CT-DNA) *in vitro* were studied utilizing absorption spectrophotometry and viscometry.

## Materials and Methods

### Cell Line and Culture Conditions

A375 human melanoma cells were grown in minimal essential medium (HiMedia, India) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (HiMedia, India), 100 µg/ml of streptomycin, and 100 U/ml of penicillin (HiMedia, India) at 37 °C in a humidified atmosphere containing 5% CO<sub>2</sub>.

### Exposure Conditions

In order to evaluate the potential risk of RFR, we used a mobile phone directly as the source of irradiation. Similar setup was previously used by other investigators [32, 33]. The cultured cells were exposed to a frequency modulated 900 MHz radiation from a Nokia C2-01 mobile (NOKIA, Finland) having maximum energy output of 495 mW. Radiation was carried out by placing the Petri dishes 2 cm away from the mobile. All the irradiation procedure was carried out at 30 °C. The irradiation setup is illustrated in Figure 1. This was monitored using a handheld power analyzer (Cafago

Personal Cell Phone Dosimeter AT441, Taiwan), previously standardized by Radio Test Set (Marconi, Model - 2955).

Exponentially growing cells were exposed to the mobile phone radiation in “talk mode” for different lengths of time to vary the doses of exposure. Parallel sets of cells were placed in the same exposure conditions with the mobile phone in “standby mode” for the maximum time, which were considered as the sham irradiated cells. Cells from the parallel set of culture that were not anywhere near the mobile phone was taken as control.



**Figure 1.** Set up for exposure of the cultured cells to mobile phone RFR

### Morphological Observation

The cells were plated in 35 mm culture dishes ( $5 \times 10^5$ ) and were incubated overnight. Following 60 min of exposure, morphology of the cells was observed under inverted phase contrast microscope (Axiscope plus 2, Zeiss, Germany) and photographs were taken for control, sham exposed, and irradiated cells.

### Cell Viability Assay

Cell viability was determined using trypan blue dye exclusion assay as described earlier [34], following Chiu et al. [35]. Cells ( $1-1.5 \times 10^5$ ) were plated in 35 mm culture dishes, incubated overnight, and then irradiated for 15, 30, and 45 min at room temperature. The control, sham exposed, and irradiated cells were all washed with phosphate buffer saline (PBS), trypsinized, and counted in a hemocytometer after staining with 0.2% trypan blue (Sigma, India) for 15 min. The surviving fraction of cells was calculated as follows:

[Total viable cells (unstained)/Total cells (stained+unstained)].

### Clonogenic Assay

Exponentially growing cells were irradiated for 15, 30, and 60 min. Irradiated cells were trypsinized and seeded in a small number in 60 mm culture dishes. After 10–12 days, the visible colonies were stained with 0.5% crystal violet (Sigma, India) before counting. Finally, the surviving fractions were determined for control, sham exposed, and irradiated cells as described earlier [36].

### DNA Damage Measured by Flow Cytometry

Breaks in DNA can be determined by detecting the accumulation of hypodiploid cell population through flow cytometry using propidium iodide (PI, Sigma, India) to stain the DNA [37]. DNA damage was estimated as described earlier [38]. Briefly,  $1 \times 10^5$  cells seeded in 35 mm Petri dishes were exposed to RFR for

45 min after incubating overnight. Post-exposure, the cells were harvested immediately by trypsinization. The harvested cells were then fixed overnight in 70% ethanol. The fixed cells were centrifuged at 1000 g for 5 min at 4 °C and the cell pellet was then resuspended in PBS and treated with 10 µg/ml RNase A (Sigma, India) for 30 min at 37 °C. Next, the cell suspension was stained for 30 min at room temperature in dark with PI (10 µg/ml). Finally, the analyses were performed in FACS Calibur (BD Biosciences, USA) using Cell-Quest Pro software (BD).

#### Cell Cycle Arrest by PI Staining

The cell cycle progression was determined as described in Ghosh et al. [38] and Ito et al. [39]. Cells ( $1 \times 10^5$ ) seeded in 35 mm culture dishes were exposed to RFR for 45 min. The cells were then harvested by trypsinization at 2 and 10 h post-exposure. Afterwards, the harvested cells were fixed overnight in 70% ethanol. The fixed cells were centrifuged at 700 g for 5 min at 4 °C. The cell pellet was then treated with RNase A (10 µg/ml, 30 min) at 37 °C and was finally stained with PI (10 µg/ml) for 30 min at room temperature in the dark. The analyses were performed in FACS Calibur (BD Biosciences, USA) using Cell-Quest Pro software (BD).

#### ROS Measurement by Flow Cytometry

The redox state of the cell can be measured by specific probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). The DCFH-DA diffuses into the cells and is acetylated by cellular esterases to non-fluorescent 2',7'-dichlorodihydrofluorescein (DCFH), which is rapidly oxidized to highly fluorescent 2',7'-dichlorofluorescein (DCF) by cellular ROS [40]. As a result, the fluorescence intensity is proportional to the ROS levels within the cytosol.

The generated ROS was estimated as described earlier [41]. Briefly,  $1 \times 10^5$  cells per 35 mm culture dishes were irradiated for 45 min. Immediately after exposure, the cells were washed, trypsinized, and suspended in PBS. Next, 10 µM of DCFH-DA (Sigma, India) was added to the cell suspension and incubated in dark for 30 min at room temperature. Flow cytometry was used to determine the fluorescence of DCF on excitation at 485 nm and the fluorescence emission was recorded at 535 nm.

Considering the fact that there would be relatively low oxidative damage under normal growth conditions, the control cells were used to arbitrarily set the window position and span. Therefore, there would be only few cells with high fluorescence of DCF. These settings were maintained for recording the fluorescence from all the other samples. The Cell Quest Pro software of FACS Caliber (BD biosciences, USA) was used for analyzing the results.

#### Determination of Cellular GSH Content

The cellular GSH content in A375 cells was estimated by measuring the total soluble thiol as described by Bose et al. [42]. About  $1 \times 10^7$  cells per 100 mm culture dishes were irradiated for 45 min. Irradiated

cells were trypsinized and suspended in PBS, followed by centrifugation at 500 g for 5 min at room temperature. The pellet was suspended in 0.5 ml potassium phosphate buffer (pH: 7) containing 2 mM EDTA (Sigma, India). The cell suspension was lysed by freeze (5 min) and thaw (5 min) for three cycles using homogenizer. Sulfosalicylic acid (Sigma, India), 10% was added to each sample and allowed to precipitate for 2-3 h on ice. The sample was centrifuged at 1000 g for 15 min at 4 °C to obtain protein free lysate. In order to estimate the total soluble thiol, the supernatant was mixed with 0.4 mM 5, 5'-dithiobis 2 nitrobenzoic acid (DTNB) (Sigma, India) in 0.2 M sodium phosphate buffer (pH: 8). Finally, the absorbance was taken at 412 nm.

#### Physico-chemical Studies

The absorption spectra of CT-DNA, 30 µg/ml (Sigma, India) was recorded in a double-beam UV-Vis spectrophotometer (Shimadzu, Japan) both pre-exposure and at different times post-exposure to RFR from a mobile phone for 45 min. The spectrum of sham irradiated DNA was also recorded.

The changes, if any, in DNA on exposure to RFR were also assessed using viscometric studies. CT-DNA (100 µg/ml) was taken in an Oswald-type viscometer of 3 ml capacity, maintained at a thermostatic oil bath at  $25 \pm 1$  °C. The flow rate was determined thrice using a manual timer that agreed within 0.2 s. The viscosity of unirradiated DNA and sham irradiated DNA was estimated immediately post-exposure while that of the RF-exposed DNA (900 MHz, 45 min) was determined at different times post-exposure.

The relative specific viscosity was calculated according to the equation

$$\eta = (t - t_0) / t_0;$$

where  $t_0$  is the flow time for the Tris NaCl EDTA (TNE), pH: 7.4 buffer and  $t$  is the observed flow time for DNA in TNE buffer. The results obtained for irradiated DNA are expressed as fold increase/decrease considering the specific viscosity of control DNA.

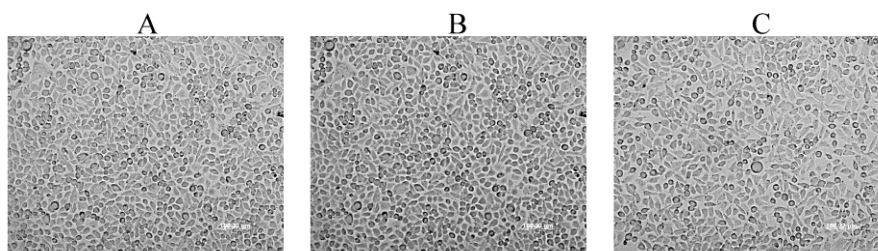
#### Statistical Analysis

The data are represented as the mean  $\pm$  standard deviation (SD) using GraphPad Prism 5 software. The DNA spectrum was plotted by OriginPro 8.5 software. Each experiment was carried out in triplicate. All the data were statistically analyzed by one-way analysis of variance (ANOVA) and Bonferroni post-hoc test using GraphPad Prism.  $P^* < 0.05$  was considered as statistically significant and 'ns' was considered as statistically non-significant.

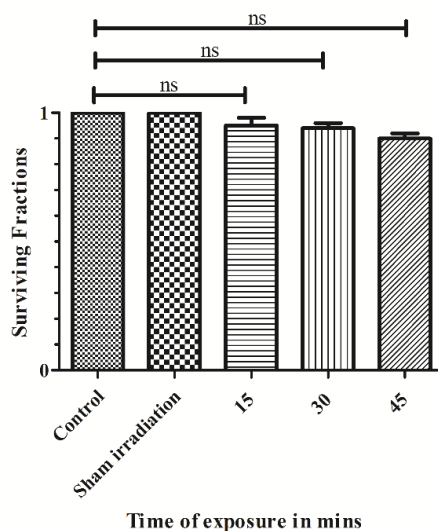
## Results

#### Effect of RFR on Cellular Morphology

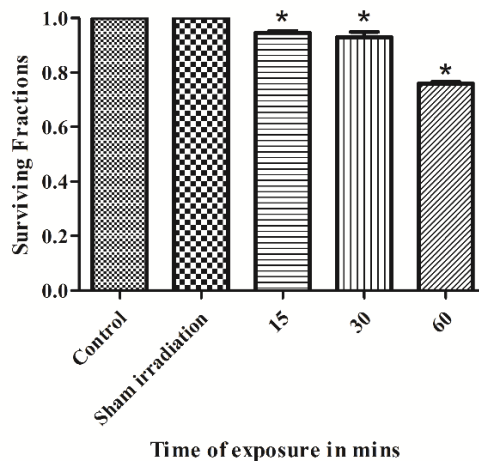
The morphology of A375 cells exposed to mobile phone radiation for 60 min is presented in Figure 2. As could be seen, there was no observable change in the cellular morphology upon irradiation.



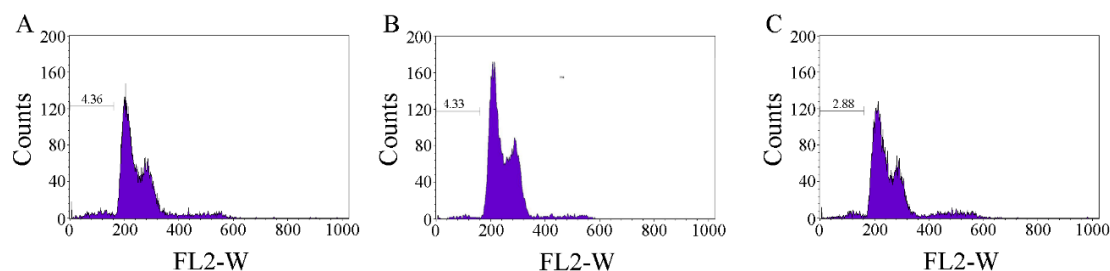
**Figure 2.** Morphology of A375 cells; (A) control, (B) sham irradiated cells, and (C) after exposure to RFR for 60 min; Magnification: 40X



**Figure 3.** Viability of A375 cells determined by trypan blue dye exclusion assay after exposure to RFR for different time periods

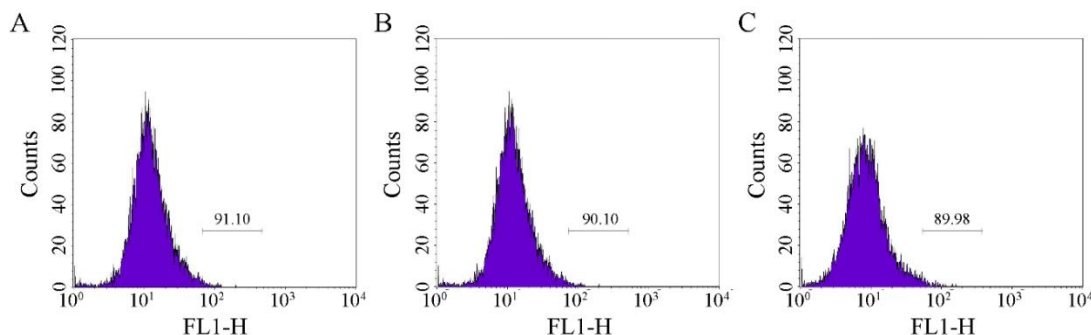


**Figure 4.** Viability of A375 cells determined by clonogenic assay after exposure to RFR for different time periods



**Figure 5.** A typical histogram plot showing DNA damage through flow cytometric assay in A375 cells stained with PI; (A) control, (B) sham irradiated cells, and (C) after 45 min RF irradiation





**Figure 6.** A typical histogram plot showing generation of ROS in flow cytometry assay using DCFH-DA for A375 cells; (A) control, (B) sham irradiated cells, and (C) after 45 min RF irradiation

#### Effect of RFR on Cell Viability

Cell surviving fractions were measured in A375 cells through trypan blue exclusion assay after exposure for different time periods. We found that viability of the cells remained unaltered even following 45 min of exposure to RFR (Figure 3).

#### Effect of RFR on Cellular Colony Forming Efficacy

We estimated delayed cell death on RFR using clonogenic assay (Figure 4). There was a small difference in cell viability of exposed cells, compared to the control or sham irradiated cells.

#### DNA Damage in RFR-Exposed Cells

In this study, we measured DNA damage in RFR-exposed cells by flow cytometry through PI staining. In a flow cytometer, the hypodiploid (sub-G0) population represents cells with damaged DNA. Exposure to RFR from mobile phone for 45 min did not result in any significant increase in the hypodiploid population compared to that of control or sham irradiated cells (Figure 5). This indicates that no DNA damage occurred upon such exposure.

#### Effect of RFR on Cellular ROS Level

Generation of cellular ROS was detected by estimating the formation of DCF in a flow cytometer. The data indicated that ROS generation in the irradiated cells was not significantly different from that of the control or sham irradiated cells (Figure 6).

#### Effect of RFR on Cellular GSH Level

The cellular tripeptide GSH is a measure of antioxidant defense in cells. The GSH content was significantly lowered in irradiated cells compared to control and sham irradiated groups. There was a recovery in GSH content of irradiated cells with time up to 18 h (Figure 7).

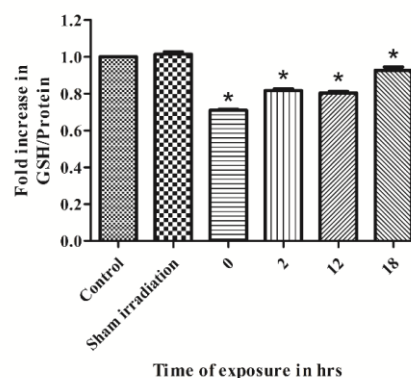
#### Effect of RFR on Cell Cycle

The distribution of cells in different phases was observed by PI staining at different times after exposure to mobile phone radiation for 45 min (Figure 8). Our findings indicated that the populations of cells in G0/G1, S, and G2/M phases measured at 2 and 10 h post-

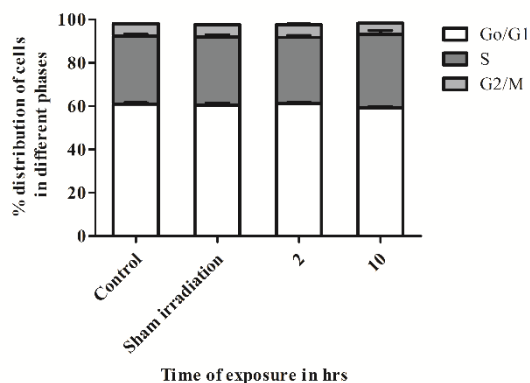
exposure were not significantly different from that in control or sham irradiated cells.

#### Spectrophotometric Evaluation

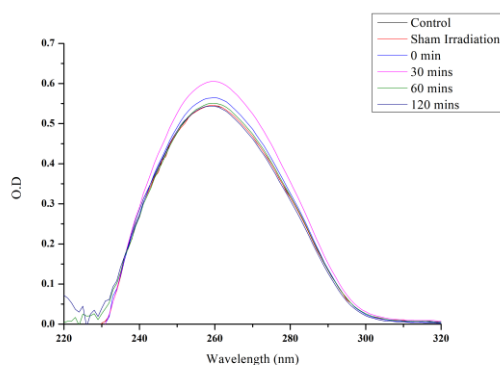
The absorption spectrum of CT-DNA exposed *in vitro* to RFR from a mobile phone for 45 min was recorded immediately and at different times post-exposure (Figure 9). Although there was no effect of sham irradiation as evidenced by the overlap of its spectra with that of the control DNA, a slight hyperchromic shift at 260 nm was observed immediately post-irradiation. The hypochromicity was found to increase gradually within 30 min after exposure. This augmentation was completely reversed in 60 min and remained the same until 120 min as the maximum time tested. The latter result indicated that there may be a small denaturation upon irradiation that was completely reversible with time.



**Figure 7.** Bar diagram showing GSH content in RFR irradiated (45 min) cells at 0, 2, 12, and 18 h post-exposure



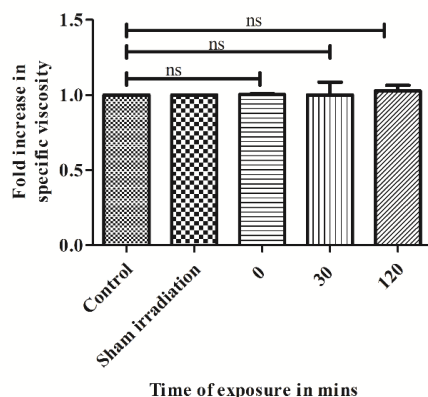
**Figure 8.** Bar diagram demonstrates the distribution of A375 cells at different phases of cell cycle at 2 and 10 h after RFR for 45 min



**Figure 9.** Absorption spectra of CT-DNA (30 µg/ml) at different time intervals post-exposure to RFR for 45 min

### Viscometric Study

The viscosity of DNA was assessed after irradiating CT-DNA for 45 min. No significant change was found in the specific viscosity of DNA immediately or even at 30 or 120 min post-exposure (Figure 10).



**Figure 10.** Influence of RFR on the specific viscosity of CT-DNA (100 µg/ml) determined at different times post-exposure

## Discussion

Cellular morphology is one of the endpoints found to be affected by RFR exposure in human lung cancer cells and embryonic fibroblast cells [43, 44]. Alteration in distribution of actin filaments was present in human

mast cells due to RF irradiation [45]. However, we did not observe any difference microscopically in the cellular morphology of irradiated A375 cells from that of the control cells even after 60 min of continuous exposure from a mobile phone handset. Using different frequencies and varied power levels no change in cellular morphology was indicated in glioblastoma, neuroglioma, or fibroblast cells from the normal fetal lung cell lines [26].

Effect on cellular viability by RFR has often been contradictory. No effect on necrotic killing was found in primary rat neocortical astroglial cells [46], human neuroblastoma, and myeloid cell lines [47]. In human lung carcinoma cell line [43] or in human peripheral blood lymphocytes viability declined after such exposures [48]. However, in transformed human epithelial amnion cells, proliferation depended on the length of exposure as well as the SAR value [49]. The present study revealed that exposure to mobile phone radiation did not have any effect on viability of A375 cells.

Although low power intensity affected cell proliferation rate, high power density had no influence on proliferation rates. In fact, at moderate to high power densities there were no observable changes in different endpoints, irrespective of cell lines used in such studies [26, 28]. Another important factor is the frequency of irradiation as both extremely low frequency (50 Hz) or high frequency (2.1 GHz) wideband code division multiple access (W-CDMA) could affect viability of human umbilical vein endothelial cells (HUVECs) and human breast fibroblast cells, respectively [22, 50].

Apoptotic cell death via mitochondrial pathway has been observed on RFR exposure by different investigators [22, 51]. In addition, chromatin condensation was found in the nucleus of human buccal epithelium cells [25]. Micronuclei formation was elevated at high SAR values on longer exposures of human blood leukocytes [52, 53], no positive effect on micronucleus formation was however observed in C3H 10 T½ cells [54]. No apoptotic cell killing was found in human primary skin cells [55]. Clonogenic assay of cell viability would estimate all forms of cell death, such as necrotic and apoptotic. Cell viability from clonogenic assay revealed a small decrease in surviving fraction with increase in time of exposure.

According to different *in vivo* and *in vitro* experiments, DNA damage resulted from different frequencies in Molt-4, T-lymphoblastoid cells, fibroblastic cells, human skin primary cells, and human lens epithelial cells [56–59]. Induction of γH2AX foci occurred without any cellular dysfunction in Chinese hamster lung cells and human skin fibroblast cells, but not in other cell lines [60]. There was no significant increase in the sub-G0 hypodiploid population of cells even after 45 min of exposure indicating that there was no damage in cellular DNA resulting from mobile phone irradiation. Similar findings were reported for mouse fibroblast cells, human glioblastoma cells, and human peripheral blood lymphocytes as well [60–63].

Furthermore, we also estimated the impact of mobile handset radiation on CT-DNA *in vitro*. Viscosity measurement did not reflect any significant change post-exposure to handset frequency. A small hyperchromic shift in absorbance of DNA until 30 min after irradiation was observed, which was completely reversed after 120 min of exposure. This could be due to some local denaturation of the DNA. RFR is known to be associated with both thermal and athermal effects [64]. Our obtained result may arise from the thermal effect of RFR. However, this was not translated to any detectable DNA damage in cells. Hekmat et al. using the base station antenna frequency of 940 MHz with SAR of 40 mW/Kg, found hyperchromic alterations, which seems to be irreversible [65]. In the 900 MHz spectrum, the frequencies used for handset and base station antenna are different. While 890-915 MHz is used for handsets, the base station antenna utilizes a different frequency band of 935-960 MHz. As a result, small changes in frequencies, as well as intensity of radiation can alter the responses to such radiation even in *in vitro* condition.

Cell cycle arrest follows damage for proficient DNA repair, where the mitotic delay allows damaged cells to recover before resuming cell replication. Although RFR did not induce any observable DNA damage it could influence the repair of damaged DNA [66]. Moreover, post-RFR cell cycle arrest was noted in human neuroblastoma cells, SRA 01/04, rabbit lens epithelial cells, and Swiss albino mice [13, 27, 58, 67, 68]. Delay in cell proliferation rate [69], lowering of mitotic index, and impaired cell cycle propagation through G2/M arrest [13] were observed in different cell lines. However, several other investigators could not find any effect on cell cycle distribution pattern or on the cell cycle regulatory proteins in various cell lines by using different radiofrequencies [47, 70, 71]. We too, could not detect any delay in cell cycle progression. Redistribution of mitochondria was found in RF irradiated cell possibly to provide more energy for autoadaptation [72]. Normal mitochondrial activities generate several ROS, including superoxide, hydrogen peroxide, hydroxyl radicals, and singlet oxygen in aerobic cells. Therefore, reorganization of mitochondria for producing more energy can lead to generation of excess ROS. Oxygen free radicals are important for biological inactivation of cellular function through damage to different biomolecules, including DNA. The involvement of ROS in RFR-induced effects has been documented by a number of investigators [23, 46, 51, 58, 73]. We however found no increment in ROS production. In the studies performed by Xu et al. and Lantow et al., the increase in cellular ROS level was not significant after exposure to GSM-1800 MHz CW or AM in Chinese hamster lung cells, as well as human umbilical cord blood-derived monocytes and lymphocytes [60,74]. Single or combined exposure of CDMA and W-CDMA had no effect on the antioxidant level in MCF10A cells [75]. Evaluation of the GSH level in cells revealed a depletion in our cell line indicating that the cellular antioxidant defense was

enough to provide protection for cells and the oxidative damage was not augmented. Various investigations demonstrate that increase in ROS generation upon RF irradiation depends more on the cell line and the inherent antioxidant status rather than the mode of exposure.

## Conclusion

According to the literature, the impacts of RFR on cells are not universal but cell type-dependent. A number of other factors, including frequency, modulation, intensity also play a role in determining the effects. It could be concluded from our findings that irradiation from a GSM mobile phone handset operating at 900 MHz has no genotoxic influence on the A375 human skin carcinoma cell line.

## Acknowledgment

The first author is supported by fellowship from University of Kalyani (KU). The authors would like to acknowledge the infrastructural support from KU and other facilities at the Department of Biochemistry and Biophysics, KU funded by DST-FIST, DST-PURSE, UGC- SAP; Government of India.

## References

1. IARC. Non-ionizing radiation, Part 2: Radiofrequency electromagnetic fields. Vol. 102, IARC monographs on the evaluation of carcinogenic risks to humans / World Health Organization. International Agency for Research on Cancer; 2013.
2. Nittby H, Grafström G, Tian DP, Malmgren L, Brun A, Persson BRR, et al. Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. *Bioelectromagnetics*. 2008;29(3):219-32.
3. Bayatiani MR, Seif F, Bayati A. The Correlation between Cell Phone Use and Sleep Quality in Medical Students. *Iran J Med Phys*. 2016;13(1):8-16.
4. Daniels WMU, Pitout IL, Afullo TJO, Mabandla M V. The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat. *Metab Brain Dis*. 2009;24(4):629-41.
5. Agarwal A, Singh A, Hamada A, Kesari K. Cell phones and male infertility: A review of recent innovations in technology and consequences. *International Braz J Urol*. 2011;37: 432-54.
6. Rad SA, Ahmad A. Effects of Mobile Phone Radiation on Surface Tension and Volume Flow Rate of Human Blood groups. *Iran J Med Phys*. 2017;14(3):122-7.
7. Shahna FG, Eshaghi M, Dehghanpour T, Karami Z. Assessment of extremely low frequency (ELF) electric and magnetic fields in hamedan high electrical power stations and their effects on workers. *Iran J Med Phys*. 2011;8(3):1-11.
8. Hamzavi G, Rastegar K, Zarifkar A-A, Movahedi M. Effects of Electromagnetic Fields with Frequencies Lower Than 50 Hz and Intensity of 50  $\mu$  T on Learning and Memory. *Iran J Med Phys*. 2009;6(2):19-26.

9. Philippova TM, Novoselov VI, Alekseev SI. Influence of microwaves on different types of receptors and the role of peroxidation of lipids on receptor-protein shedding. *Bioelectromagnetics*. 1994;15(3):183-92.
10. McNamee JP, Chauhan V. Radiofrequency Radiation and Gene/Protein Expression: A Review. *Radiat Res*. 2009;172(3):265-87.
11. Kesari KK, Siddiqui MH, Meena R, Verma HN, Kumar S. Cell phone radiation exposure on brain and associated biological systems. *Indian Journal of Experimental Biology*. 2013;51: 187-200.
12. Byus C V, Kartun K, Pieper S, Adey WR. Increased ornithine decarboxylase activity in cultured cells exposed to low energy modulated microwave fields and phorbol ester tumor promoters. *Cancer Res*. 1988;48(15):4222-6.
13. Buttiglione M, Roca L, Montemurno E, Vitiello F, Capozzi V, Cibelli G. Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. *J Cell Physiol*. 2007;213(3):759-67.
14. Zhang KD, Tong LR, Wang SM, Peng RY, Huang HD, Dong YC, et al. Apoptosis of lewis lung carcinoma cells induced by microwave via p53 and proapoptotic proteins in vivo. *Chin Med J (Engl)*. 2017;130(1):15-22.
15. Shahin S, Singh VP, Shukla RK, Dhawan A, Gangwar RK, Singh SP, et al. 2.45 GHz microwave irradiation-induced oxidative stress affects implantation or pregnancy in mice, *mus musculus*. *Appl Biochem Biotechnol*. 2013;169(5):1727-51.
16. Zhi W-J, Wang L-F, Hu X-J. Recent advances in the effects of microwave radiation on brains. *Mil Med Res*. 2017;4(1):29.
17. Zhang Y, Li Z, Gao Y, Zhang C. Effects of fetal microwave radiation exposure on offspring behavior in mice. *J Radiat Res*. 2015;56(2):261-8.
18. Belyaev III. Non-thermal biological effects of microwaves. *Mikrotałasna Rev*. 2005;13-29.
19. Ruediger HW. Genotoxic effects of radiofrequency electromagnetic fields. *Pathophysiology*. 2009;16(2-3):89-102.
20. Miyakoshi J. Cellular and molecular responses to radio-frequency electromagnetic fields. *Proceedings of the IEEE*. 2013;101: 1494-502.
21. Manna D, Ghosh R. Effect of radiofrequency radiation in cultured mammalian cells: A review. *Electromagnetic Biology and Medicine*. 2016;35: 265-301.
22. Esmekaya MA, Seyhan N, Kayhan H, Tuysuz MZ, Kurşun AC, Yağci M. Investigation of the Effects of 2.1 GHz Microwave Radiation on Mitochondrial Membrane Potential ( $\Delta \Psi_m$ ), Apoptotic Activity and Cell Viability in Human Breast Fibroblast Cells. *Cell Biochem Biophys*. 2013;67(3):1371-8.
23. Ni S, Yu Y, Zhang Y, Wu W, Lai K, Yao K. Study of Oxidative Stress in Human Lens Epithelial Cells Exposed to 1.8 GHz Radiofrequency Fields. *PLoS One*. 2013;8(8):e72370.
24. Ballard M, Tusa I, Fontana N, Monorchio A, Pelletti C, Rogovich A, et al. Non-thermal effects of 2.45GHz microwaves on spindle assembly, mitotic cells and viability of Chinese hamster V-79 cells. *Mutat Res - Fundam Mol Mech Mutagen*. 2011;716(1-2):1-9.
25. Shckorbatov YG, Pasiuga VN, Kolchigin NN, Grabina VA, Batrakov DO, Kalashnikov VV, et al. The influence of differently polarised microwave radiation on chromatin in human cells. *Int J Radiat Biol*. 2009;85(4):322-9.
26. Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, et al. 2-GHz band CW and W-CDMA modulated radiofrequency fields have no significant effect on cell proliferation and gene expression profile in human cells. *J Radiat Res*. 2010;51(3):277-84.
27. Yao K, Wang KJ, Sun ZH, Tan J, Xu W, Zhu LJ, et al. Low power microwave radiation inhibits the proliferation of rabbit lens epithelial cells by upregulating P27Kip1 expression. *Mol Vis*. 2004;10(25):138-43.
28. Cleary SF, Cao G, Liu LM, Egle PM, Shelton KR. Stress proteins are not induced in mammalian cells exposed to radiofrequency or microwave radiation. *Bioelectromagnetics*. 1997;18(7):499-505.
29. Wang KJ, Yao K, Lu DQ, Jiang H, Tan J, Xu W. Effect of low-intensity microwave radiation on proliferation of cultured epithelial cells of rabbit lens. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 2003;21(5):346-9.
30. Nylund R, Leszczynski D. Proteomics analysis of human endothelial cell line EA.hy926 after exposure to GSM 900 radiation. In: *Proteomics*. 2004: 1359-65.
31. Le Quement C, Nicolaz CN, Habauzit D, Zhadobov M, Sauleau R, Le Dréan Y. Impact of 60-GHz millimeter waves and corresponding heat effect on endoplasmic reticulum stress sensor gene expression. *Bioelectromagnetics*. 2014;35(6):444-51.
32. Das PK, Jana C, Parkunan T, Ghosh PR, Joardar NS, Pandiyan GDV, et al. In vitro Effect of Radiofrequency on hsp70 Gene Expression and Immune-effector Cells of Birds. *Adv Anim Vet Sci*. 2014;2(1):31-6.
33. Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. *Neurosci Lett*. 2007;412(1):34-8.
34. Ghosh R, Guha D, Bhowmik S, Karmakar S. Antioxidant enzymes and the mechanism of the bystander effect induced by ultraviolet C irradiation of A375 human melanoma cells. *Mutat Res - Genet Toxicol Environ Mutagen*. 2013;757(1):83-90.
35. Chiu CC, Chang HW, Chuang DW, Chang FR, Chang YC, Cheng YS, et al. Fern plant-derived protoapigenone leads to DNA damage, apoptosis, and G(2)/m arrest in lung cancer cell line H1299. *DNA Cell Biol*. 2009;28(10):501-6.
36. Ghosh R, Bhaumik G. Supernatant medium from UV-irradiated cells influences the cytotoxicity and mutagenicity of V79 cells. *Mutat Res Mutagen Relat Subj*. 1995;335(2):129-35.
37. Budzowska M, Jaspers I, Essers J, De Waard H, Van Drunen E, Hanada K, et al. Mutation of the mouse Rad17 gene leads to embryonic lethality and reveals a role in DNA damage-dependent recombination. *EMBO J*. 2004;23(17):3548-58.



38. Ghosh R, Bhowmik S, Guha D. 9-phenyl acridine exhibits antitumour activity by inducing apoptosis in A375 cells. *Mol Cell Biochem* . 2012;361(1-2):55-66.
39. Ito K, Nakazato T, Miyakawa Y, Yamato K, Ikeda Y, Kizaki M. Caffeine induces G2/M arrest and apoptosis via a novel p53-dependent pathway in NB4 promyelocytic leukemia cells. *J Cell Physiol*. 2003;196(2):276-83.
40. Park J-H, Mangal D, Tacka K a, Quinn AM, Harvey RG, Blair I a, et al. Evidence for the aldo-keto reductase pathway of polycyclic aromatic trans-dihydrodiol activation in human lung A549 cells. *Proc Natl Acad Sci U S A* . 2008;105(19):6846-51.
41. Ghosh R, Guha D, Bhowmik S. UV released factors induce antioxidant defense in A375 cells. *Photochem Photobiol*. 2012;88(3):708-16.
42. Bose Girigoswami K, Bhaumik G, Ghosh R. Induced resistance in cells exposed to repeated low doses of H2O2 involves enhanced activity of antioxidant enzymes. *Cell Biol Int* . 2005;29(9):761-7.
43. Song XL, Wang CH, Hu HY, Yu C, Bai C. Microwave induces apoptosis in A549 human lung carcinoma cell line. *Chin Med J (Engl)*. 2011;124(8):1193-8.
44. Yang L, Hao D, Wang M, Zeng Y, Wu S, Zeng Y. Cellular neoplastic transformation induced by 916 MHz microwave radiation. *Cell Mol Neurobiol*. 2012;32(6):1039-46.
45. Donnellan M, McKenzie DR, French PW. Effects of exposure to electromagnetic radiation at 835 MHz on growth, morphology and secretory characteristics of a mast cell analogue, RBL-2H3. *Cell Biol Int*. 1997;21(7):427-39.
46. Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, et al. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. *Neurosci Lett*. 2010;473(1):52-5.
47. Gurisik E, Warton K, Martin DK, Valenzuela SM. An in vitro study of the effects of exposure to a GSM signal in two human cell lines: Monocytic U937 and neuroblastoma SK-N-SH. *Cell Biol Int*. 2006;30(10):793-9.
48. Esmekaya MA, Aytekin E, Ozgur E, Güler G, Ergun MA, Ömeroğlu S, et al. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). *Sci Total Environ*. 2011;410:59-64.
49. Kwee S, Raskmark P. Changes in cell proliferation due to environmental non-ionizing radiation 2. Microwave radiation. *Bioelectrochemistry Bioenerg*. 1998;44(2):251-5.
50. Mahna A, Firoozabadi SM. Environmental 50Hz Magnetic Fields Can Increase Viability of Human Umbilical Vein Endothelial Cells (HUVEC). *Iran J Med Phys*. 2016;13(2):100-8.
51. Lu Y-S, Huang B-T, Huang Y-X. Reactive Oxygen Species Formation and Apoptosis in Human Peripheral Blood Mononuclear Cell Induced by 900 MHz Mobile Phone Radiation. *Oxid Med Cell Longev*. 2012;2012:1-8.
52. Tice RR, Hook GG, Donner M, McRee DI, Guy AW. Genotoxicity of Radiofrequency Signals. I. Investigation of DNA Damage and Micronuclei Induction in Cultured Human Blood Cells. *Bioelectromagnetics*. 2002;23(2):113-26.
53. Zotti-Martelli L, Peccatori M, Scarpato R, Migliore L. Induction of micronuclei in human lymphocytes exposed in vitro to microwave radiation. *Mutat Res - Genet Toxicol Environ Mutagen*. 2000;472(1-2):51-8.
54. Bisht KS, Moros EG, Straube WL, Baty JD, Roti JL. The effect of 835.62 MHz FDMA or 847.74 MHz CDMA modulated radiofrequency radiation on the induction of micronuclei in C3H 10T(1/2) cells. *Radiat Res* . 2002;157(5):506-15.
55. Sanchez S, Haro E, Ruffié G, Veyret B, Lagroye I. In Vitro Study of the Stress Response of Human Skin Cells to GSM-1800 Mobile Phone Signals Compared to UVB Radiation and Heat Shock. *Radiat Res*. 2007;167(5):572-82.
56. Phillips JL, Ivashchuk O, Ishida-Jones T, Jones RA, Campbell-Beachler M, Haggren W. DNA damage in molt-4 T-lymphoblastoid cells exposed to cellular telephone radiofrequency fields in vitro. *Bioelectrochemistry Bioenerg*. 1998;45(1):103-10.
57. Diem E, Schwarz C, Adlkofer F, Jahn O, Rüdiger H. Non-thermal DNA breakage by mobile-phone radiation (1800 MHz) in human fibroblasts and in transformed GFSH-R17 rat granulosa cells in vitro. *Mutat Res - Genet Toxicol Environ Mutagen*. 2005;583(2):178-83.
58. Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, et al. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. *Mol Vis*. 2008;14:964-969.
59. Pacini S, Ruggiero M, Sardi I, Aterini S, Gulisano F, Gulisano M. Exposure to Global System for Mobile Communication (GSM) Cellular Phone Radiofrequency Alters Gene Expression, Proliferation, and Morphology of Human Skin Fibroblasts. *Oncol Res* . 2002;13(1):19-24.
60. Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, et al. Cell Type-Dependent Induction of DNA Damage by 1800 MHz Radiofrequency Electromagnetic Fields Does Not Result in Significant Cellular Dysfunctions. *PLoS One*. 2013;8(1):e54906.
61. Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti JL. Measurement of DNA Damage after Exposure to 2450 MHz Electromagnetic Radiation. *Radiat Res*. 1997;148(6):608-17.
62. Malyapa RS, Ahern EW, Straube WL, Moros EG, Pickard WF, Roti JL. Measurement of DNA damage after exposure to electromagnetic radiation in the cellular phone communication frequency band (835.62 and 847.74 MHz). *Radiat Res*. 1997;148(6):618-27.
63. Khalil AM, Alshamali AM. No Significant Cytogenetic Effects in Cultured Human Lymphocytes Exposed to Cell Phones Radiofrequencies (900MHz and 1800MHz). *Jordan J Biol Sci*. 2010;147(612):1-15.

64. Elder JA, Cahill DF. Biological effects of radiofrequency radiation. Health Effects Research Laboratory, Office of Research and Development. US Environmental Protection Agency; 1984.
65. Hekmat A, Saboury AA, Moosavi-Movahedi AA. The toxic effects of mobile phone radiofrequency (940 MHz) on the structure of calf thymus DNA. *Ecotoxicol Environ Saf* . 2013;88:35-41.
66. Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifan J, Jianlin L, et al. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. *Mutat Res - Genet Toxicol Environ Mutagen*. 2010;695(1-2):16-21.
67. Pandey N, Giri S, Das S, Upadhaya P. Radiofrequency radiation (900 MHz)-induced DNA damage and cell cycle arrest in testicular germ cells in swiss albino mice. *Toxicol Ind Health*. 2017;33(4):373-84.
68. Kayhan H, Esmekaya MA, Saglam ASY, Tuysuz MZ, Canseven AG, Yagci AM, et al. Does MW Radiation Affect Gene Expression, Apoptotic Level, and Cell Cycle Progression of Human SH-SY5Y Neuroblastoma Cells?. *Cell Biochem Biophys*. 2016;74(2):99-107.
69. Velizarov S, Raskmark P, Kwee S. The effects of radiofrequency fields on cell proliferation are non-thermal. *Bioelectrochemistry Bioenerg*. 1999;48(1):177-80.
70. Chauhan V, Mariampillai A, Kutzner BC, Wilkins RC, Ferrarotto C, Bellier P V, et al. Evaluating the biological effects of intermittent 1.9 GHz pulse-modulated radiofrequency fields in a series of human-derived cell lines. *Radiat Res* . 2007;167(1):87-93.
71. Higashikubo R, Ragouzis M, Moros EG, Straube WL, Roti JL. Radiofrequency electromagnetic fields do not alter the cell cycle progression of C3H 10T and U87MG cells. *Radiat Res* . 2001;156(6):786-95.
72. Beneduci A, Chidichimo G, Tripepi S, Perrotta E, Cufone F. Antiproliferative effect of millimeter radiation on human erythromyeloid leukemia cell line K562 in culture: Ultrastructural- and metabolic-induced changes. *Bioelectrochemistry*. 2007;70(2):214-20.
73. Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872 MHz radiofrequency radiation. *Mutat Res* . 2009;662(1-2):54-8.
74. Lantow M, Lupke M, Frahm J, Mattsson MO, Kuster N, Simko M. ROS release and Hsp70 expression after exposure to 1,800 MHz radiofrequency electromagnetic fields in primary human monocytes and lymphocytes. *Radiat Environ Biophys*. 2006;45(1):55-62.
75. Hong MN, Kim BC, Ko YG, Lee YS, Hong SC, Kim T, et al. Effects of 837 and 1950MHz radiofrequency radiation exposure alone or combined on oxidative stress in MCF10A cells. *Bioelectromagnetics*. 2012;33(7):604-11.