



Direct and indirect effects of exposure to 900 MHz GSM radiofrequency electromagnetic fields on CHO cell line: Evidence of bystander effect by non-ionizing radiation

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ABSTRACT

Introduction: The rapid rise in global concerns about the adverse health effects of exposure to radiofrequency radiation (RFR) generated by common devices such as mobile phones has prompted scientists to further investigate the biological effects of these environmental exposures. Non-targeted effects (NTEs) are responses which do not need a direct exposure to be expressed and are particularly significant at low energy radiations. Although NTEs of ionizing radiation are well documented, there are scarcely any studies on non-targeted responses such as bystander effect (BE) after exposure to non-ionizing radiation. The main goal of this research is to study possible RFR-induced BE.

Material and methods: Chinese hamster ovary cells were exposed to 900 MHz GSM RFR at an average specific absorption rate (SAR) of 2 W/kg for 4, 12 and 24 hours (h). To generate a uniformly distributed electromagnetic field and avoid extraneous RF exposures a cavity was designed and used. Cell membrane permeability, cell redox activity, metabolic and mitotic cell death and DNA damages were analyzed. Then the most effective exposure durations and statistically significant altered parameters were chosen to assess the induction of BE through medium transfer procedure. Furthermore, intra and extra cellular reactive oxygen species (ROS) levels were measured to assess the molecular mechanism of BE induced by non-ionizing radiation.

Results: No statistically significant alteration was found in cell membrane permeability, cell redox activity, metabolic cell activity and micronuclei (MN) frequency in the cells directly exposed to RFR for 4, 12, or 24 h. However, RFR exposure for 24 h caused a statistically significant decrease in clonogenic ability as well as a statistically significant increase in olive moment in both directly exposed and bystander cells which received media from RFR-exposed cells (conditioned culture medium; CCM). Exposure to RFR also statistically significant elevated both intra and extra cellular levels of ROS.

Conclusion: Our observation clearly indicated the induction of BE in cells treated with CCM. To our knowledge, this is the first report that a non-ionizing radiation (900 MHz GSM RFR) can induce bystander effect. As reported

Abbreviations: RFR, radiofrequency radiation; BE, bystander effect; GSM, Global System for Mobile Communication; NTEs, Non-Targeted Effects; GSM, Global System for Mobile communication; SAR, specific absorption rate; ROS, reactive oxygen species; MN, micronuclei; CCM, conditioned culture medium; IARC, International Agency for Research on Cancer; CHO, Chinese Hamster Ovary; RPMI, Roswell Park Memorial Institute; EDTA, Ethylenediaminetetraacetic acid; PBS, phosphate buffered saline; GMSK, Gaussian filtered Minimum Shift Keying; dBm, decibel milliwatt; HFSS, High frequency simulating system; MTT, tetrazolium salt; DMSO, dimethyl sulfoxide; AO, Acridine orange; EB, ethidium bromide; AVO, vesicular organelles; CBMN, Cytokinesis-block micronucleus; DCFH-DA, 2',7'-Dichlorofluorescein diacetate; MT, medium transfer; SEM, standard error of mean; ANOVA, analysis of variance; EMR, electromagnetic radiation; H2O2, Hydrogen peroxide; OH, hydroxyl; NO, nitric oxide; DSB, double strand breaks; SSB, single strand breaks; TGF, transforming growth factor; TNF, tumor necrosis factor; COX-2, cyclooxygenase-2; mTOR, mammalian target of rapamycin; CST, Computer Simulation Technology; FIT, Finite Integration Technique; IEC, International Electrotechnical Commission; IEEE, Institute of Electrical and Electronics Engineers

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for ionizing radiation, our results proposed that ROS can be a potential molecule in indirect effect of RFR. On the other hand, we found the importance of ROS in direct effect of RFR but in different ways.

1. Introduction

Emerging telecommunication technologies including mobile phones have put us unshielded in an ocean of radiofrequency radiation (RFR)¹. Frequency of radiation emitted by mobile phones are 900 or 1800 MHz in Global System for Mobile Communication (GSM) (Mouly et al., 1992); such that, RFR exerts far too low energy to break chemical bonds so it is classified as non-ionizing radiation (Ahlbom et al., 1998; Lerchl et al., 2015). Consequently, RFR cannot lead to rapid effects on living organisms. Moreover, RFR emitted from cell phones affects independently compared to conventional thermal heating mechanisms (Manna and Ghosh, 2016). Despite these facts, International Agency for Research on Cancer (IARC) announced it as “possibly carcinogenic” (Group 2B) to humans (Organization, 2011; Shah et al., 2015). Therefore, studying biological effects induced by cell phone radiation exposure seems to be an uphill task.

Various *in vivo* and *in vitro* studies applied different endpoints to explore effects of RFR (Capri et al., 2004b; Gürbüz et al., 2018; Lin et al., 2017; Pavicic and Trosic, 2008; Verschaeve et al., 2010; Wang and Zhang, 2017). Nevertheless, observable effects in body scale and public health may be far more complicated than what cellular and molecular studies initially suggested because non-targeted effects (NTEs) of RFR were overlooked. NTEs alter the expected effects of radiation, especially in the case of low-energy exposures. As they emerge in different places (bystander effect) or times (adaptive response) compared to primarily exposure (Kadhim et al., 2013), we also call them indirect effects. In this research we studied possible RFR-induced bystander effect (BE).

Historically, BE was defined when the cells that are not exposed to ionizing radiation but in communication with irradiated cell, show features as if they have been exposed to radiation. Two major BE mechanisms have been recognized: 1) Intracellular connection through gap junctions; 2) Transmission of the chemical molecules released by irradiated cells into the culture medium (Al-Mayah et al., 2012; Azzam et al., 2001; Marin et al., 2015). Using media collected from irradiated cell cultures (so-called conditioned medium), several studies showed that direct cell-cell contact is not essential to induce BE (Peng et al., 2017). Signaling molecules such as reactive oxygen species (ROS), nitric oxide, calcium, and various kinds of cytokines released by targeted cells can evoke cells receiving the conditioned medium to present some similar or different responses to those displayed by targeted cells (Jella et al., 2018; Kostyuk et al., 2012; Lyng et al., 2011; Shao et al., 2006).

Although BE has been described to be induced by alpha, gamma, or X radiation for the first time, several studies showed that this phenomenon is not limited to ionizing radiation. BE process could be generated by other stressors, including chemotherapeutic drugs (Chinnadurai et al., 2011; Savu et al., 2015), ultraviolet light (Banerjee et al., 2005; Dahle et al., 2005), photodynamic treatment (Bazak et al., 2017; Chakraborty et al., 2009), heat (Dabrowska et al., 2005), nanoparticles (Thubagere and Reinhard, 2010), and even gene therapy (Carystinos et al., 1999). But, except the investigation conducted by our team in 2015 on the impacts of mobile GSM on BE (Mortazavi et al., 2015), this phenomenon has not been described for non-ionizing radiation including RFR.

Recently, bystander effect has gained considerable attention. This is owing to the fact that it typically employs low or medium doses of radiation (Seymour and Mothersill, 2000; Zaleska et al., 2017) which do not kill target cells but allow radiation effects to be spread around cells substantially; hence, the radiation effect is multiplied. In this study, we investigated this hypothesis that RFR can have both direct

and indirect effects like bystander effects. Furthermore, as RFR belongs to an electromagnetic radiation spectrum that act differently compared to ionizing radiation, we became curious whether probable RFR indirect effects were also occurred via a different mechanism. In this work, initially direct effects of RFR was investigated and then based on statistically significant affected endpoints, indirect effects of RFR (bystander effect) were examined.

2. Material and methods

2.1. Cell line and culture condition

Chinese Hamster Ovary (CHO) cell line was purchased from National Cell Bank of Iran (Pasteur Institute, Iran). Cells were grown in RPMI-1640 medium (Gibco, UK) supplemented with 10% fetal bovine serum (Gibco), penicillin (Sigma, USA), and streptomycin (Jaberebn-Hayan, Tehran, Iran) at 37 °C with 5% CO₂ and 95% humidity. When cells became 80–100% confluent, 0.25% trypsin and 0.03% EDTA (Ethylenediaminetetraacetic acid, Sigma) in phosphate buffered saline (PBS) solution were used to subculture.

2.2. Radiofrequency irradiation (exposure system)

Our exposure system includes 2 main sections, i.e. the 900 MHz GSM mobile phone signal generator and the cavity. Signal generator developed at Department of Medical Physics and Biomedical Engineering, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran, was used for RFR exposures in this study. The technical specifications and dosimetry of this signal generator are presented in Table 1. Briefly, this device can generate signals in two forms, single tone sinusoidal continuous wave and modulated signal. The output power in both cases can be manually adjusted between 0 and 2 Watts. In modulated form, it simulates the transmitted signal of a mobile handset. In other words, it amplifies a Gaussian Minimum Shift Keying (GMSK) modulation randomly generated digital signal in one of the standard GSM frequency channels. The device uses an RFR amplifier with maximum output power of 2 W (33 dBm) in its chain. The GSM frequency channel used in the signal generator can be selected among standard GSM frequencies between 800 and 1800 MHz. These parameters can be changed using a software through USB cable connected to the signal generator. In this research, the signal generator was tuned on a channel of 900 MHz (at a wavelength of about 33.4 cm) and power of 2 W in “Talk mode”. The signal bandwidth was 200 kHz with

Table 1
Dosimetry and physical properties of 900 MHz GSM mobile phone signal generator.

Parameter	Value
Frequency bands	935.1–959.1 MHz
Bandwidth	200 KHz
Channel	124
Modulation	GMSK
Data rate	270.833 (kbp)
Bit duration	1/270.833 k = 3.69 μs
Packet duration	577 μs
No. of bits per packet	577 μ/3.69 μ = 156.25
Packet repetition	20 ms
Packet type	Normal
	Frequency correction
	Synchronization
	Random access

modulation range of 217 Hz and duty cycle of 0.125 s like a standard GSM cell.

In order to generate a standing wave, a TE₁₀₁ cavity at the frequency of 900 MHz with dimensions of $20 \times 9 \times 45$ cm was developed. The cavity was tested in Antenna type approval laboratory at University of Tehran. Using the full-wave electromagnetic simulation with HFSS (High Frequency Structure Simulator) software, the appropriate height of the antenna was optimized to 7 cm to store electromagnetic energy inside the cavity. This software uses frequency domain finite element method (FEM) solution to solve 3D partial differential equations. The red zone in Fig. 1. A indicates the maximum total electric field distribution with vertical polarization excitation inside the cavity and is the best place for sample placement relative to its center (Fig. 1B). The incident power flow into the cell samples was 1.96 W. To calculate SARs inside the biological samples, simulation was carried out using CST (Computer Simulation Technology) microwave software. This software uses a FIT (Finite Integration Technique) method to evaluate the electromagnetic field. Moreover, by applying the IEEE/IEC 62704-1 averaging method, standard SAR values were between 0.2 and 0.4 w/kg in the biological samples. Although SAR values highly depend on the geometry and density of the samples exposed to the RFR power, the SARs achieved by simulation with both CST Microwave and also HFSS software indicated that almost 90% of the biological sample has been exposed at about 0.3 w/kg.

For easier transfer of samples, a bisected cavity was used. Some small pores were created on the cavity to allow the flow of air. In all experiments, sterilized cavity was placed in an incubator for at least about 12 h before RFR-exposure, to get the same conditions (temperature, CO₂ concentration, and humidity). Cells plated either in 35 mm plates or in 96 cell plates placed on a holder at the center of the cavity where the electric field was maximum. Control groups were placed in the same incubator.

Measurements of the reflection coefficients from ports of the cavity indicated that the structure can support electromagnetic energy and is reliable enough to be used as a RFR power exposure for our samples. Furthermore, the power leakage for both signal generator and cavity was determined by Electromagnetic Compatibility (EMC) tests. The leakage was as low as 2.13×10^{-9} W (−56.71 dBm) during exposure. Thus, no significant energy leaked out of the cavity into the incubator environment. In addition, since the body of the incubator was made of metal and due to being connected to the ground, a Faraday cage was formed, shielding the samples from stray electromagnetic fields. Also our incubator was the incubator is air-blown; it was equipped with fans that provided for constant air movement to make the temperature uniform across the environment. This was further tested by a thermocouple temperature device. Temperature in the cell cultures was constant during the exposure and was the same with controls (37.1 ± 0.1 °C). Fig. 2 shows a graphic image of RFR-exposure set-up.

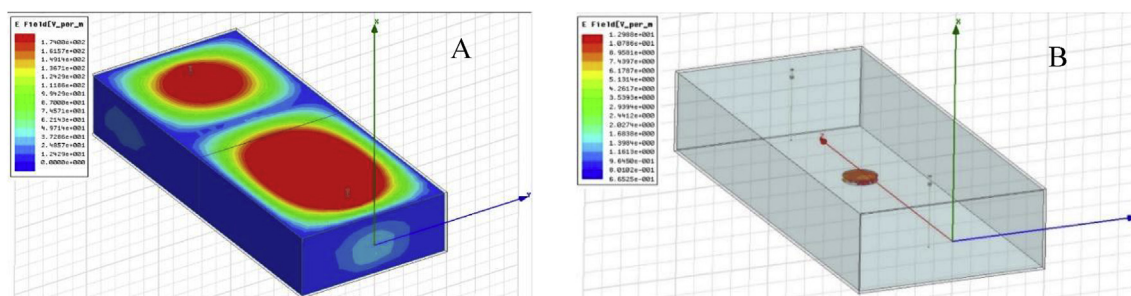


Fig. 1. Simulation of the electromagnetic field using the HFSS software. A) A screenshot of a progressive electromagnetic field inside the cavity. The red and blue zones are corresponded to the maximum and minimum homogeneity of electromagnetic field, respectively. B) A Cross-section of the electric field on the cell samples determined via HFSS simulation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

2.3. Detection and quantification of apoptosis

Acridine orange/ethidium bromide (AO/EB) staining was applied to quantify apoptosis induction by RFR exposure using flow cytometry. 6 h after RFR exposure (12 and 24 h), CHO cells were harvested, pelleted, washed twice in cold PBS, and stained with 2 ml PBS containing EB (Sigma, USA- E7637) and AO (Sigma, USA- A6014) with the final concentrations of 0.1 and 0.25 mM, respectively. All groups were stained and analyzed at room temperature. Flow cytometric analysis was performed on CyFlow Space (Parpec, Germany). Data were analyzed using FloMax software (Liegler et al., 1995; Ribble et al., 2005). In parallel, a group of cells was not exposed to RFR as negative control and a group of cells were treated with H₂O₂ at a concentration of 1.5 mM for 2 h to induce apoptosis as positive control. The experiment was repeated three times. For each group, 12,000 cells were analyzed.

2.4. Detection and quantification of autophagy

To detect the occurrence of autophagy, CHO cells were seeded in 35 mm² tissue culture dishes and exposed to RFR radiation for 12 and 24 h. After 6 h, the cells were trypsinized, harvested, and incubated with RPMI-1640 medium containing 2 µg/mL AO (Sigma, USA- A6014) for 15 min at room temperature in the dark. The number of cells with increased acidic vesicular organelles (AVO) (as a marker of autophagy) was determined by Flow cytometry (CyFlow Space, Parpec, Germany) and analyzed by FloMax software. Furthermore, a group of cells was not exposed to RFR as negative control and a group of cells were treated with 50 nM rapamycin (cat. no. C6628-25G, Sigma, Poole, UK) for 24 h as positive control. Rapamycin is an mTOR (mammalian target of rapamycin) inhibitor which induces autophagy (Jangamreddy et al., 2013; J. S. Lee and Lee, 2012). The experiment was repeated three times. For each group, 12,000 cells were analyzed.

2.5. Membrane permeability analysis

CHO cells were cultured in 24 well plates with a density of 10,000 cells/cm² one day before exposure to RFR. Exponentially growing cells were exposed to RFR for 4, 12, and 24 h. Then, Membrane permeability was measured on the base of trypan blue dye exclusion method at 0, 12, 24, 48 and 72 h after terminating the RFR exposure. Intact cell membranes do not allow cells to uptake the dye; hence, their cytoplasm were observed clear under microscope, non-stained for viable cells. On the other hand affected membrane cells or dead cells had blue or stained cytoplasm (Strober, 2015). For every group, three wells were considered. In each well, at least 400 cells were counted using a hemocytometer. At the same time, staining was performed on un-exposed control cells. Additionally, a group of cells treated with 1% triton X-100 was considered as positive control. The experiment was repeated three times.

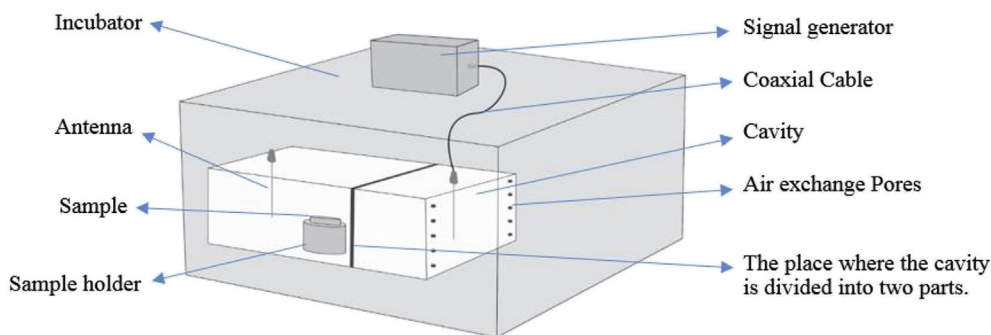


Fig. 2. A graphic picture of RFR-exposure set-up.

2.6. Redox activity analysis (MTT assay)

Cell redox activity was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide salt (MTT) method. Metabolically active cells can reduce MTT to produce water-insoluble purple formazan crystals. Cells were seeded in 96 well plates with a density of 10,000 cells/cm². Cells in exponential phase of growth were exposed to RFR for 12 and 24 h. After 4, 12, and 24 h, 10 μ L MTT solution (Sigma, USA) (5 mg/mL PBS) was added to each well. Then, cells were incubated at 37 °C for 3 h to let formazan crystals form. Afterwards, the media were removed, and 100 μ L dimethyl sulfoxide (DMSO) was added to each well to make formazan crystals soluble. Next, absorbance at 570 and 630 nm wavelengths were measured as test and reference wavelengths, respectively, by a BioTek plate reader. These analyses were also done on DMSO and medium without cells in each measurement. Difference of absorbance at the two wavelengths represented cell redox activity. The number of cells with healthy mitochondria is directly proportional to the total amount of formazan produced. In parallel, 1.5 mM H₂O₂ for 2 h was used to induce metabolic death as the positive control. As well, a group of cells was not exposed to RFR as the negative control. The experiment was repeated at least three times and 12,000 cells were analyzed for each group.

2.7. Clonogenic assay

In order to assess the ability of cells to form colonies, clonogenic assay of Puck and Marcus (Puck and Marcus, 1956) was performed. For this assay four series containing 3 groups, were considered. The day prior to experimentation, sub-confluent cells received fresh medium and were exposed to RFR for 0, 12, and 24 h. After 4 h re-incubation, cells were detached from plates by trypsinization. Detached cells were then centrifuged, followed by removal of the supernatant. Pelleted cells were re-suspended in fresh medium, and pipetted gently to produce a single cell suspension. Cells were stained with methyl green using a hemocytometer and 100 cells were plated in 35 mm plates. Then plates were placed to the incubator at 37 C, 5% CO₂, and 95% relative humidity. Following 7, 8, 9, and 10 days, a series of cells were fixed with 2% (v/v) formaldehyde solution (Sigma-Aldrich) and stained with 5% (w/v) crystal violet (Sigma-Aldrich) and colonies exceeding 50 cells were scored. Three plates were considered for each treatment. Survival fraction (SF) and plating efficiency (PE) were calculated according to the following equations:

$$SF = \frac{\text{number of colonies counted (after exposure)}}{\text{number of cells seeded} \times PE/100}$$

$$\left(PE = \frac{\text{number of colonies counted}}{\text{number of cells seeded}} \times 100 \right)$$

2.8. DNA damage

2.8.1. Alkaline single-cell gel electrophoresis (comet assay)

Comet assay was used for detection of DNA damages including single strand breaks (SSBs) and double strand breaks (DSBs). This assay was performed as described by Lee et al. (Bigdeli et al., 2016; J.-G. Lee et al., 1996). Briefly, cells were grown at a density of 10,000 cells/cm². Exponentially growing cells were exposed to RFR for 12 and 24 h. Immediately after exposure, they were trypsinized and washed with PBS. About 30,000 cells were suspended in 1% low-melting-point agarose (Sigma-Aldrich) in PBS (pH 7.4) at 37 °C. Then, 100 μ L of each sample's suspension was pipetted onto a microscopic slide already covered by a thin layer of 1% agarose. The agarose was allowed to set on ice for 10 min, then the slides were immersed in lysis buffer (2.5 M NaCl, 100 mM Na₂EDTA, 10 mM Tris, NaOH with a pH of 10.0, and 1% TritonX-100) for 1 h at 4 °C, to remove cellular proteins. After replacing the slides in the denaturation solution (0.3 M NaOH and 1 mM Na₂EDTA; pH > 13), they were placed in an electrophoresis tank (25 V, 1 V/cm, 300 mA) containing denaturation solution for 30 min. Subsequently, neutralization buffer was added and the slides were stained with 20 μ g/mL EB. Images ($\times 200$) were taken using a fluorescence microscope (Axioskop 2 plus; Zeiss, Jena, Germany). For each group, at least two hundred randomly selected representative comet images containing about 15 cells (up to 3000 cells) were analyzed. A cell with DNA damage was visualized like a comet with a head and a tail. Olive tail moment is one of the suggested parameters to quantify DNA damage; it is achieved by the following formula:

$$\text{Olive Moment} = (\text{Tail Mean} - \text{Head Mean}) \times \% \text{ of DNA in the Tail}$$

This parameter was calculated by Comet Score 1.5 software (TriTek Corp., Sumerduck, USA) and reported for statistical analysis. All this process was done for samples irradiated by 4 Gy X-ray (as positive control in study of direct effect and to check the accuracy of comet procedure in study of indirect effect) and cells without any treatment (as negative control). The experiment was repeated three times.

2.8.2. Cytokinesis-block micronucleus (CBMN) assay

Three groups of cells were seeded in 35 mm plates at low-density (at a density of 5000 cells/cm²) one day before experiment. Then, they were exposed to RFR for 0, 12, and 24 h. As positive control, a group of cells was irradiated with 4 Gy X-ray. At the end of the exposure period, the cells were treated with 6 μ g/mL cytochalasin B. Cytochalasin B inhibits the assembly of spindle, and consequently prevents cytokinesis after nuclear division causing the formation of binucleated cells. In the case of chromosomal loss, breakage, or apoptosis, one or more micronuclei (MN) were observed in cytoplasm of binucleated cells. MNs are small nucleus that are formed during the anaphase of mitosis or meiosis. Following 24 h, the cells were fixed using a 1:3 ratio of acetic acid/methanol (Merck) in 4 °C (Soleymanifard et al., 2016) and stained with Giemsa 10% (Sigma-Aldrich). For each treatment, the frequency of MNs in 1000 binucleated cells were scored using light microscopy with

the magnification of $\times 400$ (Olympus). The experiment was repeated three times.

2.9. ROS evaluation

A fluorescent probe, 2', 7'-Dichlorofluorescein diacetate (DCFH-DA), was used to detect intracellular oxidative stress. The cells were seeded at a density of 10,000 cells/cm² in 96 well plates in triplicate followed by incubation overnight. Then, the cells were exposed to RFR for 0 (as negative control), 12, and 24 h. One group was considered as positive control which treated with 100 μ M H₂O₂ for 4 h. Immediately after the

end of the exposure, the culture media was replaced with fresh media and DCFH-DA was added at a final concentration of 20 μ g/ml. Then, the cells were re-incubated for 45 min at 37 °C. Finally, the fluorescence emission of controls and RFR exposed cells were analyzed through the CyFlow Space flow Cytometer (Partec, Germany) using FloMax software at excitation and emission wavelengths of 485 and 520 nm, respectively. The experiment was repeated at least three times and 12,000 cells were analyzed for each group.

In order to observe fluorescence intensity of intracellular ROS, CHO cells were seeded in 35 mm plate. Following exposure and incubating with DCFH-DA according to the procedure mentioned above, the cells

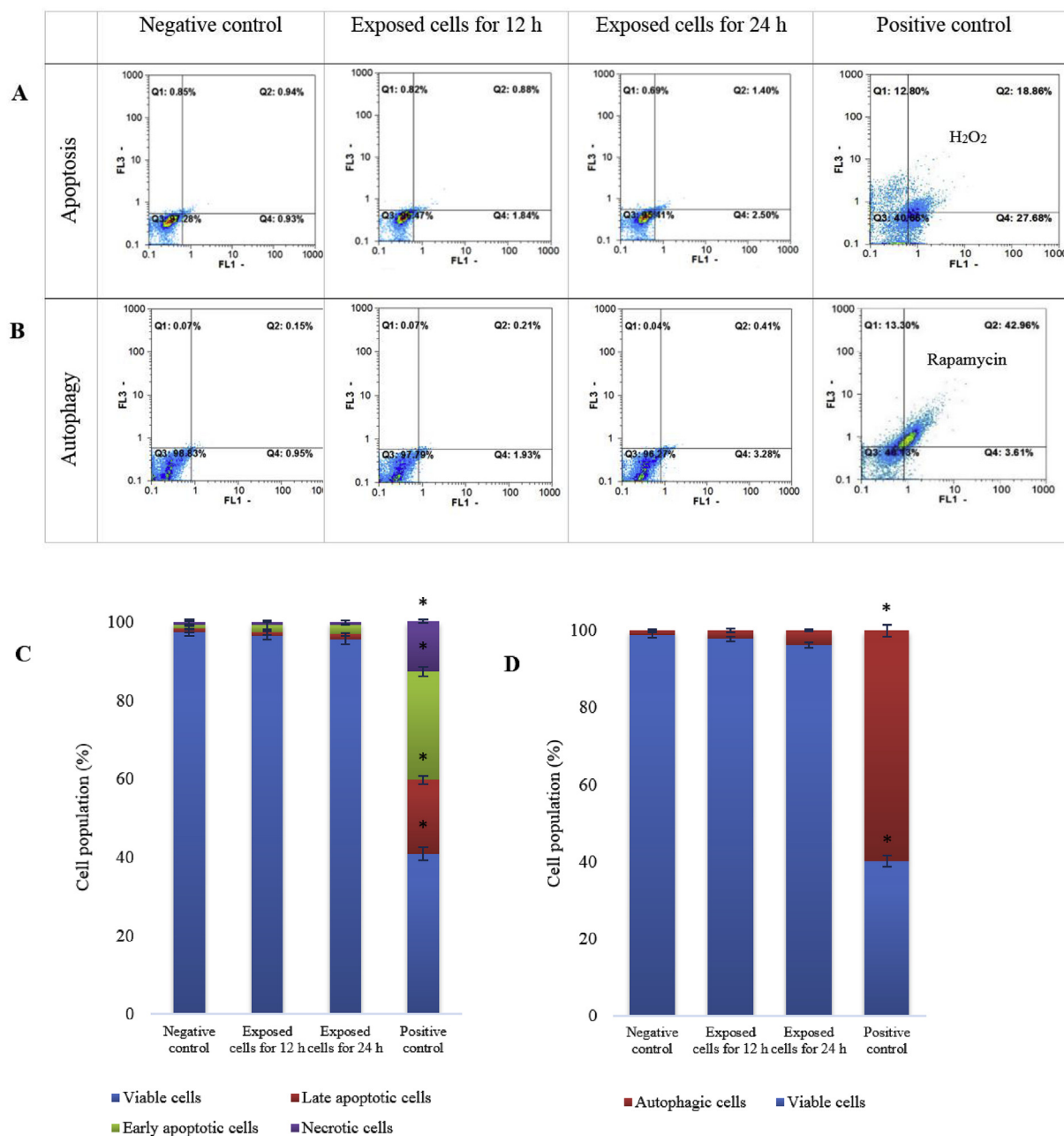


Fig. 3. Apoptosis and autophagy analysis in cells directly exposed to cells. Cells were either exposed to 900 MHz RFR emitted from cell phone for 12 and 24 h or not exposed. (A) Representative flow cytometric outputs of AO/EB double-stained cells for apoptosis. FL1-H and FL3-H channel of the flow cytometer was applied to measure green (AO) and Red (EB) fluorescence intensities, respectively. Q1, Q2, Q3, and Q4 represent necrotic, late apoptotic, viable, and early apoptotic cells, respectively. (B) Representative flow cytometric outputs of AO stained cells for autophagy. (C) Quantitative analysis of flow cytometric outputs of apoptosis results and (D) autophagy results as mean $\pm$ SEM of three independent experiments. * represents $P < 0.05$ between apoptotic or autophagic and viable cells compared to exposed cells and negative control. The experiments were repeated at least three times and 12,000 cells were analyzed for each group. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

were washed with PBS followed by analysis of fluorescence intensity of cells using fluorescence microscopy.

Furthermore, to measure the level of ROS in culture media, chemiluminescence spectroscopy was used. For each sample, 10 μ l medium was added to a well of 96-well plate contained 230 μ l sodium carbonate buffer. Next, 10 μ l luminol solution (0.1 μ M) in sodium carbonate buffer (100 mM, pH 11.0) and 10 μ l gold chloride (0.5% solution in double-distilled water) as a catalyzer were injected to the samples. Then chemiluminescence signal was recorded using a fluorescence spectrophotometer (Synergy H4 Hybrid Reader; BioTek, USA) at 425 nm. The measurements were performed after 1, 6, 12, and 24 h incubation times. All tests were repeated at least three times.

2.10. Studying bystander effect

Medium transfer (MT) procedure was applied to analyze indirect effects of radiofrequency radiation or bystander effect. Sub-confluent cells were exposed to RFR for 24 h. Then, the media were collected and filtered through a 0.22 mm filter (to remove cells from the culture media) at three time points: immediately, 12, and 24 h after RFR-exposure. These media were called CCM₀, CCM₁₂, and CCM₂₄ respectively. Then, these CCMs were transferred to cells already aspirated their media (these cells were so-called bystander) and let them remain in the incubator for 12 and 24 h. Thus, we had six experiment conditions: 00/12 which represents the condition that bystander cells received CCM₀ and were incubated for 12 h; 00/24, 12/12, 12/24, 24/12 and 24/24 were the same way.

In parallel, five other groups were considered for each condition, so there were six groups at each experiment:

- 1) 'C, RFR, MT' group: cells whose culture medium was exchanged with related CCM.
- 2) 'C, MT' group: the medium of sub-confluent cells was collected and filtered through a 0.22 mm filter to remove cells from the culture. Then, this medium was transferred to bystander cells. These groups of cells named 'C, MT'.
- 3) 'RFR, MT' group: medium (without cells) was exposed to RFR for 24 h. Then, this medium was transferred to bystander cells. These groups of cells named 'RFR, MT'.
- 4) 'MT' group: fresh medium (without cells and RFR exposure) was transferred to bystander cells. These groups of cells named 'MT'.
- 5) 'RFR' group: Cells exposed to RFR for 24 h.
- 6) 'Cont.' group: Cells not exposed to RFR.

To ensure that the observed bystander effect is related to the cells exposed to RFR and not the cells medium or medium exchange procedure, we considered groups 2, 3 and 4 as control groups. Our experiments run under a blind condition for three times. All samples were coded and decoding was performed after evaluation.

2.11. Statistical analysis

Results were analyzed using SPSS 22 software. Data were indicated as means $\pm$ standard error of mean (SEM). For continuous variables, the means were compared by one way analysis of variance (ANOVA) and Tukey's post hoc testing. P value of 0.05 was considered as the level of statistical significance. Three independent experiments were performed for each endpoint.

3. Results

3.1. Cell apoptosis

Flow cytometric results of AO/EB stained cells showed that RFR exposure failed to induce apoptosis. As displayed in Fig. 3. A and C, percentage of apoptosis in RFR-exposed cells for 12 and 24 h were

$1.86 \pm 1.4\%$ and $3.54 \pm 1.6\%$, respectively, which were not statistically significant ($p > 0.05$) compared to that of non-exposed cells ($3.96 \pm 1.3\%$). However, cells treated with 100 mM H₂O₂ as positive control presented statistically significant amount of apoptotic or dead cells in comparison with control or RFR-exposed cells.

3.2. Autophagy

Fig. 3B displays the percent of cells undergone autophagy after RFR exposure. Moreover, quantitative analysis of flow cytometric data is presented in Fig. 3D. The highest exposure time in our experiment (24 h) caused 3.75% death which was not statistically significant ($p > 0.05$) compared to cells receiving 12 h RFR ($2.2 \pm 1.02\%$) or to control cells ($1.7 \pm 1.3\%$). While 0.25 μ M rapamycin as positive control increased red fluorescence intensity ($p < 0.05$) indicating increased AVO (a sign of autophagy).

3.3. Membrane permeability

Trypan blue dye exclusion method was performed to examine membrane permeability. Intact cell membranes do not allow trypan blue to penetrate the cell; hence, they have clear and unstained cytoplasm. The frequency of unstained cells in RFR-exposed groups for 12 h and incubation times of 0, 12, 24, and 72 h (97.83 ± 4.5 , 95.83 ± 3.7 , and $94.73 \pm 2.3\%$, respectively) are equal to their frequency in control groups (98.4 ± 3.5 , 98.33 ± 5.1 , and $96.86 \pm 2.9\%$, respectively). Besides, data obtained from 24 h of RFR-exposure were similar to data of the 12 h experiments ($p > 0.05$). These results indicate that the RFR in our exposure condition did not statistically significant change the permeability of the cell membrane. Whereas 11.3% of cells treated with 1% triton X-100 (as positive control) presented stained cytoplasm which is higher than that in exposed or control groups ($p > 0.05$).

3.4. Cell redox activity (MTT)

To evaluate cell redox activity, MTT assay was applied. Cells with healthy mitochondria reduce yellow MTT to purple formazan which has specific absorbance at 570 nm. Data obtained spectrophotometrically showed that cells exposed to RFR for 12 and 24 h have the ability to produce formazan immediately after or 12, 24, 48, and 72 h post exposure. Difference of absorbance at the 570 and 630 nm wavelengths in RFR-exposed cells in all experiments were the same as that of controls ($p > 0.05$). While cells treated with 1.5 mM H₂O₂ (as positive control) exhibited statistically significant inhibition in reduction of MTT and showed lower absorbance value at 570 nm compared to controls ($p < 0.05$) (Data not shown).

3.5. Clonogenic ability

According to the principles of the colony formation technique, colonies with the same number of cells and the same size involving at least 50 cells are considered to count; in this way, after seven days, Frequency of colonies of colonies were $64.94 \pm 0.8\%$ and $39.17 \pm 0.8\%$ in cells exposed to RFR for 12 and 24 h respectively which was lower than that compared to control group ($P < 0.05$). The SF of cells exposed to RFR for 12 and 24 h has been displayed for day 7th in Fig. 4. Also after nine days, the frequency of colonies in cells exposed to RFR for 12 and 24 h were $70.34 \pm 6.2\%$ and $45.25 \pm 5.1\%$ respectively which was lower than that compared to control group ($P < 0.05$).

In the same time, we counted the number of colonies at each group with cell population greater than 50 ones and ignored the similarity of size or cell population. Based on these criteria, the number of colonies after 9 days were $92.85 \pm 4.8\%$ and $57.74 \pm 5.5\%$ in 12 and 24 h RFR-groups, respectively.

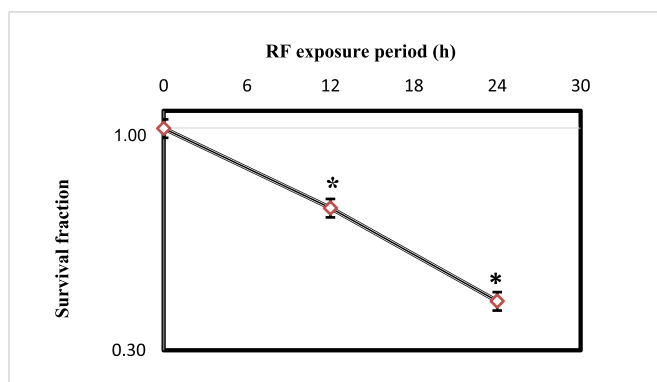


Fig. 4. Survival curves for cells exposed to RFR for 0, 12, and 24 h according to clonogenic assay. The data are shown as mean $\pm$ SEM for at least three independent experiments containing 3 sample in each group. Statistical analysis between the groups was determined by ANOVA; * represents $P < 0.05$ in comparison with the control group.

Furthermore, we checked the size of colonies and the cell number in each colony at different groups. There were more cells in colonies of control group compared to those in exposed groups in ninth day.

We selected 24 h exposure time period to analyze indirect effect of RFR (bystander effect). The percentage of colony formation in the various media transfer conditions (described in material and method) are presented in Fig. 5. Statistically significant lower number of colonies were found in both RFR exposed cells ('RFR' group) ($39.17 \pm 0.8\%$) and the group treated for 12 h with CCM 12 h after the end of RFR exposure (12/12 condition of 'C, RFR, MT' group) ($46.21 \pm 1.3\%$). In contrast, these numbers stood statistically significant higher at $72.68\% \pm 9.7$ for 'C, MT' group, $87. \pm 9.6\%$ for 'RFR, MT' group and $82.42 \pm 10.89\%$ for 'MT group' which did not show statistically significant difference compared to 'Cont.' group (Fig. 6). These results are evidence of occurrence of BE and showed that reduction of colony formation ability was not due the media (without cells) which exposed to RFR or media transferring process but it was because of CCM. Clearly, 12/12 condition caused MT bystander effect, while no other conditions induced that.

00/12: The cell culture media after 24 h RFR exposure immediately transferred to bystander cells and remained in contact with them for 12 h (the first and second two digits represent the time of media transfer after RFR exposure and time of treatment, respectively).

'C, RFR, MT' group: cells whose culture medium was exchanged with related CCM. 'Cont.' group: Cells not exposed to RFR. 'RFR' group: Cells exposed to RFR for 24 h 'C, MT' group: the medium of sub-confluent cells was collected and filtered through a 0.22 mm filter to remove cells from the culture. Then, this medium was transferred to bystander cells. These groups of cells named 'C, MT'. 'RFR, MT' group:

medium (without cells) was exposed to RFR for 24 h. Then, this medium was transferred to bystander cells. These groups of cells named 'RFR, MT'. 'MT' group: fresh medium (without cells and RFR exposure) was transferred to bystander cells. These groups of cells named 'MT'.

'C, RFR, MT' group: cells whose culture medium was exchanged with related CCM. 'Cont.' group: Cells not exposed to RFR. 'RFR' group: Cells exposed to RFR for 24 h 'C, MT' group: the medium of sub-confluent cells was collected and filtered through a 0.22 mm filter to remove cells from the culture. Then, this medium was transferred to bystander cells. These groups of cells named 'C, MT'. 'RFR, MT' group: medium (without cells) was exposed to RFR for 24 h. Then, this medium was transferred to bystander cells. These groups of cells named 'RFR, MT'. 'MT' group: fresh medium (without cells and RFR exposure) was transferred to bystander cells. These groups of cells named 'MT'.

3.6. DNA damages

3.6.1. Alkaline single-cell gel electrophoresis (comet assay)

In our experiments, no significant difference was found in the olive moment between non-exposed cells and cells exposed to RFR for 12 h ($p > 0.05$) (data not shown). While increasing the exposure time to 24 h caused higher olive moment ($p < 0.05$) (Fig. 7).

As RFR exposure for 24 h had statistically significant effect on olive moment, we chose this period of time to analyze indirect effect of RFR (bystander effect). Moreover, according to colony formation results, we discovered that just 12/12 state (transfer of culture media to bystander cells 12 h after RFR exposure and remained for 12 h) reduced colony formation and caused the same effect as those directly exposed to RFR, so just 12/12 state was performed for this endpoint. As it is shown in Fig. 7 and 24 h RFR exposure caused statistically significant increase in the olive moment in alkaline comet assay results; this increment was also found in non-exposed cells which received conditioned media (bystander cells) with 12/12 procedure (Fig. 7).

3.6.2. Cytokinesis-block micronucleus (CBMN) assay

The frequency of micronuclei (MN) in 12 and 24 h RFR exposed cells increased to 12.78% and 13.4%, respectively. But this increment was not statistically significant in comparison with the frequency of MN in non-exposed cells (10.36%). All these frequencies had statistically significant difference compared to those irradiated with 6 Gy X-ray (as positive control). X-ray increased MN frequency to 43.7%. It showed that RFR failed to cause detectable DSB.

3.7. ROS production

The intracellular level of ROS increased statistically significant after exposing cells to RFR for 12 h (1.3 folds of control) compared to non-exposed cells. Furthermore, ROS level was higher following 24 h RFR-

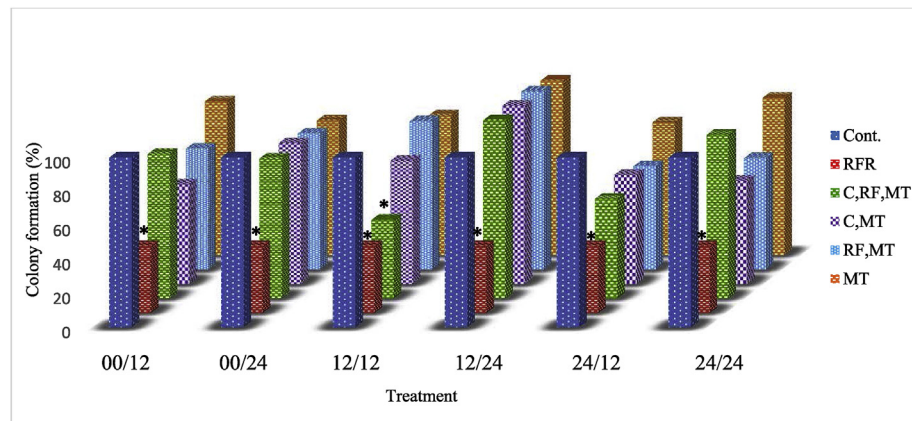


Fig. 5. Representative of colony formation results of the 6 experimental condition (different interval and interface times) with their relative groups. Each group contained 3 samples. The experiment was done for at least three independent experiments. Statistical analysis between the groups was determined by ANOVA; * represents statistically significant difference compared to control groups ($p < 0.05$).

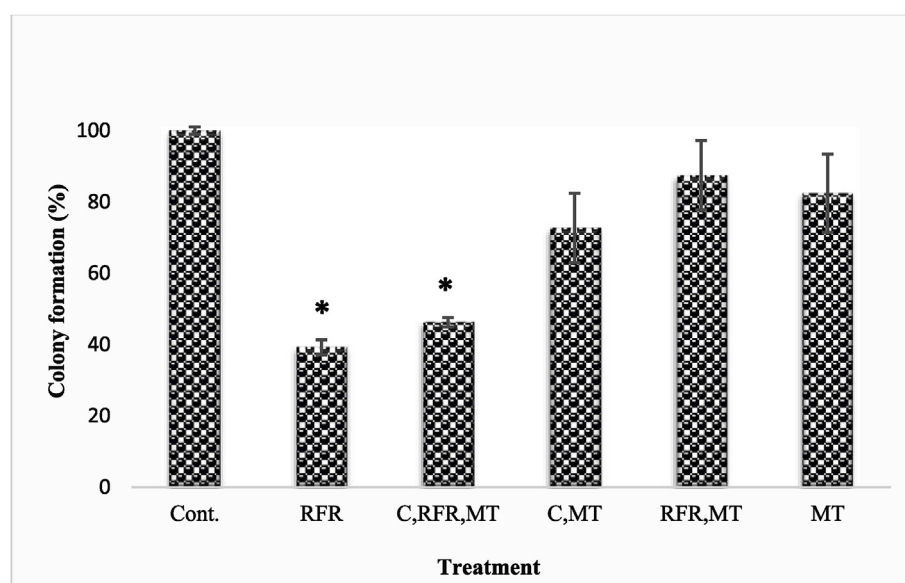


Fig. 6. Representative of colony formation results of 12/12 condition. The data are shown as mean $\pm$ SEM for three independent experiments. Each group contain 3 samples. Statistical analysis between the groups was performed by ANOVA; * represents statistically significant difference compared to control group ($p < 0.05$).

exposure than control group. (1.5 folds of control) ($P < 0.05$) as it is shown in Fig. 8 A and C. Besides, $100 \mu\text{M}$ H_2O_2 elevated the level of ROS more than 10 fold greater than control.

Fig. 8. B shows fluorescence microscopy of four examined cell groups. In the control group, no obvious fluorescence images were found, whereas the bright green fluorescence images were observed in the group exposed to RFR for 12 h. More shining green fluorescence images and more cells with bright dye fluorescence were visible in the cell group receiving 24 h RFR. Forth group was the cells treated with $100 \mu\text{M}$ H_2O_2 as positive control.

As RFR had statistically significant effect on ROS level, that was also assessed in bystander cells. We chose 24 h exposure time period to analyze indirect effect, because it had stronger effect on ROS level. We found that treatment of bystander cells with CCM for a period of 12 or 24 h, neither immediately nor 12 h after RFR exposure caused increase in ROS production.

Moreover, level of ROS in CCM was measured, chemiluminescence spectroscopy results showed statistically significant increase in ROS level in CCM after 24 h RFR exposure compared to the control group (nearly 2 folds of control). RFR exposure for 12 h elevated the ROS level in CCM as well, but that was not statistically significant in comparison to non-exposed cells. This elevation remained stable in different time points till 12 h after incubation (Fig. 9).

4. Discussion

In this study, we applied the cell culture media transfer method to induce BE and colony formation assay at the first step. Reduction in colony formation was observed in both directly RFR exposed cells and those cells treated with 12/12 CCM. This observation is indicative of BE which is in line with those conducted for X-ray radiation by Faqih and coworkers. They showed statistically significant decrease in plating efficiency in bystander cells that received media from the X-ray irradiated cells (Faqih et al., 2015). However, our results contradict a number of published papers indicating that irradiation with gamma ray and Fe ions do not induce a bystander response. Inverse effects have also been observed. Baskar et al. showed enhancement of colony-formation efficiency in bystander GM637H cells (Baskar et al., 2007). To the best of our knowledge, before this study, no attention has been paid to the indirect effects of RFR on cell colony formation ability, while reduction of the colony formation ability in cells directly exposed to 2.45 MHz RFR for 24 h has been reported by f-Kubiczek and Harrison (Balcer-Kubiczek and Harrison, 1985). However, Pavicic et al. did not report alteration in colony-forming ability of V79 cell line exposed to 864 MHz continuous wave for 1, 2 and 3 h (Pavicic and Trosic, 2008; Pavicic et al., 2006). It was likely that they would have seen different results if the cells were exposed to RFR for more period time.

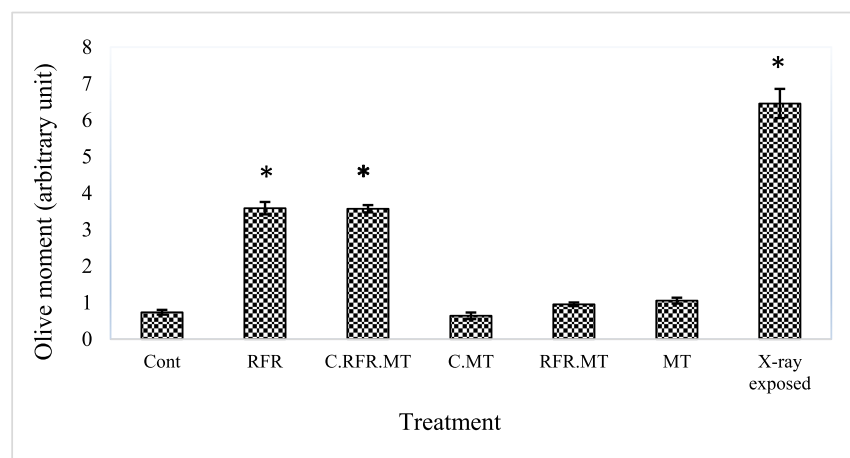


Fig. 7. Results of olive moment after transferring conditioned media to non-exposed cells, 12 h following 24 h RFR exposure, 12/12 condition; * represents statistically significant increase in comparison to control determined by ANOVA ($p < 0.05$); The data are shown as mean $\pm$ SEM for at least three independent experiments. Up to 3000 cells were analyzed for each group. 'C, RFR, MT' group: cells whose culture medium was exchanged with related CCM. 'Cont.' group: Cells not exposed to RFR. 'RFR' group: Cells exposed to RFR for 24 h 'C, MT' group: the medium of sub-confluent cells was collected and filtered through a 0.22 mm filter to remove cells from the culture. Then, this medium was transferred to bystander cells. These groups of cells named 'C, MT'. 'RFR, MT' group: medium (without cells) was exposed to RFR for 24 h. Then, this medium was transferred to bystander cells. These groups of cells named 'RFR, MT'. 'MT' group: fresh medium (without cells and RFR exposure) was transferred to bystander cells. These groups of cells named 'MT'.

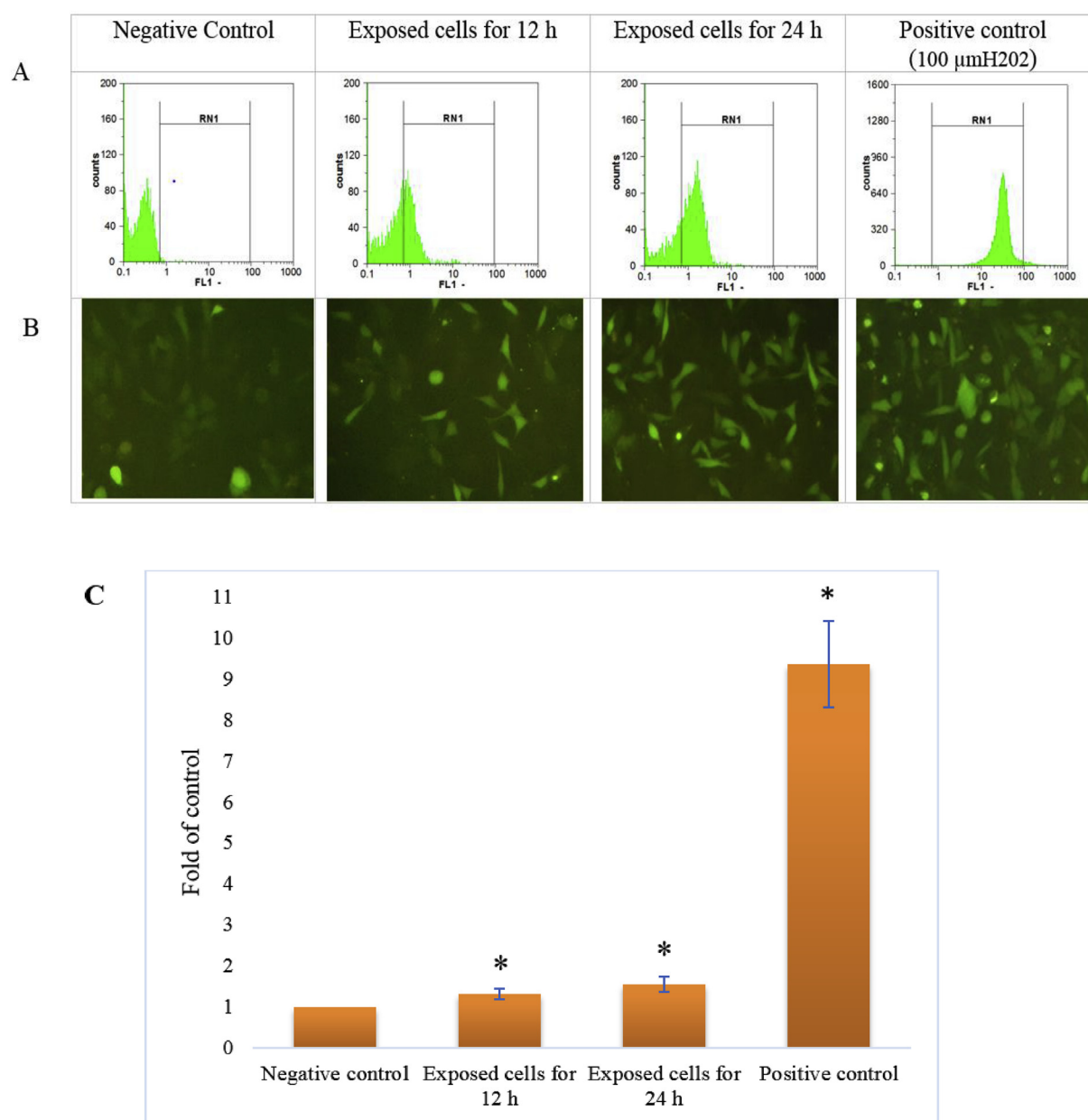


Fig. 8. ROS measurement in RFR directly exposed cells. A: Representative flow cytometric images of intracellular ROS in CHO cell line. FL1-H of the flow cytometer was applied to measure green (DCFH-DA) fluorescence intensities (12,000 cells were analyzed for each experiment), B: Fluorescence microscopy images of intracellular ROS in CHO cell line. C: Quantitative results of ROS measurement. * represents statistically significant increase in comparison to control determined by ANOVA ($p < 0.05$); The data are shown as mean $\pm$ SEM. 12 and 24 h RFR exposures increased ROS production; non-exposed cells did not show ROS induction. All experiments were repeated at least three times. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

One of the reasons for the reduction of the colony formation ability is genomic instability including DNA breaks (Ferrara et al., 2007). In our study, comet assay data showed that both direct and indirect RFR exposures caused DNA break damages. There are several *in vivo* and *in vitro* evidences indicating that RFR emitted from cell phones damages DNA in cells that have been directly exposed to RFR (Lai and Singh, 1995; Luukkonen et al., 2009; McNamee et al., 2002; Paulraj and Behari, 2006; Tice et al., 2002). But, genomic instability in indirectly exposed cells (bystander cells) has only been studied for ionizing radiation (Al-Mayah et al., 2012; Redmond et al., 2014; Sokolov et al., 2005; Vorob'eva et al., 2011). This the first study addressing the DNA damages due to non-ionizing radiation including RFR in bystander cells.

In general, reduction in colony formation occurs as a result of stopping the cell cycle progression. One of the following reasons could

explain this issue: apoptosis, entering in the aging phase or staying quiescent until cell recovery occurs and DNA replication and cell division begins again (Ferrara et al., 2007). In the current study, RFR-exposed groups represented statistically significant more colonies when they were incubated more than 7 days; it showed that some clusters (collection of cells including less than 50 cells) grown and established new colonies. This increase also happened in control groups but it was not statistically significant. This illustrated that RFR has not resulted in permanent block in cell growth or apoptosis, but only stopped the proliferation of some cells to repair themselves. Radiation-induced division delay is a phenomenon that has been defined for many years (Schneiderman and Hofer, 1980).

In parallel to the previous discussion, the data of flow cytometry showed that RFR did not induce apoptosis or autophagy in RFR-exposed

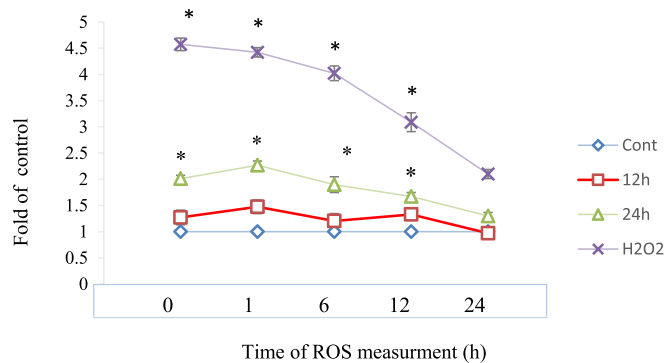


Fig. 9. ROS level in CCM of cells exposed to RFR for 12 and 24 h, immediately, 1, 6, 12 and 24 h after exposure. ROS is illustrated as a proportion of each parallel non-exposed sample. Statistical analysis between the groups was determined by ANOVA. * represents statistically significant increase in comparison to corresponding controls ($p < 0.05$). Three independent experiments were done.

cells. In addition, results obtained from trypan blue assay did not show any increase in the permeability of cell membrane. Any change in cell membrane permeability is a sign of cell death. This finding is in line with the results obtained from human peripheral blood (Capri et al., 2004a) and lymphoblastoid cells (Hook et al., 2004b) which did not show cell death after RFR exposure. On the contrary, some studies reported apoptosis in primary neuronal cultures (Joubert et al., 2008), human colon cancer cells (Maeda et al., 2004) and human epidermoid cancer cells (Caraglia et al., 2005) after RFR exposure. Moreover, we did not detect autophagy in RFR-exposed cells. Liu and coworkers showed that autophagy prevents cells from apoptotic cell death in the case of the exposure the cells to 1800 MHz GSM exposure at SAR values of 4 W/kg for 24 h (K. Liu et al., 2014). Moreover, Ju Hwan Kim et al. displayed that 835 MHz RFR exposure at a SAR of 4.0 W/kg for 12 weeks induced autophagy. These authors proposed that autophagy happens to protect neurons of mice brain tissues against chronic RFR (Kim et al., 2017). In our study, the power and time of RFR-exposure were not suitable to trigger autophagy or apoptosis pathways but stimulated the cells to spread the message of damages (DNA break or inability to form colony) to other cells; hence, BE was occurred. In confirmation of our results, Liu et al. found a reduction in colony formation both in exposed and bystander cells at neutron doses which were not lethal for cells (Z. Liu et al., 2006). Controversial results might be related to exposure conditions or vulnerability of cells to RFR.

To detect the kind of the DNA break, CBMN assay was done. CBMN assay is a technique revealing occurrence of just DSB. Our results did not show any increase in MN frequency in RFR-exposed groups compared to control group. In this way, Vijayalaxmi and Prihoda. in a meta-analysis of 88 publications did not observe statistically significant differences between the frequencies of MN in RFR-exposed and control groups (Vijayalaxmi and Prihoda, 2012); however, there are some results which show increase in frequency of MN in RFR-exposed cells. For example, Tice et al. reported statistically significant elevation in the frequency of MN in lymphocytes exposed to 837 MHz RFR with an average SAR value of 5.0 or 10.0 W/kg for 24 h. Furthermore, exposure to 2.45 GHz RFR at SAR value of 100 W/kg resulted in increment of frequency of MN (Koyama et al., 2003). Exposing to a high power SAR is not directly comparable with our data because induction of MN in these studies was a result of temperature increase. Considering CBMN and comet results together guided us to suppose SSB as the dominant DNA damage induced by RFR at exposure protocol used in this study. This result is confirmed by the previous results that RFR-exposure is not lethal.

According to previous studies, the most prominent factor to induce DNA breaks by RFR is ROS including $O_2^{\cdot -}$, H_2O_2 , $OH^{\cdot}$, and $NO^{\cdot}$

(Luukkonen et al., 2009). RFR can increase the activity, concentration, and lifetime of ROS through changes in the energy level and spin orientation of electrons, loss of efficiency of antioxidant mechanisms and alterations in the mitochondrial electron transfer chain (Cig and Naziroglu, 2015). Our data of flow cytometry assay and fluorescence microscopy showed increase in intracellular ROS after both 12 and 24 h exposure period which is consistent with several reports confirming RFR effects on biological activity via oxidative stress (Hook et al., 2004a; Ilhan et al., 2004). Manta and coworkers showed a 2 fold elevation in ROS levels in *Drosophila melanogaster* body after 6 h of electromagnetic fields exposure (Manta et al., 2014). However there are some studies that did not find any alteration in level of ROS *in vivo* or *in vitro* by RFR (Brescia et al., 2009; Lantow et al., 2006; Silva et al., 2016). Concurrent with increased DNA damage in exposed cells, increasing RFR exposure time induced more ROS production. These results provide evidence for induction of DNA strand break by ROS which is in parallel with previously reported studies (Monks et al., 2006; Storch et al., 2016). Thus, RFR energy has the ability to elevate ROS production, but does not tend to be localized along its track as described for ionizing radiation (Hall and Giaccia, 2012). Ionizing radiation can form local regions of ion pairs, free radicals, and solvated electron. These regions are spatial distributions of energy deposition in biological structure. There is a relationship between the energy deposited and the number of single strand breaks. Two single strand breaks on opposite strands of a DNA molecule were separated by a distance less than ten base-pair produce DNA double strand break (Goodhead and Brenner, 1983; Hall and Giaccia, 2012). Therefore, we hypothesized that sufficient amount of radicals and energy were not produced by RFR (in our exposure condition) to cause two close SSBs on opposite strands of a DNA molecule to create a DSB.

Different kinetics of the directly and indirectly induced DNA damages has been suggested for ionizing radiation (Smilenov et al., 2006). Besides, our results suggested that these mechanism may be different by RFR, although they are not lethal in both directly exposed and bystander cells. Because by increasing the RFR exposure period time to 24 h, ROS levels increased in directly exposed cells which is consistent with increment of DNA vulnerability; while DNA damages in bystander cells was observed without enhancement of intracellular ROS level.

Although mechanisms behind BE have not been definitely elucidated (Peng et al., 2017), it is clear that generating clastogenic factors is associated with induction of BE. In this light, irradiated cells secrete signaling molecules which impact bystander cells (Morgan, 2003). Signaling molecules include a variety of cytokines, calcium ions, nitric oxide (NO), the long-living radicals, and especially ROS (Lyng et al., 2011; Lyng et al., 2006; Shao et al., 2006). To examine whether ROS can be a mediator of BE induced by RFR, we evaluated ROS level in cell culture media. ROS level was higher in media of exposed cells compared to that of the control group. Although ROS level began to decrease after the end of RFR exposure, it was still higher than that in the corresponding control sample at different interval times and returned to level of unexposed cells after 24 h (Fig. 9). There are evidences that ROS remain in higher level than basic in exposed groups several hours after electromagnetic fields exposure (Manta et al., 2014).

However, in our study, increase of ROS in media did not evoke ROS production pathways in bystander cells. Accordingly, it seems that ROS can be a mediator molecule responsible for triggering molecular signals which cause DNA damages or reduction in colony formation ability. Extra cellular ROS oxidizes the lipids in cell membranes and consequently activates a process called lipid peroxidation and the arachidonic-acid cascade. In this process, malondialdehyde (MDA) and 4-hydroxynonenol (4-HNE) are produced. MDA is a highly reactive compound which reacts with deoxyadenosine and deoxyguanosine in DNA, forming DNA adducts (Hussain et al., 2003; Marnett, 1999). DNA adducts can result in DNA strand breaks either by chemical cleavage of the DNA backbone through direct alkylation of the phosphodiester bond or through disrupting DNA repair during replication (Swenberg

et al., 1985).

According to the results presented in Fig. 5, BE did not happen by CCM separated immediately after exposure but was induced by those separated 12 h after exposure. Therefore, we found that the interval time between the MT from exposed cells to bystander cells and the time period of cell incubation in this medium, plays major roles in development of bystander effect which means that RFR-exposed cells need time to produce signaling molecules and release them in the surrounding environment. As well as ROS, interleukin 8, interleukin 6, interleukin 33, transforming growth factor (TGF- β) (Gow et al., 2010; Pasi et al., 2010; Shao et al., 2008; Widel et al., 2014), ERK, MAPK, tumor necrosis factor receptor alpha (TNF α), and cyclooxygenase-2 (COX-2) (Hei et al., 2008; Zhou et al., 2008) are known to be mediators to induce BE by ionizing radiation. However, the same phenomenon for RFR is being studied in our laboratory.

5. Conclusion

In conclusion, RFR emitted from cell phones as a non-ionizing radiation have inevitable effects on cells such as DNA damage, reduction of colony formation ability, ROS production, etc. These effects have been reported as a result of direct exposure. Although RFR have low energy and do not have strong impacts on cells, they are able to have indirect effects or NTEs such as BE. In the current study, for the first time, we showed that RFR can induce bystander phenomenon. In the case of ionizing radiation, this phenomenon has two dissident consequences; first, damaging neighboring normal cells, and second, detecting tumor cells not directly hit by radiation. This phenomenon in non-ionizing radiation also may have clinical applications which need further investigation. Although exact mechanism of BE is not totally understood, it is assumed that the main signal mediator involved in this phenomenon either in ionizing or non-ionizing radiation is the same and mediated via oxidative stress.

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Conflicts of interest

None.

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