Exposure to mobile phone radiations at 2350 MHz incites cyto- and genotoxic effects in root meristems of *Allium cepa*

Shikha Chandel¹ · Shalinder Kaur¹ · Mohd Issa² · Harminder Pal Singh² · Daizy Rani Batish¹ · Ravinder Kumar Kohli^{1,3}

Received: 18 July 2018 / Accepted: 3 December 2018 / Published online: 5 January 2019
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Abstract

Background The exponential increase of electromagnetic field radiations (EMF-r) in the natural environment has raked up the controversies regarding their biological effects. Concern regarding the putative capacity of EMF-r to affect living beings has been growing due to the ongoing elevation in the use of high frequency EMF-r in communication systems, e.g. Mobile phones.

Methods In the present study, we tried to examine the cyto- and genotoxic potential of mobile phone EMF-r at 2350 MHz using onions (*Allium cepa* L.). Fresh adventitious onion roots were exposed to continuous EMF-r at 2350 MHz for different time periods (1 h, 2 h and 4 h). The evaluation of cytotoxicity was done in terms of mitotic index (MI), phase index and chromosomal aberrations. Genotoxicity was investigated employing comet assay in terms of changes in % HDNA (head DNA) and % TDNA (tail DNA), TM (tail moment) and OTM (olive tail moment). Data were analyzed using one-way ANOVA and mean values were separated using post hoc Tukey's test.

Results The results manifested a significant increase of MI and chromosomal aberrations (%) upon 4 h, and ≥ 2 h of exposure, respectively, as compared to the control. No specific changes in phase index in response to EMF-r exposure were observed. The % HDNA and % TDNA values exhibited significant changes in contrast to that of control upon 2 h and 4 h of exposure, respectively. However, TM and OTM did not change significantly.

Conclusions Our results infer that continuous exposures of radiofrequency EMF-r (2350 MHz) for long durations have a potential of inciting cyto- and genotoxic effects in onion root meristems.

Keywords Electromagnetic field radiations · Mitotic index · Chromosomal aberrations · DNA damage

Introduction

Along with the pre-eminent dependency of man on various communication mediums and modern-day gadgets, the exposure to non-ionizing radiations is also increasing at a very fast pace. Most of the electronic gadgets being used today on daily bases are based on non-ionizing radiations and have led to an elevation in the concentration of radiofrequency electromagnetic field radiations (EMF-r) in the natural environment [1]. Of the numerous gadgets, cellular phones are the ones that have become an inevitable part of day to day life. Owing to the various

beneficial services that the mobile phones provide to the users, there has been an unprecedented upsurge in the usage of mobile phones [2]. However, increased number of mobile phones has consequently led to exponential increase in number of base stations for the maintenance of communication, which further favors the augmentation of EMF-r in the environment. According to a recent report, globally, there are 7.377 billion mobile phone subscriptions, averaging 99.7 subscriptions per 100 inhabitants [3]. At the same time, the unregulated use of mobile phones during the last few years has grabbed the attention of people towards the tendency of non-ionizing radiations especially, EMF-r, to make an impact on health of living beings. Many previous studies have documented the biological effects of radiofrequencies on humans [4, 5], animals [6], insects [7], and plants [8]. Moreover, radiofrequency EMF-r have been classified in group 2B, i.e., a "possible human carcinogen" [9]. However, in most of the EMF-r studies, major focus has been put on animals, and plants have been an outcast. Plants hold an important position in the ecosystem as they are the primary

✉ Shalinder Kaur
shalinderkaur@hotmail.com

¹ Department of Botany, Panjab University, Chandigarh 160014, India

² Department of Environment Studies, Panjab University, Chandigarh 160014, India

³ Central University of Punjab, Mansa Road, Bathinda 151 001, India

producers and major source of oxygen. Therefore, plants deserve a major share of our attention as these are static and thus, are always under the influence of radiations. Moreover, the studies on impact of EMF-r on plants have shown contrasting results and have been inconclusive as few studies have documented the negative impact of EMF-r [2, 10, 11], while the other few have documented the positive effects [12, 13]. Therefore, it is important to analyze the impacts of EMF-r on plants.

Researchers around the world have reported various biological consequences of EMF-r exposure and have attributed them to be thermal effects [14, 15]. At the same time, few studies have also brought into notice the potential of EMF-r exposure in inciting the non-thermal effects [16–19]. Thus, there is still lot of ambiguity regarding the effects of EMF-r. Amongst the various cellular targets, DNA has always been an important molecule and widely analyzed for potential EMF-r damage [20]. However, in plant systems, scanty studies are available on DNA damage and most of the studies have been done at lower frequencies. Nevertheless, with the advent of new technology, higher frequencies (3G, 4G) are being used for better communication system. In the light of these contemplations, we aimed at analyzing the cyto- and genotoxic effects of 2350 MHz EMF-r in the root meristematic cells of onion (*Allium cepa* L.; $2n = 16$). Due to inadequate evidences on cytotoxic and genotoxic effects of EMF-r on plants, the present work has been conducted. Studying damage to chromosomes or DNA is of significant contemplation as it may lead to permanent alterations. Mitosis is widely used to study cytotoxic and genotoxic effects of a substance. Cytotoxicity was analyzed in terms of mitotic index, frequencies of chromosomal abnormalities and phase index. For the study of genotoxicity (DNA damage) comet assay was employed. It involves analysis of movement of DNA fragments from the nucleus with the help of electrophoresis. For the present investigation, we selected onion as a test plant as it serves an excellent model for evaluating mutagens [21] due to presence of higher percentage of dividing cell that stains well.

Materials and methods

Materials

Uniform sized onion bulbs were acquired from a local market. Outer dry scales and roots were carefully scrapped with a razor blade. Onions were then set for rooting for 2–3 days in beakers filled with distilled water in dark conditions at room temperature ($25 \pm 1^\circ\text{C}$). Chemicals of analytical grades were used for the experimentation.

EMF-r treatment

For the EMF-r treatment, we followed the methodology of Kumar et al. [22] with some modifications. We exposed the test

plants to EMF-r using Agilent N9310A radio frequency signal generator (Keysight Technologies, USA) connected to a power amplifier (ZHL-5 W-2GX+; Minicircuits, USA) and a power supply. To avoid any external source of electromagnetic field radiations, the exposure system was kept in a room coated with RF shielding paint (Y-shield HSF54). Onion roots, on attaining the length of 2–3 cm, were irradiated to EMF-r for different durations. Group 1 was sham exposed, while group 2, 3 and 4 were treated with 2350 MHz EMF-r for 1 h, 2 h and 4 h, respectively. Group 5 was treated with methyl methane sulfonate (MMS) at 0.01 mg/ml concentration for 4 h and it served as positive control. The output densities were recorded with the help of ScanEM®-C Probe (CTK015; 3 M Technologies, USA) coupled with a Spectran RF power density meter (HF-4060, Aaronia AG, Germany). The average power density was recorded to be $492.3 \pm 21.43 \text{ mW m}^{-2}$ with specific absorption rate (SAR) of $3.13 \times 10^{-1} \text{ W kg}^{-1}$ at 5 cm from the antenna. SAR had been calculated roughly as it is quite tough to estimate SAR on irradiated tissues directly [23]. The values were evaluated by taking data for tissue density (ρ) and electrical conductivity (σ) for the dielectric properties of tissue at 2350 MHz ($\rho = 1030 \text{ kg cm}^{-3}$ and $\sigma = 1.739 \text{ S m}^{-1}$) from database of Institute of Applied Physics, Sesto Fiorentino, Italy [24].

Cytogenetic analysis (*Allium cepa* test)

After the exposure, onion root apices were excised and immersed in a fixative (aceto-alcohol; 1:3; v/v) for 24 h. After fixation, roots were stored in 70% ethanol at 4°C . Roots were then hydrolyzed in 1 N HCl for 1 min with intermittent heating. Afterwards, roots were rinsed with distilled water and stained in acetocarmine. Slides were prepared using squash technique as per the methodology of Armbruster et al. [25] with slight modifications. Three slides were made for each treatment and around 750–850 cells were scored from individual slide using a light microscope (Olympus CX21i) attached with a camera (Magnus-Microscope Digital Camera) and LCD. Various parameters like MI (percentage of cells in dividing state), % chromosomal aberrations (percentage of aberrant cells) and phase index (percentage of cells in a particular mitotic phase) were evaluated to analyze the cytotoxic potential of EMF-r.

Comet assay

Immediately after the exposure (0 h), we examined DNA damage using Comet assay by following the protocol given by Tice et al. [26]. One-fourth frosted end microscopic slides were first of all prepared by coating them with 1% normal melting point agarose at 50°C . Nuclei were then isolated by slicing the onion roots tips with sharp razor blade in Phosphate Buffer Saline (PBS, pH 7.4) in a watch glass placed over ice. Nuclei suspension (100 μl) was added to low melting point agarose (50 μl) in PBS at 37°C in Eppendorf tube and pipetted over coated slides

and covered with coverslips. The slides were then placed on iced surface for 10 min, followed by removal of coverslips and immersion in lysis solution for approximately 1 h under dark conditions. Thereafter, the nuclei were incubated in the freshly prepared cold electrophoretic buffer (0.3 M NaOH and 1 mM EDTA; pH $\geq$ 13) for half an hour in an electrophoretic tank prior to electrophoresis (at 25 V, 300 mA, for 25 min). Subsequently, slides were rinsed thrice in distilled water, followed by staining with ethidium bromide ($20 \mu\text{g ml}^{-1}$) in dark for approximately 5 min. Coverslips were placed after removing the excess stain by dipping slides in cold water. Slides were then examined using a fluorescence microscope (excitation filter of BP 546/10 nm; barrier filter of 590 nm). About 5 roots were used for making each slide and for each treatment 3 slides were prepared to serve replication. From each slide at least 50 nuclei were scored.

Quantification of DNA damage was done in terms of % HDNA (head DNA), % TDNA (tail DNA), TM (tail moment) and OTM (olive tail moment) using an Image Analysis Software (CASP 1.2.3b comet assay package).

Statistical analysis

We performed the experiment in a completely randomized manner with 3 replicates per treatment. Each replicate comprised of a single onion bulb. For cytotoxicity test as well as comet assay, one slide was made from each replicate. Data were analysed by one-way ANOVA and mean values were separated using post hoc Tukey's test at $P \leq 0.05$. All statistical analyses were performed using SPSS ver. 16.

Results

Cytogenetic effects

EMF-r treatment altered the mitotic division in onion root tip cells. The effect of EMF-r on mitotic index (MI) and

chromosomal aberrations (%) is presented in Fig. 1. Treatment of EMF-r increased the MI in a dose-dependent manner. However, significant changes in the number of dividing cells upon EMF-r exposure were observed only at higher exposure duration, i.e., 4 h (Fig. 1a). Compared to the control, MI increased significantly by $\sim 29\%$ on EMF-r exposure of 4 h, whereas a decrease of $\sim 41\%$ was observed in MMS treated group.

Microscopic analysis also revealed that increase in MI was also accompanied by enhanced % chromosomal aberrations (Fig. 1b). A significant increase of ~ 1.0 and ~ 1.4 fold in the aberrations (%) was observed in root meristem cells exposed for 2 and 4 h, respectively, over the control. Likewise, positive control displayed a significant increase of ~ 1.9 fold as compared to the control. Upon EMF-r exposure, several chromosomal aberrations like stickiness, *c*-mitosis, laggards, vagrants, polyploidy, chromosomal ring, mitotic bridge and fragments were observed (Fig. 2). Among these aberrations, the most frequent were stickiness, followed by *c*-mitosis, mitotic bridge and spindle disturbances (Table 1). Similarly, in MMS treated group, mitotic bridge, *c*-mitosis and stickiness were the most frequent aberrations.

Exposure to EMF-r did not alter the phase index of different mitotic stages (Fig. 3). It was observed that in all the EMF-r exposed groups as well as controls (sham control and positive control) majority of the cells were in prophase while, least number of cells was observed in telophase.

EMF-r exposure disrupts DNA integrity

DNA integrity in onion root tip cells exposed to EMF-r for 1 h, 2 h and 4 h at 2350 MHz was analyzed using comet assay. Parameters like, % HDNA, % TDNA, TM and OTM were examined to quantify the DNA damage. Treatment of MMS (0.01 mg/ml) for 4 h was used as positive control which showed maximum DNA damage. The results are illustrated in Fig. 4a-d.

Fig. 1 Effect of EMF-r exposure at 2350 MHz on (a) Mitotic index and (b) Chromosomal aberration (%) in *Allium cepa* root meristem cells ($n = 3$ onions for each treatment group). Data presented as mean $\pm$ SE. Different alphabets represent significant difference among them at $P \leq 0.05$, applying Tukey's test

Fig. 2 Normal stages of mitosis: a-e, (a) Interphase, (b) Prophase, (c) Metaphase, (d) Anaphase, and (e) Telophase; Different aberrations observed in *Allium cepa* root meristem cells exposed to EMF-r at 2350 MHz: f-s, Chromosomal aberrations, (f) c-mitosis, (g)

Chromosomal fragment, (h) Chromosomal rings, (i) Polyploidy, (j) Laggard, (k) Vagrant, (l) Stickiness, (m) Mitotic bridge, (n) Spindle disturbance at anaphase, and (o) Morphological alteration of cell

Analysis of the results obtained from Comet assay demonstrated a dose-dependent DNA damage. With increase in exposure duration, decrease in % HDNA accompanied with increase in % TDNA was observed. A significant decrease in HDNA by ~2% and ~4.3% was observed in cells exposed for 2 h and 4 h, respectively, over the control, whereas a decline of ~11.1% was observed in MMS treated cells (Fig. 4a). Likewise, % TDNA increased significantly by ~0.3, ~0.7 and ~1.9 fold upon EMF-r exposure of 2 h, 4 h, and MMS, respectively, compared to the control (Fig. 4b). No significant changes in TM and OTM were observed in any of the EMF-r exposed groups with respect to control (Fig. 4c, d). However, in comparison to control, MMS treatment significantly increased tail moment and olive tail moment by ~4.7 and ~1.9 fold.

Discussion

Exploring the literature regarding the biological effects of EMF-r revealed indecisive conclusions, as few studies have reported negative implications of EMF-r [22, 27] while, other few stated no or positive effects [28, 29]. The present study thus tried to inspect the cyto- and genotoxic potential of 2350 MHz EMF-r using *A. cepa* as the test plant.

In the current work, cytotoxicity was assessed by evaluating parameters like MI, and frequencies of various chromosomal aberrations in response to EMF-r exposure. The results manifested an increase in MI and chromosomal aberrations (%) in a dose-dependent manner. This observed increase is corroborated by earlier studies that have also reported a

Table 1 Different types of Chromosomal aberrations observed in *Allium cepa* root meristem cells exposed to EMF-r at 2350 MHz ($n = 3$ onions)

Treatment	Types of chromosomal aberrations								
	CM	SD	P	S	MB	CF	V	L	CR
Sham control	2	2	—	4	—	—	—	—	—
EMF-r exposed									
1 h	—	—	—	3	3	1	1	—	—
2 h	4	2	—	6	3	—	1	1	—
4 h	4	4	1	8	2	2	2	—	1
MMS (0.01 mg/ml)	3	1	—	3	5	—	1	—	1

CM, c-mitosis; SD, Spindle disturbance; P, Polyploidy; S, Stickiness; MB, Mitotic bridge; CF, Chromosomal fragment; V, Vagrant; L, Laggard; CR, Chromosomal ring

Fig. 3 Effect of 2350 MHz EMF-r on Mitotic phases in *Allium cepa* root meristem cells. Data presented as mean $\pm$ SE; ($n = 3$ onions for each treatment group). Different alphabets represent significant difference among them at $P \leq 0.05$, applying Tukey's test

similar effect [11, 30, 31]. A similar trend of increase upon EMF-r exposure has also been reported for human cells by many research cohorts [32, 33]. This increase in MI could be attributed to (a) increase in proliferation rate or (b) delayed mitosis. Tkalec et al. [11] analyzed the effect of 400 MHz and 900 MHz EMF-r at 41 and 120 V/m on *A. cepa* and reported increase in MI due to delayed mitosis. EMF-r exposure

induced several chromosomal aberrations such as stickiness, *c*-mitosis, laggards, vagrants, polyploidy, chromosomal ring, mitotic bridge and fragments in the root meristem cells of onion. Chromosome stickiness observed in the present study occurs when chromosomes clump together which could be the result of denaturation of nuclear proteins or degradation/depolymerization of DNA [34], whereas laggards appear due to improper spindle functioning [35]. Further, chromosomal fragments may perhaps be the consequence of inversion of acentric chromosome or deleterious effect of enhanced reactive oxygen species generation in response to EMF-r [36]. The occurrence of all these abnormalities advocates the impact of EMF-r exposure on spindle function [37]. The spindle disturbance upon EMF-r exposure could be linked to commotion of calcium-ion homeostasis which disrupts microtubule polymerization, thereby altering spindle formation [11].

Further, in this study, the quantitative appraisal of DNA damage was also done with the help of comet assay by exploring parameters like % HDNA, % TDNA, TM and OTM. The % TDNA represents the content of the damaged DNA in an individual cell [38]. The parameters, TM and OTM link the extent of tail to the quantity of DNA present in tail [38]. In our experiment, TM and OTM did not show any significant change in comparison to the control. However, a significant increase in both TM and OTM was recorded in MMS treated cells. Our results, thus,

Fig. 4 Influence of EMF-r exposure at 2350 MHz on mean values of (a) Head DNA (%), (b) Tail DNA (%), (c) Tail moment, and (d) Olive tail moment in *Allium cepa* root meristem cells as determined by comet assay ($n = 3$ onions). Data presented as mean $\pm$ SE. Different alphabets represent significant difference among them at $P \leq 0.05$, applying Tukey's test

demonstrate that EMF-r exposure of higher durations has a damaging effect on DNA molecule. Our observations are further supported by the findings of Mihai et al. [38], who analyzed DNA integrity in Vero cells with the help of comet assay and reported that extremely low frequency EMF-r (100 Hz and 5.6 mT) treatment of 45 min had genotoxic impact. Similar to our findings, Franzellitti et al. [20] also reported significant changes in the % TDNA in the trophoblast HTR-8/SVneo cells irradiated for 4 h, 16 h and 24 h to GSM-217 Hz and also upon exposure to GSM-Talk modulation scheme. However, tail length and TM were found to increase significantly only in cells irradiated for 16 h and 24 h to GSM-217, whereas, the cells exposed to GSM-Talk modulation scheme showed a significant increase for tail moment only when exposed for 24 h [20]. Authors have also reported recovery of the high frequency EMF-r induced alterations within incubation period (absence of radiations) of 30 and 120 min [20]. These findings are further corroborated by other studies reporting the potential role of EMF-r in inciting DNA damage to the cells [39–42].

Verschaeve et al. [43] reported an upsurge in the DNA single stranded breaks in human blood lymphocytes samples on exposure to 954 MHz radiofrequency radiations for 1–2 h. Our observations are parallel to the studies conducted by Çam and Seyhan [44] who reported a significant increase in the DNA damage in human hair root cells on EMF-r exposure of 15 and 30 min at 900 MHz. Further, the results of our experiment are corroborated by the findings of Hekmat et al. [45] who reported the toxic biological effects of 45 min exposure of mobile phone radiations at a frequency of 940 MHz on the calf thymus DNA.

The exact mechanism of DNA damage caused by EMF-r exposure is still ambiguous. However, the interaction of EMF-r with the delocalized electrons in DNA bases, leading to non-uniform flow of charge and thus resulting in DNA helix bending and initiation of transcription, is considered as one of the possible reasons for genotoxicity [46]. Moreover, it is also suggested that as radiofrequency EMF-r do not hold enough energy to directly cause breakage of chemical bonds in DNA molecules therefore, there must be some indirect mechanisms that lead to genotoxic effects. Reactive oxygen species (ROS) generated during oxidative stress cannot be ruled out as a potential factor for DNA damage. Many researchers have reported the generation of ROS in plants in response to EMF-r exposure [2, 47]. Lai and Singh [27] documented that free radical might play a role in DNA damage induced by 60 Hz magnetic field in rat brain cells. The authors also reported that free radical scavengers blocked the deleterious effects of EMF-r exposure on DNA. Earlier, De Iuliis et al. [48] demonstrated generation of ROS and consequently, DNA damage in human sperms in response to EMF-r. Other than these, disturbance in the processes of DNA repair in response to EMF-r exposure, is also suggested as a potential mechanism of DNA damage [39, 49]. Thus, it can be suggested that EMF-

r act as an abiotic stress to the plants and produce cyto- and genotoxic effects [11, 36]. Also, the results of our study have shown that the effects of EMF-r are more pronounced at the higher exposure durations. Thus, the results support the notion of health risks of higher exposure to EMF-r.

Conclusions

In conclusion, our study provided evidences that similar to MMS, mobile phone EMF-r induce chromosomal aberrations and DNA damage, particularly at longer exposures. Although the exact mechanism of action is still ambiguous, it might be important to consider that cell phone should not be used for longer duration or used with handsfree away from the body so as to avoid damage to DNA. Further, these should not be used for gratification. Nevertheless, more studies are required to explore the mechanisms of action of these radiations at molecular level. Thus, advance research is obligatory in this field for better understanding and formulation of effective protective measures for mitigation of the harmful effects of these radiations.

Acknowledgements Authors are highly grateful to Science and Engineering Research Board, Department of Science and Technology, New Delhi, India, for financial assistance.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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