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ORIGINAL ARTICLE

Appraisal of immediate and late effects of mobile phone radiations at 2100 MHz on mitotic activity and DNA integrity in root meristems of *Allium cepa*

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Abstract
The present study evaluated the potential of 2100 MHz radiofrequency radiations to act as cytotoxic and genotoxic agent. Fresh onion (*Allium cepa* L.) roots were exposed to electromagnetic field radiations (EMF-r) for different durations (1 h and 4 h) and evaluated for mitotic index (MI), phase index, chromosomal aberrations, and DNA damage. DNA damage was investigated with the help of the comet assay by assessing various parameters like % head DNA (HDNA), % tail DNA (TDNA), tail moment (TM), and olive tail moment (OTM). Effects of EMF-r exposure were also compared with that of methyl methanesulfonate (MMS; 90 μM), which acted as a positive control. The post-exposure effects of EMF-r after providing the test plants with an acclimatization period of 24 h were also evaluated. Compared to the control, a significant increase in the MI and aberration percentage was recorded upon 4 h of exposure. However, no specific trend of phase index in response to exposure was detected. EMF-r exposure incited DNA damage with a significant decrease in HDNA accompanied by an increase in TDNA upon exposure of 4 h. However, TM and OTM did not change significantly upon exposure as compared to that of control. Analysis of the post-exposure effects of EMF-r did not show any significant change/recovery. Our data, thus, suggest the potential cytotoxic and genotoxic nature of 2100 MHz EMF-r. Our study bears great significance in view of the swiftly emergent EMF-r in the surrounding environment and their potential for inciting aberrations at the chromosomal level, thus posing a genetic hazard.

Keywords Electromagnetic field radiations · Onion · Chromosomal aberrations · Genotoxicity · Recovery

Introduction

Mobile phones and the other electronic devices used in the communication systems are based on non-ionizing radiations that fall in the radiofrequency spectrum (Cermak et al. 2018). The dramatic increase in the use of mobile phones over the past few years has heightened the public concern regarding the biological effects of electromagnetic field radiations (EMF-r). Due to an unparalleled intensification of EMF-r in the environment, researchers around the world are investigating mobile phone radiations for their potential ill-effects on living organisms. From the literature, it is quite evident that there are inconclusive/questionable results regarding the bio-impacts of EMF-r (Cucurachi et al. 2013; Vijayalaxmi and Scarfi 2014; Vian et al. 2016). In general, the observed bio-effects of radiofrequency radiations have been linked to increase in the temperature upon radiation exposure (Alfieri et al. 2006; Bernardini et al. 2007; Verschaeve et al. 2010). However, several studies have demonstrated non-thermal biological effects of EMF-r (Diem et al. 2005; Friedman et al. 2007; Kivrak et al. 2017; Klonowski 2018). Plants growing in the natural environment face different kinds of stresses (salt, water, metal, pathogen, etc.) that affect their normal growth and functioning. With the unprecedented

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augmentation of EMF-r in the environment, it indeed has become quite important to assess the effects of EMF-r on plants. Moreover, plants act as an exceptional model for experimentation as they are immovable and an excellent reporter of any disturbances in the environment (Beaubois et al. 2007). Most of the earlier studies have documented that EMF-r elicit negative effects in plants (Tkalec et al. 2013; Singh et al. 2012; Stefi et al. 2017, 2018). Notwithstanding, there are a few reports demonstrating the positive impacts in plants (Jinapang et al. 2010; Bulak et al. 2018). EMF-r affect plants at morphological (Cretescu et al. 2013; Stefi et al. 2017), physiological (Kumar et al. 2016), biochemical (Singh et al. 2012), and molecular (Roux et al. 2008) levels. EMF-r have also been documented to alter the oxidative metabolism in plants (Sharma et al. 2009; Chandel et al. 2017). Recently, Stefi et al. (2018) demonstrated accumulation of secondary metabolites, decreased photosynthetic pigment content, induction of oxidative stress, and a significant rise of L-DOPA decarboxylase in myrtle leaves exposed to 1800 MHz radiations for 30 min at intervals of 48 h for 50 days.

Mobile phones use electromagnetic radiation in the microwave range (450–3800 MHz and 24-80 GHz in 5G mobile). In the past, several studies have investigated the effect of 450–1800 MHz EMF-r in plants (Tkalec et al. 2005; Roux et al. 2008; Sharma et al. 2009; Gustavino et al. 2016; Kumar et al. 2016). However, not much has been done to unravel the biological effects, including the cyto- and genotoxic effects, of 2100 MHz EMF-r in plant systems. Nucleic acid or DNA being one of the most vital cellular molecules plays various important roles in a biological system, and thus, any alterations can induce damage. Thus, the present study aimed to ascertain whether EMF-r of 2100 MHz frequency can evoke cyto- and genotoxic effects in onion (*Allium cepa* L.) root meristems.

Materials and methods

Test plant

For analysis, onion (*Allium cepa* L., $2n = 16$; *Amaryllidaceae*) was used as a test plant because of its sensitivity as well as large size of the cells with fewer numbers of easily stainable chromosomes. The effects of EMF-r exposure were compared with that of methyl methanesulfonate (MMS) which served as a positive control in the study. We procured onion bulbs of uniform size from a local market. The bulbs were set for rooting with their basal part dipped in distilled water in beakers under dark conditions at room temperature (24 ± 2 °C). Once the freshly emerged roots reached the length of 2–3 cm, they were exposed to treatments. Chemicals of analytical grade purchased from Sisco Research Laboratory (India), Hi-Media (India), and Sigma-Aldrich (USA) were used for the experiments.

Exposure system

The exposure setup involved Agilent N9310A RF signal generator (Keysight Technologies, USA) connected to an amplifier (ZHL-5W 2GX+; Minicircuits, USA) and a power supply for the generation of homogenous EMF-r of 2100 MHz. Output densities were logged with the help of Scan EM®-C Probe (CTK015; 3 M Technologies, USA) coupled with a radiofrequency (RF) power density meter (Spectran, HF-4060; Aaronia AG, Germany). The exposure system was placed in a room painted with Y-shield (HSF54) RF shielding paint. Power density and specific absorption rate (SAR) at 5 cm from the antenna were recorded to be ~489.7 ± 18.15 mW m^{-2} and 2.82×10^{-1} W kg^{-1}, respectively. Calculation of SAR on the exposed tissue directly is quite difficult (Çenesiz et al. 2011). Therefore, it was calculated roughly using the values of tissue density ($\rho = 1030$ kg cm^{-3}) and electrical conductivity ($\sigma = 1.574$ S m^{-1}) for dielectric properties of body tissue at 2100 MHz from the database of Institute of Applied Physics, Sesto Fiorentino, Italy (Andreuccetti et al. 1997).

Exposure design

Fresh onion roots (2–3 cm) were exposed to 2100 MHz EMF-r for different time durations. Onion bulbs were divided into four groups, each consisting of three bulbs. Group 1 was sham exposed, while groups 2 and 3 were exposed to 2100 MHz EMF-r for 1 and 4 h, respectively. Group 4 served as a positive control and was treated with 90 μM of MMS for 4 h. Upon exposure, half of the roots of each bulb were excised immediately, i.e., at 0 h and used for cyto- and genotoxic analyses. Afterward, the bulbs with remaining roots were then kept in a growth chamber set at 24 ± 2 °C, 80 ± 2% relative humidity, and 18/16-h light/dark photoperiod of 240 μ mol m^{-2} s^{-1} photon flux density. After an acclimatization period of 24 h, the remaining roots were excised and used for cyto- and genotoxic analyses.

Allium cepa test

For reviewing cytotoxic effects of EMF-r, various parameters like mitotic index (MI; percentage of dividing cells), phase index (percentage of cells in a particular mitotic

stage), and percentage and types of chromosomal aberrations were analyzed in onion root tips using squash technique, according to the protocol given by Armbruster et al. (1991) with slight modifications. Onion root apices were excised and immersed in a solution of ethanol: glacial acetic acid (3:1; *v*/*v*). After 24 h, roots were transferred and stowed in 70% ethanol at 4 °C. Thereafter, root apices were hydrolyzed in 1 N HCl (instead of 5 N HCl used by Armbruster et al. 1991) with sporadic heating for ~ 1 min at room temperature (25 °C). Roots were then bathed several times with distilled water followed by staining with 1% acetocarmine for 30 min (instead of Feulgen reagent used by Armbruster et al. 1991). After staining, 2–3 root tips were removed and macerated in a drop of glacial acetic acid (40%; *v*/*v*) and covered with coverslip. For each treatment, three slides (one slide per replicate) were prepared and about 800–900 cells were scored from each slide. Slides were analyzed with the help of a light microscope (Olympus CX21*i*) coupled with a camera and an LCD monitor. All the analyses were done at two stages: firstly, immediately after the exposure (i.e., 0 h), and secondly, after providing the plants with an acclimatization or recovery period of 24 h under control conditions (i.e., 24 h post-exposure).

Appraisal of DNA damage

The genotoxic potential of EMF-r was assessed in terms of the effect on DNA integrity with the aid of the comet assay. We followed the technique given by Tice et al. (2000). Prior to the comet assay, glass slides (with one-fourth frosted ends) were coated with 1% normal melting point agarose dissolved in a phosphate buffer saline (PBS, pH 7.4) at 50 °C. Onion root tips were then sliced gently in PBS (pH 7.4) over an ice base to isolate nuclei. Fifty microliters of 1% low melting point agarose in PBS was mixed with a nuclei suspension (100 μl) at 37 °C and pipetted over agarose-coated slides. The slides were then covered with coverslips and placed over an ice base. After 10 min, coverslips were removed and slides were placed in a lysis solution for 1 h under dark conditions. Afterward, slides were sited in an electrophoresis tank holding electrophoretic buffer (pH ≥ 13, 300 mM sodium hydroxide and 1 mM ethylenediaminetetraacetic acid) for 30 min. These were incubated to enable unwinding of DNA prior to electrophoresis (at 300 mA, 25 V for 25 min). Thereafter, electrophoresed slides were rinsed three times with distilled water and stained with ethidium bromide (20 μg ml^{-1}) for 5 min under dark conditions. Slides were then dipped in cold water to remove excess stain followed by covering the slides with coverslips. The stained slides were examined under a fluorescence microscope (Nikon Eclipse 80*i*) at an excitation filter of BP 546/10 nm and a barrier filter of 590 nm. A total of three slides (1 slide per replicate/bulb) were made for each treatment and about five roots per onion bulb were used for preparing each slide. For each treatment, almost 150 nuclei were scored.

DNA damage was quantified employing a computerized image analyzing software (CASP 1.2.3b; comet assay package) and four different parameters: %HDNA (percentage of DNA in head), %TDNA (percentage of DNA in tail), TM (TDNA × tail length), and OTM (a product of TDNA and distance between the intensity centroids of the head and tail along *x*-axis of the comet), were determined.

Statistical analysis

The treatments were organized in a completely randomized manner. For each treatment, three onion bulbs were maintained, each representing one replicate. Data were represented as mean ± SE. Analysis of data was done using one-way ANOVA and means were separated using post hoc Tukey's test at the significance level of $P \leq 0.05$. Student's *t* test was used for determining the statistical significance between the two sets, 0 h and 24 h post-exposure, at $P \leq 0.05$. All the statistical analyses were performed using SPSS ver. 16.

Results

Putative cytotoxic and genotoxic effects upon exposure to mobile phone radiations at a frequency of 2100 MHz were evaluated in root tips of onion in terms of the mitotic index (MI), frequencies of different chromosomal aberrations, phase index, and DNA damage (Figs. 1, 2, and 3).

Exposure to 2100 MHz EMF-r increased the number of dividing cells in the onion root meristems (Fig. 1a). Immediately after exposure for 4 h (i.e., 0 h post-treatment), MI increased by 25% over the control. However, exposure for a shorter duration, i.e., 1 h, did not cause any significant difference in the values of MI between the exposed and control samples. In root meristems treated with MMS, MI showed a significant decrease of ~ 45% over that in the control (Fig. 1a).

Exposure to EMF-r also induced a number of chromosomal aberrations in the root meristem of onion (Figs. 1b, 2). These included vagrant and laggard chromosomes, mitotic bridge, stickiness, and chromosomal fragments (Fig. 2). Among these, in EMF-r-treated groups,

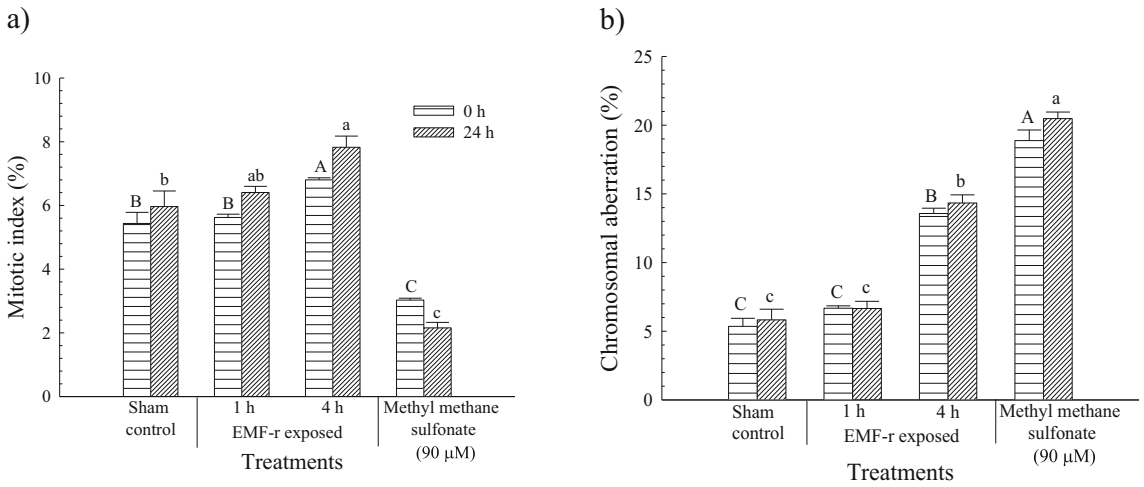

Fig. 1 Mitotic index (**a**) and % chromosomal aberrations (**b**) in root meristem cells of *Allium cepa* exposed to 2100 MHz electromagnetic field radiations (EMF-r), determined at 0 h and 24 h post-exposure. Data presented as mean ± SE ($n = 3$). Different alphabets (upper case at 0 h and lower case at 24 h post-exposure) represent significance among treatments at $P \leq 0.05$, applying Tukey's test

stickiness and *c*-mitosis were the most frequent aberrations, whereas *c*-mitosis and chromosomal fragments were observed most frequently in MMS-treated group (Table 1). Analysis of the root tips cells showed that there was an increase in the count of aberrant cells with an increase in the duration of exposure (Fig. 1b). Percent chromosomal aberrations increased by ~1.52-fold in response to EMF-r exposure of 4 h, when determined immediately after treatment, compared to the control. Similarly, ~2.5-fold increase in chromosomal aberrations

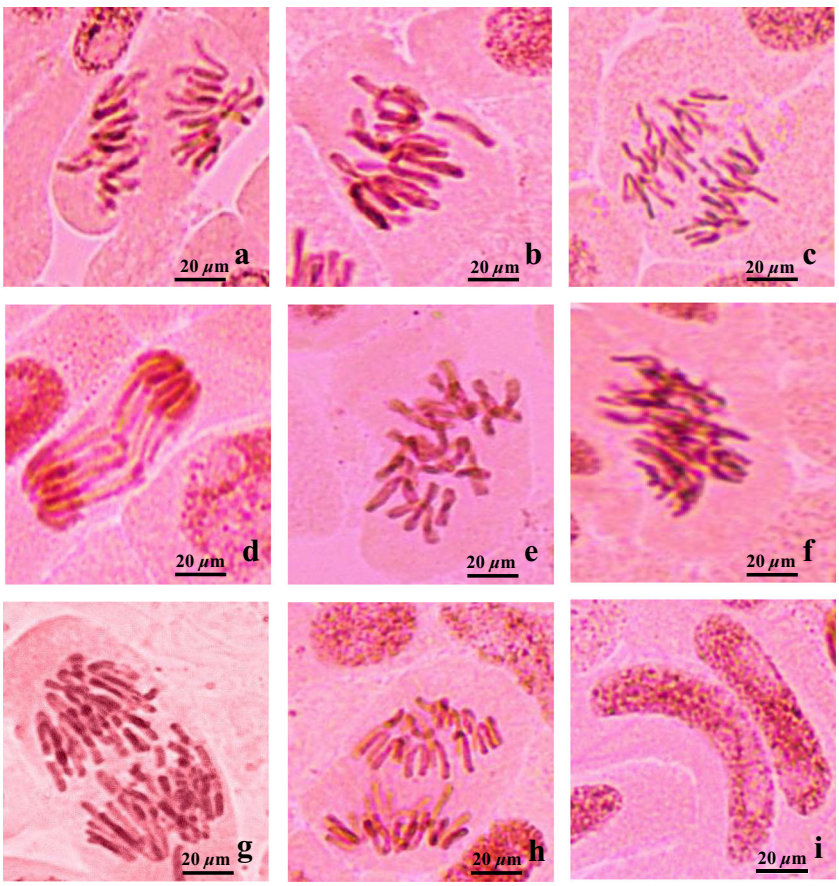

Fig. 2 Types of chromosomal aberrations in root meristem cells of *Allium cepa* exposed to 2100 MHz electromagnetic field radiations (EMF-r). **a** Vagrant, **b** laggard, **c** vagrant and laggard, **d** mitotic bridge, **e** *c*-mitosis, **f** stickiness, **g** chromosomal fragment, **h** spindle disturbance, **i** morphological alteration in the cell. Bars represent 20 μm

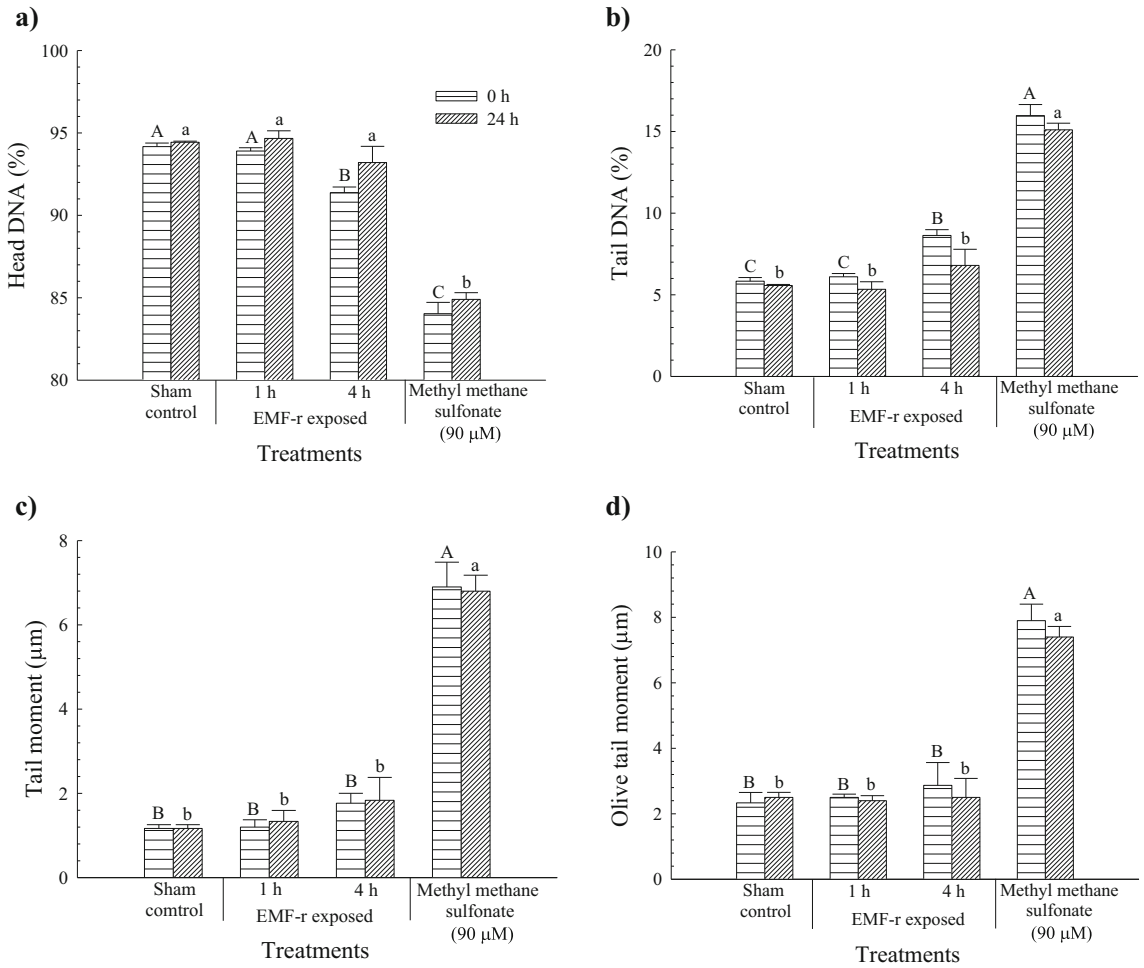

Fig. 3 DNA damage in root meristem cells of *Allium cepa* exposed to 2100 MHz electromagnetic field radiations (EMF-r). **a** Head DNA (%), **b** tail DNA (%), **c** tail moment (μm), and **d** olive tail moment (μm). For the explanations for the statistical analysis, see Fig. 1

was observed when onion roots were treated with MMS (Fig. 1b). However, no specific changes in the phase index were observed in response to the treatment of 2100 MHz EMF-r (Table 2).

At 24 h post-exposure, a similar trend of changes was observed in MI and chromosomal aberrations. However, the differences were found to be insignificant compared to the observations made immediately after exposure, i.e., 0 h (Fig. 1a, b).

Table 1 Chromosomal aberrations in root meristems of *Allium cepa* exposed to 2100 MHz electromagnetic field radiations (EMF-r), determined at 0 h and 24 h post-exposure

Treatment		Chromosomal aberrations						
		Vagrant	Laggard	Mitotic bridge	*c*-Mitosis	Stickiness	Chromosomal fragment	Spindle disturbance
Sham control	0 h	–	–	–	2	4	–	1
	24 h	1	–	1	3	3	–	1
EMF-r exposed								
1 h	0 h	1	–	–	3	3	–	2
	24 h	1	1	3	2	2	–	2
4 h	0 h	2	1	3	4	6	3	3
	24 h	3	2	2	4	9	2	5
Methyl methanesulfonate (MMS; 90 μM)	0 h	1	2	1	3	2	3	2
	24 h	–	1	1	2	3	1	2

Table 2 Phase index in root meristems of *Allium cepa* exposed to 2100 MHz electromagnetic field radiations (EMF-r), determined at 0 h and 24 h post-exposure

Treatment	Phase index (%)							
	Prophase		Metaphase		Anaphase		Telophase	
	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Sham control	53.1 ± 0.47a	40.2 ± 0.55c,*	21.5 ± 0.31b	34.3 ± 0.66a,*	17.7 ± 0.47c	16.4 ± 0.63c	7.7 ± 0.53b	9.1 ± 0.84c
EMF-r exposed								
1 h	43.7 ± 0.75b	47.9 ± 0.82a	24.4 ± 0.66a	21.2 ± 0.50b	20.0 ± 0.51bc	24.9 ± 0.54a,*	11.9 ± 0.79a	6.0 ± 0.43d,*
4 h	45.7 ± 0.62b	42.4 ± 0.68bc	21.6 ± 0.32b	19.5 ± 0.57b	20.3 ± 0.63ab	21.2 ± 0.43b	12.4 ± 0.85a	16.9 ± 0.44a
Methyl methanesulfonate (MMS; 90 μM)	44.5 ± 0.82b	44.8 ± 1.10ab	20.3 ± 0.74b	20.5 ± 0.48b	22.9 ± 0.66a	20.5 ± 0.48b	12.2 ± 0.45a	14.2 ± 0.48b

Data presented as mean ± SE ($n = 3$). Different letters in a column represent a significant difference at $P \leq 0.05$, applying Tukey's test

*Represents significance between 0 and 24 h post-exposure at $P \leq 0.05$, according to Student's *t*-test

DNA damage (analysis of genotoxicity)

DNA integrity in root meristematic cells of onion irradiated to EMF-r at 2100 MHz for 1 h and 4 h was investigated immediately after the treatment and after acclimatization, i.e., at 0 and 24 h post-exposure, respectively, with the help of the comet assay. The analysis was done in terms of %HDNA, %TDNA, TM, and OTM (Fig. 3). Immediately after exposure (0 h), with an increase in the duration of exposure, a decrease in %HDNA accompanied by an increase in %TDNA was observed. HDNA decreased significantly in the groups exposed for 4 h to EMF-r, and MMS by 2.97% and 10.83%, respectively, as compared to the control (Fig. 3a). Similarly, in comparison to control, TDNA increased significantly by ~ 0.48-fold in the roots irradiated to EMF-r for 4 h and by ~ 1.76 fold upon MMS treatment (Fig. 3b). Compared to the control, no significant differences in TM and OTM were observed in EMF-r exposed groups (Fig. 3c, d). However, in MMS-treated cells, TM and OTM increased significantly by ~ 4.75- and ~ 2.43-fold, respectively, with respect to control (Fig. 3c, d). Analysis of the DNA integrity done at 24 h post-treatment revealed no significant difference in the values of HDNA, TDNA, TM, and OTM over that in 0 h stage post-exposure (Fig. 3a–d).

Discussion

The current study examined the potential of mobile phone EMF-r at a frequency of 2100 MHz to incite cytotoxic and genotoxic effects in *A. cepa* root meristems exposed for different time periods (1 and 4 h). We observed an upsurge in the count of dividing cells in a dose-dependent manner in the EMF-r-exposed samples, thereby suggesting alterations in mitotic rate. The effect was more pronounced in root meristems exposed to EMF-r for longer durations (4 h). Our findings are corroborated by earlier studies reporting increased MI and chromosomal aberrations in *A. cepa* root meristems upon exposure to 400 MHz (Tkalec et al. 2009) and 900 MHz EMF-r (Tkalec et al. 2009; Pesnya and Romanovsky 2013). This abnormal mitotic activity could be accredited to spindle impairment upon EMF-r treatment (Prokhorova et al. 2008; Tkalec et al. 2009) or be an after effect of delayed mitosis (Tkalec et al. 2009).

EMF-r exposure incited different chromosomal aberrations such as vagrant and laggard chromosomes, *c*-mitosis, mitotic bridge, chromosome stickiness, fragments, and spindle disturbances, in onion meristematic cells. Among these, stickiness and *c*-mitosis were the most frequent ones observed in EMF-r-exposed cells. The occurrence of chromosome stickiness could be ascribed to the denatured nucleoproteins or degraded/depolymerized DNA upon EMF-r treatment, which lead to chromosomal clumping (Kumar et al. 2003; Choudhary et al. 2012). Similarly, *c*-mitosis may be a consequence of microtubule disassembly (Rieder and Cole 2000). Thus, the occurrence of different chromosomal abnormalities could be attributed to spindle disturbances incited by EMF-r exposure (Pesnya and Romanovsky 2013). EMF-r have also been reported to increase micronuclei frequencies in *Vicia faba* (Gustavino et al. 2016) and *Tradescantia* (Haider et al. 1994).

The impact of EMF-r on DNA integrity in onion root meristematic cells was investigated with the help of comet assay in terms of HDNA, TDNA, TM, and OTM. The comet assay is one of the most sensitive techniques used to investigate DNA damage at the primary level (Tice et al. 2000). The observations made from the present study indicate that 2100 MHz EMF-r have a potential to disturb the DNA integrity in *A. cepa* root meristems as indicated by a significant decrease in HDNA along with an increase in TDNA. TDNA indicates the content of damaged DNA in a cell. Migration of DNA from the nucleus leads to the formation of the tail. However, TM and OTM did not show any statistically significant effect upon EMF-r exposure. Our results are supported by many of the earlier studies. For example, TDNA increased significantly in

trophoblast HTR-8/SVneo cells exposed to GSM-217 Hz and GSM-Talk modulation for 4–24 h (Franzellitti et al. 2010). However, a significant increase in TM and OTM was found only in cells exposed for ≥ 16 h to GSM-217, and 24 h to GSM-Talk modulation scheme (Franzellitti et al. 2010). In agreement with the results of our study, toxic biological effects were reported in the calf thymus DNA in response to 940 MHz EMF-r exposure of 45 min (Hekmat et al. 2013). Similarly, single-stranded DNA breaks were observed in human blood cells irradiated to 954 MHz EMF-r for 1–2 h at a SAR of 1.5 W kg^{-1} (Verschaeve et al. 1994). Our results are further supported by the findings of Çam and Seyhan (2012), who reported DNA damage in human root hair cells on exposure to 900 MHz EMF-r with SAR of 0.974 W kg^{-1} for a short duration of 15 and 30 min. Nevertheless, both the frequency and the exposure durations, used by the authors, were very low as compared to the ones used in the present study.

From the literature, it is relatively apparent that the exact mechanism of interaction between a plant and EMF-r is still abstruse, although many researchers have tried to explain the genotoxic effects of EMF-r. Blank and Goodman (2011) suggested that EMF-r interacts with the delocalized electrons of DNA bases and results in the non-uniform flow of charge leading to bending of the DNA helix and initiation of transcription. Oxidative damage is also considered as one of the possible reasons for DNA damage as researchers hold the consensus that there must be some indirect mechanism of DNA damage upon EMF-r exposure since these radiations do not hold enough energy required to directly break a chemical bond (Vian et al. 2016). Aitken et al. (1993) and Agarwal and Saleh (2002) suggested that reactive oxygen species (ROS) may be responsible for the damage of sperm DNA and some other biomolecules (lipids and proteins) resulting into male infertility. Similarly, De Iuliis et al. (2009) documented the production of ROS and DNA damage in human spermatozoa upon EMF-r exposure. Lai and Singh (1997) pointed out the role of free radicals in inducing DNA damage upon EMF-r exposure and found that such genotoxic effects could be blocked by the compounds with free radical scavenging properties. Along with these, interference of EMF-r with the DNA repair process has also been suggested as a possible mechanism of DNA damage upon EMF-r exposure (Phillips et al. 1998; Sykes et al. 2001). However, in our study, we could not make out the exact reason for the observed biological effects of EMF-r.

Through these experiments, we also tried to investigate the extent of recovery shown by the plants exposed to EMF-r. DNA repair mechanisms are of utmost importance for the survival as well as to upkeep different cell functions. Moreover, different cells hold different repair capabilities because of genetic differences (Franzellitti et al. 2010). Therefore, in the current experiment, after treatment, plants were allowed to recover under control conditions in the absence of radiation for 24 h. After 24 h post-recovery, although slight signs of recovery were observed; however, these were not significant, thereby suggesting that alterations persisted with time after exposure.

Conclusions

It is apparent from the present study that radiofrequency radiations at 2100 MHz have a potential for inciting cytotoxic and genotoxic effects in *A. cepa* root meristems. The observed biological effects were dependent on the duration of exposure, and the maximum alterations were found upon 4 h of exposure. As the concentration of EMF waves by man-made devices like mobile phones is increasing in the environment at a very fast rate, we cannot afford to ignore the biological effects of these radiations. Our study holds great significance in view of this rapidly emergent EMF-r in the surrounding environment and their potential for inciting aberrations and damage at the chromosomal level, thus posing a genetic hazard. The study calls for a proper risk assessment in terms of impacts on the environment and public health of the increasing electromagnetic smog and development of strategies to reduce EMF-r pollution in the natural environment.

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Compliance with ethical standards

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

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