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Cytotoxicity and Genotoxicity of Radiofrequency Electromagnetic Field in Onion Root Tip Cells and Mouse Polychromatic Erythrocytes

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ABSTRACT

Introduction: Mobile phone users and base stations have increased exponentially in recent decades. These expansions have extended worries about the potential risk of long-lasting Radiofrequency Electromagnetic Fields (RF-EMF) exposure on human health and environmental quality. The current study was designed to explore the cytogenetic consequences of subjecting two biological systems to RF-EMF at a frequency of 1800MHz and a specific absorption rate of 0.27 W/kg.

Material and Methods: Chromosome aberration test (onion meristematic cells) and micronucleus assay (mouse erythrocytes) were used to evaluate the potential cytotoxic and genotoxic effects of the in vivo exposure to RF-EMF at a frequency of 1800 MHz. The two living systems were subjected to RF-EMF for 0, 0.5, 1, 2, and 4 hours daily for seven successive days. We recorded the percent aberrant cells (%Abc), the percentage of micronuclei formation in erythrocytes (%MN), and the percentage of micronucleated polychromatic erythrocytes (%MNPCE).

Results: It was demonstrated that the short- and intermediate-term exposure to RF-EMF may cause a gradual time-dependent boost in root growth. However, significant growth inhibition was observed following 4-hour exposure. Exposure to RF-EMF did not change mitotic indices of onion meristematic cells. Significant increases in Abc, MN, and MNPCE percentages were recorded.

Conclusion: The outcome of this study proposes that unlimited exposure of living organisms to RF-EMF may lead to adverse effects. Therefore, unnecessary use of mobile phones should be avoided.

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Introduction

With the emergence of the fourth and fifth generations (4G and 5G) network technologies, there has been a rapid growth in the use of smartphones. The percentage of households with internet access increased from 18% in 2005 to 46% in 2015 [1]. As of 2022 report [2], there are 8.6 billion mobile phone subscriptions worldwide, averaging 107.5 per 100 people. To meet the growing communication demands, the global number of base stations installed is about 6.5 millions. Most of the reported studies correlate the adverse effects with pulse-modulated signals employed in the second-generation (2G) global system for mobile communication (GSM) or in the wideband code-division multiple access (WCDMA)/3rd Generation Universal Mobile Telecommunication system (WCDMA/3G UMTS) [1,2]. The 1800 MHz band provides an attractive option for network operators and device constructors. Much less is known regarding the biological influences of radiofrequency (RF) signals (1800 MHz) utilized in the 4G Long-term evolution (LTE) mobile services [1-3].

Though phone companies, regulatory agencies, and service providers warrant the safety of mobile phones, there are overwhelming debates and controversies over the health risks of these products [3]. An absence of proof does not mean a lack of consequence but rather the existence of misconduct that monopolizes evaluation to reinforce the no-risk paradigm. Another source of the negative findings is the shortage of the appropriate measurement of parameters necessary to draw conclusive conclusions.

Various body tissues absorb electromagnetic radiation (EMR) according to the places where the mobile is carried, especially the liver and the kidney [4,5]. Practically, the degree of absorption and spreading of EMF in tissue differs depending on several factors, such as relative water content of the tissue, dielectric properties and transmission of the tissue, as well as duration and frequency of the phone used, shape, geometry, distance from the source, presence of obstacles, etc.) [6,7].

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The biological effects of exposure to RFR range from impact on behavior, memory, movement, and reproduction to the products on the molecular structure and function [8-10]. Even if the exposure is below the threshold levels of RF-EMF estimated by health organizations [11], it is predominantly contemplated that mobile communication technology can exert subtle, non-thermal alterations on the living tissues [12, 13]. The exact mechanism of action of EMR on biological systems is not fully understood. The production of reactive oxygen species (ROS) has been suggested as a possible mechanism of the non-thermal effects of RFR-EMF on living organisms [14]. Scientists found that low-intensity microwave radiation emitted by mobile phones caused DNA damage in the liver cells of rats, as indicated by increased levels of the biomarker 8-Hydroxy-2'-deoxyguanosine (8-OHdG) [15,16].

In the current study, we examined the cyto- and genotoxic potential of mobile phone RF-EMF at 1800 MHz using onions (*Allium cepa* L.) and Balb/C mice. One disadvantage of plant and animal models is that their results are not directly applicable to humans. On the other hand, however, they rule out the psychological effect, which suggests that people develop symptoms only because they are anxious by the antenna masts they can see [6]. We hope this research will help provide additional data to the existing ones in monitoring and regulating the wise usage of mobile phone applications.

Materials and Methods

Chemicals

Phosphate-Buffered Solution (PBS) of Colcemid (CAS number 477–30-5) was obtained from Life Technologies/Gibco (USA).

Exposure settings

The exposure settings in the present experimental design were simulated to be close to human everyday mobile use (LG model G7 thin Q, SAR 0.244 W/kg)

Plant study

The method was reported before [17]. Briefly, the base of the onion (*A. cepa*) bulb (25 – 30g) was submerged in tap water and placed in a 50 ml glass beaker. When the roots reached a length of 1 – 1.5 cm, while intact on the bulb, the cups were arranged on top of a plastic circular tray (27.5 cm in diameter) at a distance of 7.5 cm from its center (Fig. 1a). The rooted bulbs were randomly divided into five groups (5 bulbs each). Group 1 served as sham-control (bulbs in baker, mobile phone jammer switched off; no EMF to evaluate possible stress factors. Groups 2 to 5 were exposed to an EMF transmitter at 1800 MHz and SAR value of 0.27 W/ kg for 0.5, 1.0, 2.0, and 4.0 h daily for seven consecutive days. Experimental practices were carried out in the same period of the day between 9:00 a.m. and 1:00 p.m. to ensure uniform exposure; the tray was rotated clockwise every 24 hours so that it returned to its starting position by day seven.

At the end of the exposure period, the mean root length of the sham-control was considered 100%, and the percent change in average root length resulting from exposure to RF-EMF was calculated.

For cytogenetic analysis, chromosome spreads from root tips were prepared as described previously [17]. The root tips were harvested and transferred to Petri plates for a 2.5-h pretreatment with 0.05% Colcemid solution (Colcemid 0.5% stock solution: PBS; 1:9) in the dark at (25 ± 0.5) ºC. Then, root tips were fixed in a freshly prepared Carnoy solution (absolute ethanol and glacial acetic acid; 3:1). Cells were stained with 2% aceto-carmine at 25 ºC. The Mitotic Index (MI) was determined by calculating the percentage of dividing nuclei among 2,000 screened nuclei from the control and the experimental treatments. Five hundred well-defined mitotic meristematic cells from the control and experimental treatment were examined for chromosome abnormalities in the same preparations, and the percent of cells carrying these abnormalities (%Abs) was determined.

Animal study

Ethical approval

All procedures and experiments followed the guidelines established by the European Communities Council Directive (2010/63/EU Council Directive Decree). The protocols were approved by the Institutional Animal Care and Use Committee at Yarmouk University (IACUC/2021/4).

Calculation of Specific Absorption Rate

In the present study, we quantified the average SAR value from EMF using the following expression [18].

$$SAR = \frac{p}{\rho} = \frac{\sigma E^2/2}{\rho} = \frac{\sigma E^2}{2\rho} \quad (1)$$

Where σ=Tissue electric conductivity, ρ=Mass density, and E=Peak value of an internal electric field

$$E = \sqrt{S\eta} \quad (2)$$

Where S=Power density=PtGt/(4πd^2), Pt=Transmitted power from the jammer =10dBm

Gt=Gain of transmitted antenna =9dB

$$\eta = \mu o \sqrt{\frac{\mu_r}{\varepsilon_r}} \quad (3)$$

Where η: the medium impedance, μo: The free space impedance 377 Ω, μ_r: the relative permeability, ε_r:The dielectric constant

A comparison was made between SAR levels in a human head exposed to a handset operating at 1800 MHz (250 mW output power and those obtained in the mouse tissues with a 100-mW input power at the antenna's connector. In this case, the simulation showed that the ratio of the maximum local SAR in the mouse versus the human was 1.3/0.6 sPI plusmn in the brain and 1.0/0.5 sPI plusmn in the skin, respectively.

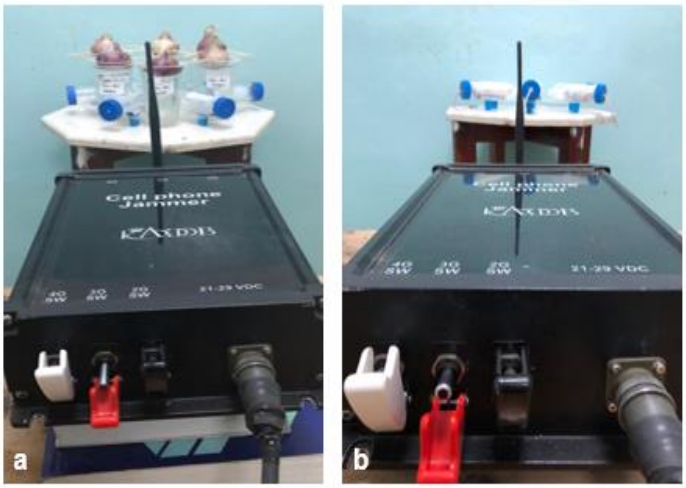

Figure 1. The mobile phone jammer and the circular plastic tray show the experimental setup and the principle of exposure to mobile phone electromagnetic radiofrequency field at 1800 MHz. and a SAR value of 0.27 W/ kg. a. Each onion bulb is placed in the mouth of a 50 ml glass baker. b. Each mouse was placed inside a separate perforated 50 ml polypropylene tube with the head oriented towards the center

Experimental design

Twenty-four Balb/C male mice (six weeks old, average weight 25g/mouse) raised under standard animal house conditions were used. Mice were randomly divided into six groups (4 animals per group). Each mouse was individually positioned in a 50 ml polypropylene tube fixed on a circular plastic tray in the complete absence of any unwanted source of EMF (Figure 1b). Each tube had circular drilled ventilation holes 3 mm in diameter at all sides to reduce the stress, allow air passage, and prevent overheating. The animals' heads were at an equal distance (7.5 cm) from the center of the experimental tray, which was 1 cm above the level of the transmitter. Experimental animals (groups 3 to 6) were exposed to RF-EMF generated from the antenna of an EMF transmitter at 1800 MHz and SAR value of 0.27 W/ kg) for 0.5, 1.0, 2.0, or 4.0 hours daily for seven consecutive days. Exposure sessions were accomplished at a fixed time every day. A clockwise rotation of the experimental tray was performed daily to ensure uniform exposure.

Animals in the first group (the control) remained in a plastic cage without any unwanted source of EMF. Animals in the second group were used as a sham-control (mouse in a tube, on the plastic tray, while the mobile phone jammer was switched off; no EMF for possible stress factors).

After the termination of the experiment, peripheral blood was collected, smeared, and stained as described previously [19]. Under blind code, cytotoxicity was followed by determining the percentage of polychromatic erythrocytes (%PCE) in a sample of 2000 red blood cells. As an indicator of in vivo genotoxicity, 2000 PCE per mouse were screened for the presence of MN, and the percent of micronucleated PCE (% MNPCE) was recorded.

Statistical analysis of data

The results were evaluated using SPSS version 25 (IBM Inc., USA). An independent Student's t-test was used for plant data to determine the significance of the standards. One-way ANOVA analysis was used to find significant differences between the means of % MN and % MNPCE at different exposure times. LCD-Post hoc test for multiple comparisons was followed to evaluate differences between the control groups and the other exposure times. A P -value < 0.05 was considered significant.

Results

The results of the seven-day RF-EMF exposure of *A. cepa* roots growth are shown in Table (1) as well as in Figure 2 and Figure 3a. Compared to the control (18.7 ± 0.87 cm), there were progressive increases in the average root length and reached a statistically significant maximum at 2-h exposure, where the mean of root length was 26.7 ± 1.76 cm. Roots exposed to four hours had the shortest roots (15.0 ± 0.00 cm). Exposure of roots to RF-EMF for 0.5, 1.0, or 2.0 daily for seven continuous days did not result in any regular or significant (P <0.05) increases in mitotic activity of onion root tip meristematic cells, in comparison to the control group (Table 2 and Figure 3b). Only exposure of the onion roots for four h reduced, but not significantly, MI in the meristem tissue of onion.

Following EMF exposure at various periods for seven consecutive days, at all stages of cell division, we encountered both clastogenic aberrations like bridges and fragments as well as physiological abnormalities such as chromosome stickiness, c-mitosis, laggards, and vagrants (Figure 4). Although short and intermediate exposure times did not cause significant elevations in the % of aberrant root meristem cells, a ~2.0-fold increase in this percentage over the sham-control was recorded following exposure to RF fields for four hours (8.8% versus 14.1%, respectively) (Table 3 and Figure 3c). The most commonly observed

types of CAs were chromosome fragmentation and chromosome stickiness (Table 3).

Table 1. Percent change in onion root growth measured after exposure to radiofrequency electromagnetic field at 1800 MHz for different durations for seven consecutive days. Five onion bulbs were used for each exposure time

Exposure Time (hour/day)	Average Root Length (cm) Mean ± SEM	% Change in Root Length	*P*-value*
0.0 (Sham control)	18.67±0.866	-	-
0.5	20.33±0.882	+ 8.89	0.208
1.0	24.00±0.000	+ 28.55	0.002
2.0	26.67±1.764	+ 42.85	0.000
4.0	15.00±0.000	- 19.66	0.028

* Independent Student's t-test at 95% confidence interval.

Figure 2. Representation of the onion root growth in response to exposure to electromagnetic radiofrequency field at 1800 MHz from left to right: 0, 0.5, 1.0, 2.0, and 4.0 h every day for seven consecutive days

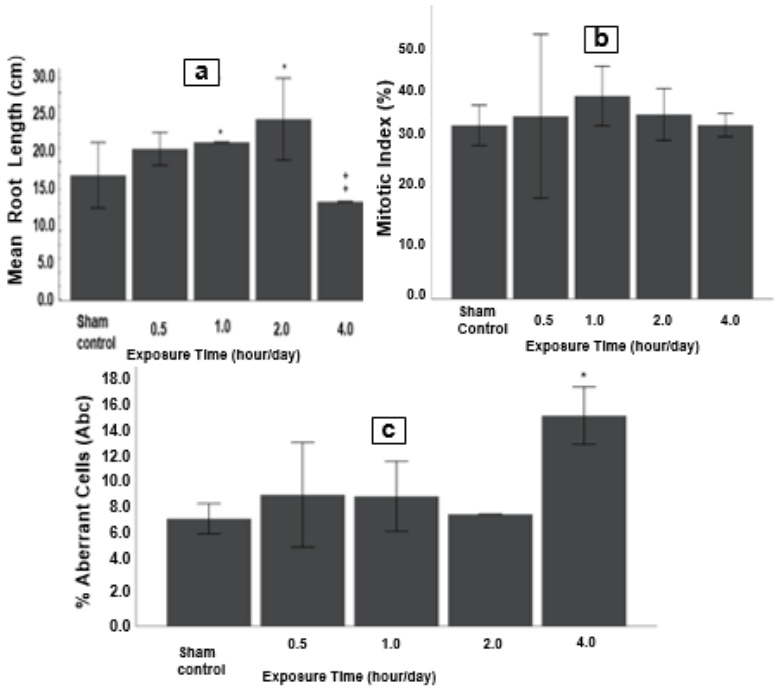

Figure 3. The effect of onion (A. cepa) roots exposure to electromagnetic radiofrequency field at 1800 MHz at the specified daily time for seven consecutive days. a. Average length of onion roots. b. Percent MI. c. Percent aberrant cells. * Significantly higher at p-value < 0.05. ‡ Significantly lower at p-value < 0.05. Data represent means ± SEM for groups of 5 bulbs.

Table 2. Cytotoxic response of onion root meristem cells to exposure to to radiofrequency electromagnetic field at 1800 MHz for seven consecutive days. For each control and experimental treatment, 2000 nuclei were screened for mitotic division

Exposure Time (Hour/day)	Mitotic Index ± SEM	% Change in Mitotic Index	*P*-value*
0.0 (Sham Control)	28.75±2.69	-	-
0.5	30.25±10.95	5.21	0.673
1.0	33.60±3.98	16.87	0.184
2.0	30.55±3.47	6.26	0.617
4.0	28.80±1.54	0.17	0.982

* Independent Student's *t*-test at 95% confidence interval.

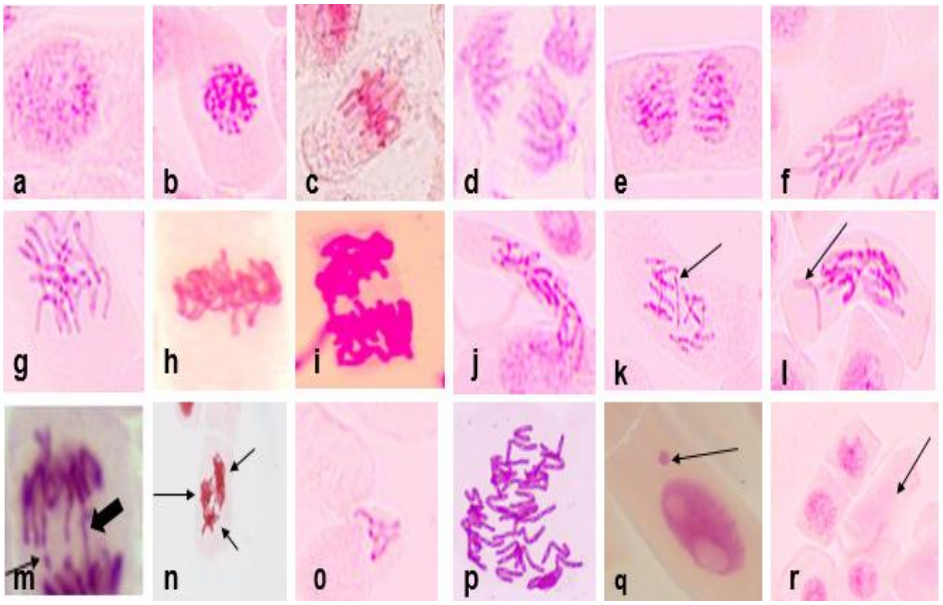

Figure 4. Photomicrographs of squash preparations from root mitotic meristem cells of onion (*Allium cepa* L.; 2n=16) stained with aceto-carmine. Typical phases of mitosis: a-e: interphase, prophase, metaphase, anaphase, and telophase, respectively. Representative chromosome aberrations observed following exposure to electromagnetic radiofrequency field at 1800 MHz are shown. C-mitosis (2n=32) (f); spindle disturbance at metaphase (g); sticky metaphase (h); sticky anaphase (i); extended metaphase (j); laggard chromosome (arrow) at metaphase (k); vagrant chromosome (arrow) (l); anaphase bridge (thick arrow) and chromosome fragment (thin arrow) (m); tripolar cell (n); hypoploid (aneuploid) cell (o); polyploid cell (p); interphase micronucleus (q); enucleated (ghost) cell (r). Magnification: 1000 X

Table 3. Genotoxic response of onion root meristem cells to exposure to to radiofrequency electromagnetic field at 1800 MHz for seven consecutive days. For each control and experimental treatment, 2000 nuclei were screened for chromosome aberrations

Exposure Time (h/day)	Type of Chromosome Aberration												TNA	% Abc	*P*-value**
	AB*	CF	CM	EM	LC	MN	RC	SC	PC	TC	VC	Others			
0.0 (Sham Control)	-	60	-	-	-	-	4	16	-	-	-	64	144	7.2	-
0.5	-	56	2	2	2	2	-	48	-	2	-	62	176	8.8	0.154
1.0	6	80	8	-	-	-	2	28	6	-	-	44	174	8.7	0.180
2.0	1	60	2	2	-	2	1	37	-	-	-	45	150	7.5	0.784
4.0	8	100	9	2	4	-	1	36	4	-	2	116	282	14.1	0.000

* % Abc: Percent aberrant cells; AB: Anaphase bridge; CF: Chromosome fragment, CM: c-mitosis; EM: Extended metaphase, L: Laggard chromosome; MN: Micronucleus; P: Polyploid cell; RC: Ring chromosome; Sticky chromosome; TNA: Total number of aberrations; Tripolar cell; V: Variant chromosome. ** Independent Student's t-test at 95% confidence interval.

Table 4. Frequency of micronuclei, percent of micronucleated polychromatic erythrocytes, and echinocytes peripheral blood from Balb/C mice exposed to radiofrequency electromagnetic field at 1800 MHz. At the specified daily exposure time for seven consecutive days. Five animals were used for each exposure time

Exposure Time	MN* Mean ± SEM	% MN Mean ± SEM	MNPCE Mean ± SEM	% MNPCE Mean ± SEM
Unexposed Negative Control	32.92 ± 4.31	1.65 ± 0.22	7.67 ± 0.26	0.38 ± 0.01
Sham-exposed control	35.50 ± 0.65**	1.78 ± 0.03	10.50 ± 0.19	0.53 ± 0.01
0.5 h	44.40 ± 7.36	2.22 ± 0.37	14.80 ± 2.95	0.74 ± 0.15 [a]
1.0 h	48.27 ± 8.93	2.41 ± 0.45	12.67 ± 1.87	0.63 ± 0.09
2.0 h	39.27 ± 3.56	1.96 ± 0.18	14.40 ± 2.72	0.72 ± 0.14 [a]
4.0 h	78.33 ± 11.72	3.92 ± 0.59 [a]	17.00 ± 2.53	0.85 ± 0.13 [a]

* MN: Micronuclei/Micronucleated; NCE: Normochromatic erythrocytes; PCE: Polychromatic erythrocytes.
** Data from four animals only.
*** a Statistically significant compared with the control groups ($P < 0.05$). b Significant relative to the unexposed control ($P < 0.05$).

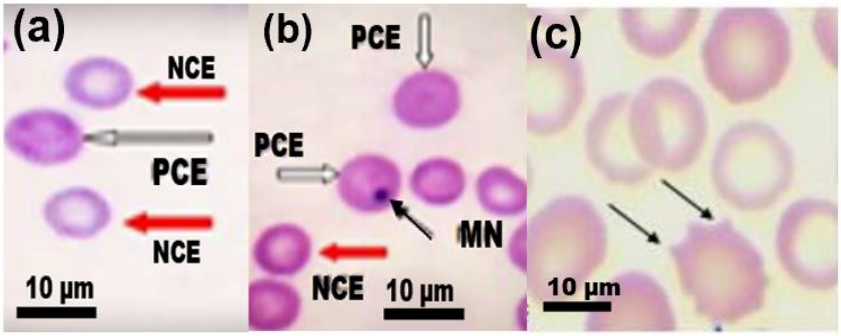

Figure 5. Photomicrographs of the peripheral blood erythrocytes of Balb/C mice exposed to electromagnetic radiofrequency field at 1800 MHz for different exposure times. (a) White arrow: polychromatic erythrocyte (PCE) and red arrow: normochromatic erythrocytes (NCE). (b) PCE with a micronucleus (MN). (c) An echinocyte (Burr cell) with characteristic morphological appearance; speculated border over the entire cell surface: Hematoxylin and Giemsa stain. Magnification: 1000 X

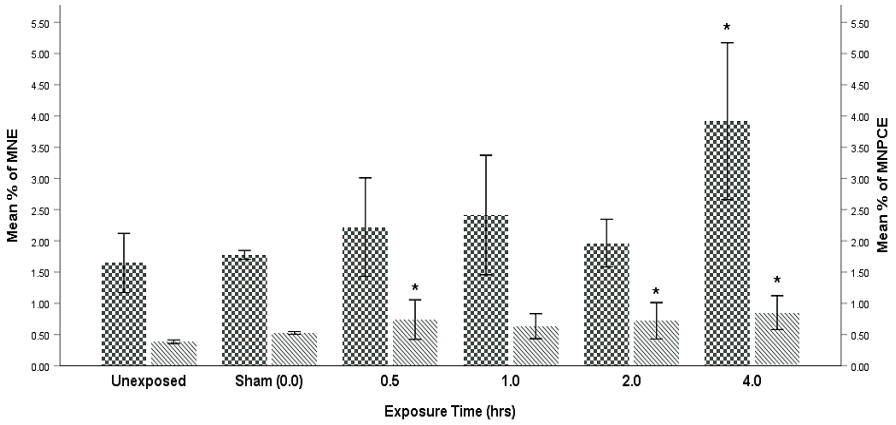

Figure 6. Mean percent of micronuclei and percent of micronucleated polychromatic erythrocytes in peripheral blood from Balb/C mice exposed to the electromagnetic field at 1800 MHz. at the specified daily exposure for seven consecutive days. Data represent means ± SEM for groups of 4 animals.

At the end of the experiments, all mice appeared normal. Photomicrographs of NCE and PCE with MN are shown in Figure (5a and 5b). Although most cells exhibited a single micronucleus, two micronuclei were encountered in some cells. Figure 6 and Table 4 show the percentage of MN and MNPCE in the blood of different animal groups. ANOVA analysis followed by Post Hoc tests of MNPCE for multiple comparisons demonstrated that, relative to the control groups, significance values of the percentage of MN of mice exposed to EMF for four hours.

On the other hand, except for the one-hour- exposure period, the percentage of MNPCE from the experimental groups was significantly different from the control groups ($P < 0.05$). All microscopically examined blood smears showed the presence of echinocytes (also called Burr cells) (Figure 5C). The blood of EMF-exposed animals showed

significantly (P <0.05) higher echinocyte incidences than the control groups (Data not shown).

Discussion

It is well documented that specific end-points frequently used in evaluating the safety of environmental factors (CAs and MN) are reliable biomarkers of DNA damage [20]. Long-term continuous or daily repeated EMF exposure has been found to result in cellular stress responses at non-thermal power levels that lead to an accumulation of DNA errors [6]. Due to the scarcity of human studies, plant and animal models were used in the present investigation to follow the potential of RF-EMF to cause cytotoxic and genotoxic effects. In the current research, RF-EMF significantly altered the root growth of *A. cepa* in an exposure time-dependent fashion. Although the short and intermediate exposure (up to 2 h/day) for seven consecutive days stimulated root growth, the longest exposure time (4h/day) resulted in a 20% decline in the root length. In support of this action of the EMF were the results observed with root number/bulb, where this number increased to 2 h/day exposure, then declined following four-hour exposure. A decrease in root growth of over 40% indicated the presence of toxic agents [21] having sublethal effects on the plant [22]. The impact on this parameter is observable after 3-5 days of treatment [23]. This effect could be due to the changes in the microenvironment of the *Vigna radiate* (mung bean) [24]. Alternatively, as reported before in *A. cepa* root cells, the effect could be attributed to the formation of ROS [25] or excessive increases in intracellular calcium concentration [14, 26]. These changes may lead to various responses, such as alterations in gene expression or enzymatic activities [14, 26-29]. This response could ultimately interfere with average plant growth, nodule formation, and production. Effects of EMF on plants and animal life, such as diminished radial growth of pine trees, have been demonstrated [6].

In this study, increases in MI in the exposed meristem cells were not statistically significant at all exposure periods examined. The mitotic index, which measures the proportion of cells in the mitotic phase of the cell cycle, is used as a biomarker for cell proliferation. When the MI significantly exceeds the control value, it can harm cells, leading to disordered expansion and even malignant transformation [30]. Thus, whether the increased MI should be considered beneficial or harmful is not apparent.

Although insignificant, a slight depression in MI calculated in the present investigation after four hours of exposure to RF-EMF may reflect a direct genotoxic effect. Therefore, genotoxicity was measured following the induction of CA by EMF exposure at 1850 MHz. It was found that short and intermediate exposure times did not cause significant elevations in the % of aberrant root meristem cells. However, a ~2.0-fold increase in this percentage over the sham-control (8.8% versus 14.1% in sham-control and 4-h exposure, respectively) was recorded. The most frequent abnormality recorded in the present study was chromosome fragmentation, followed by chromosome stickiness.

In plant systems, few studies on the effects of to RF-EMF on DNA are available in the literature. Our observations verify and extend previous reports and demonstrate that RF-EMF can induce chromosome anomalies in onion (*A. cepa*) cells [31, 32]. Also consistent with our data are those presented by others [33], which demonstrated a significant increase of approximately 1 to 1.4-fold in % aberrations in root meristem cells from onion exposed to RF-EMF at 2350 MHz for 1 and 4 h, respectively. Similarly, significant several-fold elevations of MN frequency above the sham value were recorded in the meristematic cells in the root tips of broad bean (*Vicia faba*) [34] and chickpea (*Cicer arietinum*) [35] following exposure to mobile phone transmission in the 900-915 MHz frequency range. Contradictory data were presented regarding MI. While it was increased in one study [33], it decreased in another [34].

Most CA observed in the meristem cells of *A. cepa* are lethal, but many corresponding aberrations are viable and can cause genetic effects [36]. Breaks may occur, and subsequent inhibition of repair mechanisms may lead to base mismatches, mutation, and CA, such as fragmented chromosomes and DNA breaks [37]. Anaphase chromosome fragments and bridges help obtain information on clastogenic activity, whereas vagrant chromosomes and c-metaphases increase the risk of aneuploidy [30, 38]. Chromosome stickiness may be due to the defective functioning of one or two types of specific nonhistone proteins involving chromosome organization that connects the chromatids by sub-chromatid bridges [37]. It may also occur through immediate reactions with DNA during its inhibition periods, causing DNA–DNA or DNA–protein cross-linking. Sticky chromosomes indicated a highly toxic, irreversible effect, probably leading to cell death [21]. A toxicant may affect DNA and telomeres, which protect chromatids and chromosomes from sticking together. Thus, as previously reported, chromosomes aggregate together in a sticky mass [39].

Since a single assay system cannot assess the potential genotoxicity or mutagenicity of a chemical/physical factor, we carried out the MN formation assay in PCE from mice exposed to RF fields to obtain more information and to reach a more definitive conclusion. Erythrocytes are particularly well suited to the analysis of MN because, during the maturation of erythroblast to PCEs (a period of about six h following final mitosis), the nucleus of the cell is extruded, making detection of MN easier because any MN that has been formed may remain behind in the otherwise anucleated cytoplasm [19, 40]. Furthermore, the PCE still contains rRNA, so it stains blue-grey with Giemsa, allowing differentiation from the smaller, nonspherical, mature, hemoglobin-containing erythrocytes (NCEs), which stain less blue with Giemsa.

Previous works [41-43] reported no significant relationship between MN frequency in exfoliated buccal

cells of healthy mobile phone users and duration of mobile phone use. Consistent with these results are those that reported no evidence for induction of MN in cultivated human lymphocytes [44, 45] and human glioblastoma cell lines [46], as well as in cultured C3H 10T½ mouse fibroblast cells [47].

One limitation of our study is the small number of onions and mice used in all groups because we made all efforts to minimize the number of animals used and their suffering. Another limitation is that it did not follow the chronic effects of exposure to RF-EMF. So, the long-term impact of exposure to RF-EMF should be considered. It should be recalled that the measurement of SAR is uncertain for animals or plants [48].

Conclusion

In conclusion, extrapolating the findings in one species to those in another is difficult. However, the results of the current investigation using two biological systems (onion and mouse) suggest that under the present experimental continuous, exposure to LTE signal fields may reduce vegetative growth and have a cytotoxic and genotoxic impact. Because of marching towards 5G frequency, the consequences of exposure to such radiation on plants and animals should be examined thoroughly.

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