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ARTICLE

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An Evaluation of the Genotoxic Effects of Electromagnetic Radiation at 900 MHz, 1800 MHz, and 2100 MHz Frequencies with a SMART Assay in *Drosophila melanogaster*

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ABSTRACT

With the development of today's technology, the electromagnetic radiation spread by mobile phones and base stations is also rapidly increasing, and this causes serious concerns about the environment and human health. The *Drosophila* model organism is widely used in genetic toxicology studies because its genome is highly similar to the genes identified in human diseases. In this study, the genotoxic effects of radiofrequency electromagnetic radiation were evaluated by the wing Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* at 900 MHz, 1800 MHz, and 2100 MHz. The SMART method is based on the observation of genetic changes occurring in the trichomes of the *Drosophila* wings appearing as mutant clones under the microscope. Throughout the study, total clone parameters were evaluated by exposing the *Drosophila* larvae to electromagnetic fields for two, four, and six hours per day for two days. As a result of the study, it was observed that the number of mutant clones was statistically increased according to the negative control group in all applications except for the six-hour application at 1800 MHz.

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Electromagnetic fields; radio frequency; nonionizing radiation; Drosophila; genotoxicity

Introduction

In recent years, researchers have begun to investigate the dosimetric effects of electromagnetic fields over a wide frequency range, from the extremely low frequency bands to the radio frequency bands, in order to determine the possible biological effects (Lee et al. 2008; Ozen 2008a; Ozen 2008b; Kos et al. 2011; Akpinar et al. 2012; Fedele et al. 2014; Hidisoglu et al. 2016; Seada et al. 2016; Gokcek-
Sarac et al. 2017a; Masumnia-Bisheh et al. 2017; Hidisoglu et al. 2018). Cellular phone exposure has been a significant part of these studies. The electromagnetic energy that is absorbed by any biological tissue exposed to electromagnetic fields at frequencies greater than 100 kHz and that causes thermal effects depends on many factors such as the frequency, power density, electrical properties of the involved tissue, possible attenuation and orientation of the incident electromagnetic field (Verschaeve 2014). The thermal effects of electromagnetic radiation (EMR) exposure can include ocular damage such as cataracts, corneal damage and retinal damage (Vander Vorst et al. 2006). Also, EMR may cause burns to the tissues (Ozen et al. 2011). In addition, exposure to electromagnetic fields can cause many hazards such as DNA damage, cell death, changes in ion concentrations at the cellular level, cell proliferation and biomolecule synthesis disorders (Aitken et al. 2005; Fitzsimmons et al. 1989; Ozawa et al. 1989; Remondini et al. 2006). On the positive side, in the hyperthermia method used in the treatment of cancerous tissues, targeted EMR is utilised to clear the tumour by increasing its temperature to 43–45°C (Vander Vorst et al. 2006).

It is known that exposure to RF fields can lead to detrimental impacts on various biological functions. These adverse effects can include a reduction in cognitive functions, an increase in brain tumour occurrence, and adverse effects on the hormone secretion of the pituitary gland (Vijver et al. 2014). The behaviour of occurred electromagnetic fields, radiation scenarios and corresponding exposure values at RF have been identified by the World Health Organization (WHO) as a new and developing high-priority area because of the negative effects of electromagnetic exposure on biological systems (World Health Organization 2010). The International Commission on Non-Ionizing Radiation Protection (ICNIRP) has published limits for exposure to the electric fields at frequencies used by cellular communications systems for both public and occupational (International Commission on Non-Ionizing Radiation Protection (ICNIRP) 2020). The

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possible effects of electromagnetic fields on animals and plants are also being investigated (Balmori 2009; Cucurachi et al. 2013; Grémiaux et al. 2016; Odemer and Odemer 2019; Senavirathna et al. 2014).

Mobile phone systems are widely used around the world. While they utilise different frequency bands such as 900 MHz, 1800 MHz and 2100 MHz, they have the same pulse repetition frequency value of 217 Hz (Panagopoulos et al. 2007a; Yahya et al. 2016). Although numerous articles have been published about the RF-EMR in the cellular communication frequency bands, limited studies have been performed in *Drosophila melanogaster* (*D. melanogaster*) to investigate the genotoxicity levels at the mentioned frequencies. This study aims to investigate the genotoxic effects of RF-EMR absorption at three cellular communications frequencies (900 MHz, 1800 MHz and 2100 MHz) in *D. melanogaster* somatic cells through the wing SMART method. Therefore, *D. melanogaster* larvae were exposed to electromagnetic radiation under controlled environmental conditions for two days at different frequencies with power levels. Assessments of electromagnetic dosimetry levels in *D. melanogaster* were carried out through the experimental results.

Materials and Methods

Exposure System

Third instar larvae were divided into 11 groups indicated as two-hour exposed, four-hour exposed and six-hour exposed. Exposure experiments were carried out under controlled environmental conditions (25°C and ~60% humidity). Experiments with the negative control group were carried out in the same environment while the signal generator was turned off. *D. melanogaster* larvae were exposed to the electromagnetic fields under the same environmental conditions during the two days. During the experiments, the generator was turned on for 60 minutes and turned off for 20 minutes to complete the planned exposure period.

The exposure system is depicted in Figure 1. The experiments were carried out in an anechoic chamber which has internal dimensions of 7 meters × 3.8 meters × 4 meters and which is covered inside with microwave absorbers. A styrofoam carousel holder was used to hold the *Drosophila* larvae-filled pyrex tube. An RF signal generator emitting the electromagnetic field for each operating frequency was used. A signal generator was placed on a wooden table which was outside of the anechoic chamber. A monopole antenna, fed by a signal generator, was placed inside at the middle point of the styrofoam holder to radiate the electromagnetic field uniformly. The connection between the signal generator and the antenna was made with coaxial cable.

A 50-ohm (Ω) matched signal generator was used to perform the experiments at different frequencies. The carrier frequencies were 900 MHz, 1800 MHz and 2100 MHz. The signal of each frequency was pulse modulated, the pulse width was 0.577 milliseconds (msec) and the modulation frequency was 217 Hz. The maximum power levels for 900 MHz, 1800 MHz and 2100 MHz were 10 W, 2 W and 2 W, respectively. The electric field amplitudes, the source powers, and the spans between the antennas and the tubes (represented as in Figure 1) for each frequency are shown in Table 1. The amplitudes of the electric fields were measured by

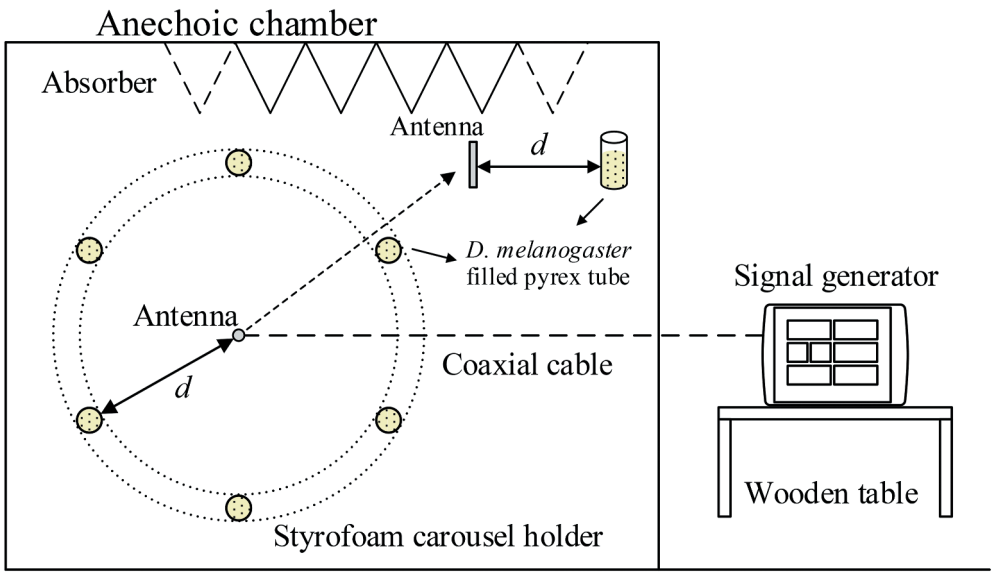


Figure 1. Radio frequency electromagnetic radiation exposure system.

Table 1. Exposure system parameters for each frequency.

Parameters	900 MHz	1800 MHz	2100 MHz
Radiated electric field (V/m)	35.2	41	41
Source power (W)	5	1.5	1.5
d dimension (cm)	3.5	8	8

NBM-550 Broadband Field Meter with an appropriate isotropic probe (Narda, Germany).

SMART Test

The SMART assay carried out according to Graf et al. (1984) method. Third instar larvae were divided into 11 groups indicated as two-hour exposed, four-hour exposed and six-hour exposed. Exposure experiments were carried out under controlled environmental conditions, and the temperature was 24°C. Experiments with the negative control group were carried out in the same environment while the signal generator was turned off. *D. melanogaster* larvae were exposed to the electromagnetic fields for the same duration over the course of two days. During the experiments, the generator was turned on for 60 minutes and turned off for 20 minutes to complete the planned exposure period.

In the SMART analysis, the clone frequency values between the application and control groups were evaluated by using Kastenbaum and Bowman (1970) binomial conditional test at significance level p: 0.05. Positive (+), negative (-), weak positive (z) and negligible difference (z) evaluations of the applied frequencies were performed according to the Frei and Würgler (1988) multiple decision procedures. If the frequency of the applied clones is as high as the multiplication factor (m) from the control group, they are considered positive. Because small spots and total spots had high spontaneous frequencies and were considered m=2, large spots and twin spots showed the low spontaneous frequency and were considered to be m=5 (Graf and Singer 1992).

Results and Discussion

Mobile phones, base stations, and power transmission lines make life easier in the developing and developed worlds. Exposure to radiofrequency (RF) electromagnetic fields (EMF) is increasing due to the increase in the density of base stations and power transmission lines (Bodera et al. 2015, 2013). Diagnostic procedures such as magnetic resonance imaging (MRI) applications in medicine, as well as hyperthermia applications for cosmetic procedures also create additional exposure (Bodera et al. 2013). In addition, the increase in the production and usage of electronic and telecommunications devices in the last 30 years has led to increased exposure to daily electromagnetic radiation (Hanci et al. 2013; Gurbuz et al. 2014).The frequencies of mobile phones have been between 600 MHz and 800 MHz since their inception. Today's third-generation phones use the 1800–2100 MHz microwave range, and the second-generation phones use 900 MHz (Him et al. 2018). When frequencies of 900 MHz and 1800 MHz wavelengths are absorbed at the molecular level, they can increase the intracellular temperature and directly affect the macromolecules (Ertilav et al. 2018). Electromagnetic fields (EMF) have two different effects on the biological system: thermal effects and non-thermal effects. As a result of the thermal effect, the transfer of energy in the tissue increases oxidative stress (Husni et al. 2013). Due to the increased exposure to radio frequency EMR (RF-EMR), many international organisations have initiated different projects to investigate the impacts on human health (Gurbuz et al. 2014). In addition, RF-EMR has been included in the Group 2B carcinogen category by the International Agency for Research on Cancer (International Agency For Research On Cancer (IARC) 2013).

Ionising radiation, ultraviolet rays, and EMR can cause DNA damage, necrosis, atrophy, cell death, oedema, and inflammation in tissues (Brugarolas et al. 1995; Cadet et al. 2005; Lai 1996; Matsumura and Ananthaswamy 2004; Stone et al. 2003). Furthermore, it is thought that EMF exposure may be related to an increase in reactive oxygen species, genotoxicity, and cancer, as well as disturbances of the nervous system and reproductive systems (Koyu et al. 2005; Marjanovic et al. 2012; Vijayalaxmi and Prihoda 2014). As a result of the rapid development of the mobile phone industry, the potential impacts on human health, particularly the brain, are concerning. The possible potential effects of electromagnetic radiation in the brain are of great concern because mobile phones are used close to the head area (Hidisoglu et al. 2016).

In the first stage of the study, the genotoxic effects of 900 MHz RF-EMR were investigated by the *Drosophila* SMART method, and the genotoxic effects were observed in total clones in all exposure times (2, 4, 6 hours) as compared to the control group (Figure 2A). Moreover, it was found that genotoxicity increased significantly in small single, large uniform, and total *mwh* clones with six hours of exposure (Table 2). In the MDA-MB-231 breast cancer cell line, selenium decreased oxidative stress and mitochondrial membrane damage as well as reduced cell viability and oxidative cell damage produced by 900 MHz EMR (Kahya et al. 2014). The survival potential of MCF-7 and human adipose mesenchymal stem cell lines (HADSCs) was assessed by the MTT test with different parameters (6, 21, 51, and 101 min/day, 3 and 5 days, 10 and 20 cm distance) at 900 MHz RF exposure. No decrease in cell viability was observed in the MCF-7 cell line at the increased duration

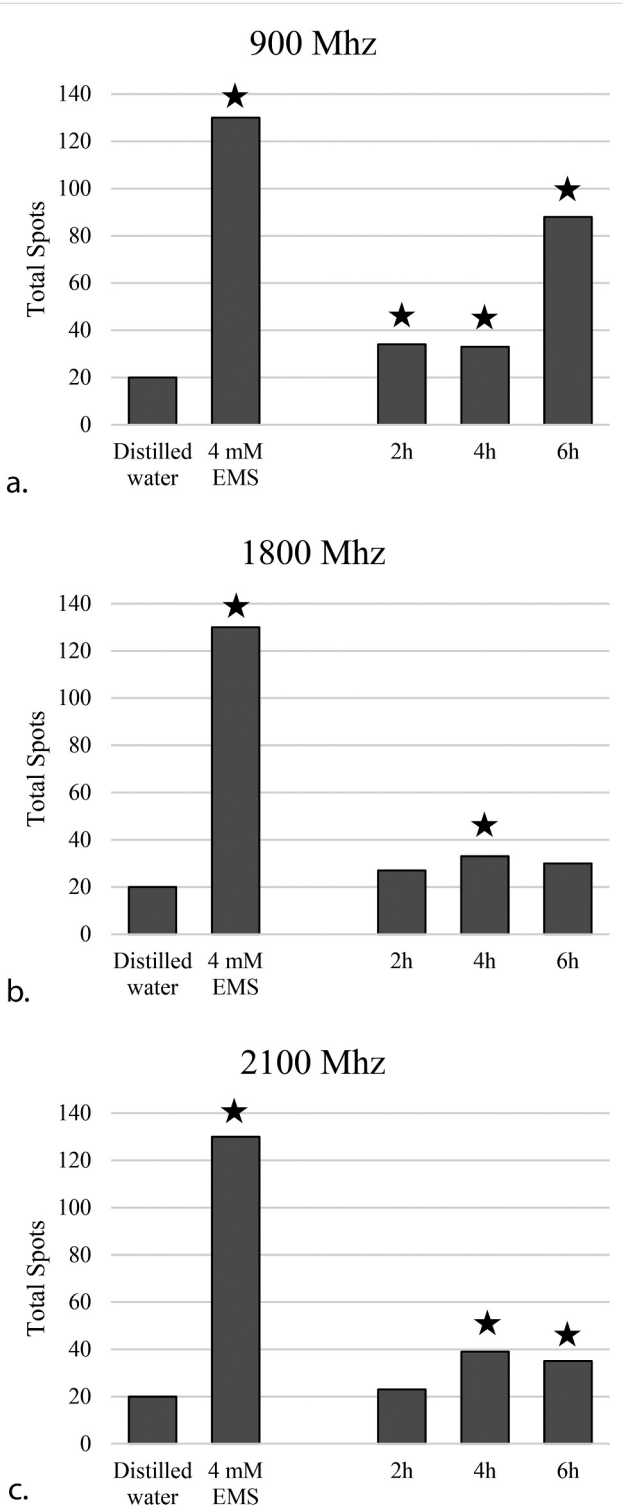


Figure 2. Genotoxicity obtained in the *Drosophila* SMART assay after 900 MHz (A), 1800 MHz (B) and 2100 MHz (C) frequency band treatment.

of exposure. In the HADSC line, it was observed that cell viability decreased significantly at all durations of exposure except six minutes. In addition, the distance to the antenna in both cell lines did not have a significant effect on cell viability (Shahbazi-Gahrouei et al. 2018).

It has been determined that garlic powder in rat brain tissue and blood samples causes decreases in oxidised protein and MDA levels (Bilgici et al. 2013). In a study that investigated the antioxidant effects of green tea against EMR exposure at a frequency of 900 MHz, it was determined that oxidative stress parameters in the rat hippocampus and striatum decreased (Ahmed et al. 2017). In another study, there was no significant effect on memory and learning abilities as a result of 900 MHz exposure in rats, while decreased locomotive behaviour and increased corticosterone levels were observed (Daniels et al. 2009). Histopathological changes and apoptosis were induced in Sprague Dawley rat kidney cells as a result of 900 MHz exposure for 25 days, at one hour per day (Okatan et al. 2018).

In Sprague Dawley rats, the oxidative stress markers MDA and 8-hydroxydeoxyguanosine (8-OHdG) levels increased with 900 MHz exposure for six hours per day for six months as compared to the control group, while no significant genotoxic damage was observed in the DNA damage examination via the COMET assay (Alkis et al. 2019). The COMET test was performed in male Swiss albino mouse germ cells for 35 days at 900 MHz per day, and an increase in DNA damage was observed (Pandey and Giri 2018). Seada et al. (2016) investigated the effects of mobile phone frequencies on the movements of *Drosophila*; 900 MHz mobile phone signals applied in one-hour and two-hour periods have been shown to cause disturbances in larval movement and cause hyperactivity in the flight behaviour of flies. Also, 900 MHz RF significantly reduced the reproductive capacity of *Drosophila* in six minutes/five-day applications (Panagopoulos et al. 2004). In the EMR exposure in *Eisenia fetida*, an increase in heat shock protein levels and genotoxicity was observed as a result of two hours and four hours of exposure to 900 MHz (Bourdineaud et al. 2017). Panagopoulos et al. (2007b) observed that DNA damage occurred in *Drosophila* oocytes with 900 MHz and 1800 MHz radiation exposure in the terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) experiment. Today, there are different opinions on the evaluation of the toxicological effects of EMR. In this study, it was observed that 900 MHz EMR increased genotoxicity, and this effect dramatically increased in six hours. In some studies, 900 MHz EMR does not cause any significant toxic effects, but there are also some opinions about the need to take in the 2A carcinogen group of EMR exposure, which is in the 2D carcinogen group, according to the International Agency for Research on Cancer (IARC) classification (Danese et al. 2017; Hardell 2018;

Table 2. Genotoxicity of 900 MHz frequency band in *D. melanogaster* somatic cells.

		Small single spots (1–2 cells) (*m* = 2)			Large single spot (> 2 cells) (*m* = 5)			Twin spots (*m* = 5)			Total *mwh* spots (*m* = 2)		
	Number of wings (N)	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.
Distilled water (72 h)	80	19	0.24		1	0.01		0	(0.00)		20	0.25	
4 mM EMS	10	78	7.80	+	47	4.70	+	5	0.50	+	123	13.00	+
900 Mhz													
2 h	80	28	0.35	i	6	0.08	i	0	0.00	i	34	0.42	+
4 h	80	28	0.35	i	3	0.04	i	2	0.02	i	33	0.41	+
6 h	80	80	1.00	+	7	0.09	+	1	0.01	i	88	1.10	+

Fr., frequency D., statistical diagnosis; +, positive −, negative i, inconclusive; m = multiplication; probability levels = 0.05.

Morgan et al. 2015; Scarfi et al. 2006). In this context, the importance of studies with different exposure frequencies and doses in different organisms is increasing.

In the results obtained from this study, 1800 MHz slightly increased the genotoxic effect in terms of total mwh clones and total clones in only four hours of treatment in *Drosophila* somatic cells. An increase in genotoxicity as compared to the control group in distilled water was observed in two-hour and six-hour applications, but no statistically significant results were obtained in the analyses (Figure 2B and Table 3). The 900 MHz and 1800 MHz frequencies applied to *Drosophila* adult individuals for a total of 20 minutes per day for five days resulted in decreases in egg laying efficiency (Geronikolou et al. 2014). Lameth et al. (2017) observed a change in the central nervous system genes, receptors, and neurotransmitter functions as a result of a two-hour exposure to 1800 MHz EMR administered to rat heads and suggested that these changes may be the triggers of neuroinflammation in the central nervous system. In a study of 24 adult rats, organisms were divided into 900 MHz and 1800 MHz groups and were exposed to 60 minutes per day for one year. According to the results obtained, it was observed that intracellular calcium (Ca) intake, ROS, apoptosis, and caspase-3 and caspase-9 activity increased (Ertilav et al. 2018). Because cell phones are used close to the eye area, DNA damage and oxidative stress were investigated in the eyes of rats. EMR was applied for two hours per day for eight weeks, and caspase-3 and p38MAPK gene expression levels increased (Eker et al. 2018). To investigate the effects of RF radiation on sperm DNA, samples from 22 donors were exposed to 1800 MHz EMR. The samples showed a decrease in the mobility and viability of the sperm and an increase in oxidative DNA damage (De Iuliis et al. 2009). As a result of 1800 MHz RF application for 15 minutes per day for five days, a significant increase in lipid peroxidation was observed in rat brain and blood tissues (Bodera et al. 2015). It was determined that 1800 MHz applied to 3T3 cells for between 0.5 hours and eight hours increased ROS levels but did not induce DNA damage (Hou et al. 2015). Liu et al. (2013) investigated the damage caused by 1800 MHz in male mouse germ cells via an enzyme-applied COMET test and determined that DNA damage was caused by oxidative stress.

In vivo and in vitro studies show that an adaptive response may occur in organisms as a result of electromagnetic field exposure (Jiang et al. 2012; Gherardini et al. 2014; He et al. 2017). It is thought that RF radiation can produce small but not statistically significant genotoxic damage, and that this increase can trigger an adaptive response by activating the protection/repair mechanisms (Sannino et al. 2009). In our study, it is thought that the decrease in genotoxicity observed in 1800 MHz RF application for six hours is a result of the repair mechanism which started to operate at low doses.

In this study, we observed statistically significant genotoxicity in small single clones, total mwh clones, and total

Table 3. Genotoxicity of 1800 MHz frequency band in *D. melanogaster* somatic cells.

		Small single spots (1–2 cells) (*m* = 2)			Large single spot (> 2 cells) (*m* = 5)			Twin spots (*m* = 5)			Total *mwh* spots (*m* = 2)		
	Number of wings (N)	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.
Distilled water (72 h)	80	19	0.24		1	0.01		0	(0.00)		20	0.25	
4 mM EMS	10	78	7.80	+	47	4.70	+	5	0.50	+	123	13.00	+
1800 Mhz													
2 h	80	22	0.28	i	4	0.05	i	1	0.01	i	27	0.34	i
4 h	80	29	0.36	i	3	0.04	i	1	0.01	+	33	0.36	+
6 h	80	26	0.32	i	3	0.04	i	1	0.01	i	30	0.38	i

Fr., frequency D., statistical diagnosis; +, positive −, negative i, inconclusive; m = multiplication; probability levels = 0.05.

clones in applications of 2100 MHz for four and six hours (Figure 2C and Table 4). A slight increase in genotoxicity was observed in two-hour applications, though the results obtained from the two-hour applications were not found to be statistically significant. In rats treated with 2100 MHz for two hours per day for 10 weeks, an increase in hippocampal protein kinase levels affecting the memory and learning functions of their brains was observed (Gokcek-Sarac and Er 2017b). It was found that Wistar albino rats exposed to 2100 MHz for 30 minutes for six days experienced low levels of sperm density, structural defects in epithelial cells, and oedema formation in the intercellular area (Erdemli et al. 2017). Increased DNA damage in the brain, kidney, liver, and skin tissues in rats exposed to 2400 MHz radiation was determined by the COMET method (Akdag et al. 2016). Celiker et al. (2017) observed that the apoptotic index increased in Wistar albino rats treated with 2100 MHz EMR for 30 days. Furthermore, degenerations were observed in histopathological investigations. Micronucleus formation was observed in rats exposed to radiofrequency radiation between 1800 MHz and 2100 MHz for 30 minutes per day, six days per week, for one month (Gurbuz et al. 2014). Charles-Foster albino rats were exposed to a total of 24 hours of RF at a rate of one hour per day for 28 days, and dysfunctions, cytochrome-c leakage, and caspase-3 and caspase-9 activity were observed in mitochondria in their brain tissue (Gupta et al. 2018).

Mobile phone systems operating at cellular communication frequencies can lead to serious effects that have been investigated by researchers, according to the well-known international organisations. In the literature, frequencies of 900 MHz, 1800 MHz, and 2100 MHz are classified as cell phone frequencies, and both children and younger adults are more susceptible to non-ionising radiation at these frequencies than adults are (Alkis et al. 2019). Base stations and mobile phones with 900 MHz antennas have twice the output power of 1800 MHz ones (Panagopoulos et al. 2007b). The results of this difference were reflected in our study, and the highest genotoxic effect was observed at 900 MHz.

Toxic substances, infections, extreme temperatures, and radiation can cause free radicals (Halliwell 1999). Epidemiological studies and laboratory studies have shown that EMR causes effects such as impaired neural impulses and increased permeability in the blood-brain barrier . As a result, the risk of brain tumours rises (Chen et al. 2014). The brain is an organ where more than half of the oxygen is consumed in the human body, and the high oxygen capacity increases the risk of ROS formation (Ozmen et al. 2007). One of the biological effects of radiofrequency emissions is the increase in reactive oxygen species, and it has been determined that RF-EMR causes ROS production in both in vivo and in vitro studies (Bodera et al. 2015; Koyu et al. 2009; Liu et al. 2013). As a result of EMR exposure, the brain is considered to be the most at-risk organ. ROS production, mitochondrial dysfunction, intracellular Ca traffic degradation, and extreme heat shock protein changes in specific gene regions of brain tissue are adversely affected. However, the mechanisms of EMR exposure conditions are not fully understood (Kivrak et al. 2017).

Conclusion

The use of mobile telephones and wi-fi devices that radiate electromagnetic fields have reached high levels in the 21st century worldwide. These devices improve the quality of life by facilitating data transmission and communication. Unfortunately, mobile devices may cause adverse effects such as neuronal damage, pain, and cancer. People are worried about public health as these devices can lead to exposure in all stages of life, including childhood, adulthood, and old age (Ertilav et al. 2018). To summarise, this article includes data on Drosophila larvae exposure to RF-EMR at 900 MHz, 1800 MHz, and 2100 MHz for evaluating genotoxicity by virtue of the SMART test. Even if the six-hour experiment's results at 1800 MHz showed no statistical significance, the rest of the obtained results showed that the number of mutant clones was increased as compared with the negative control group. Also, experimental results showed that the effects of RF-EMR depend on

Table 4. Genotoxicity of 2100 MHz frequency band in *D. melanogaster* somatic cells.

		Small single spots (1–2 cells) (m = 2)			Large single spot (> 2 cells) (m = 5)			Twin spots (m = 5)			Total *mwh* spots (m = 2)		
	Number of wings (N)	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.	No.	Fr.	D.
Distilled water (72 h)	80	19	0.24		1	0.01		0	(0.00)		20	0.25	
4 mM EMS	10	78	7.80	+	47	4.70	+	5	0.50	+	123	13.00	+
2100 Mhz													
2 h	80	23	0.29	i	1	0.01	i	0	0.00	i	22	0.28	i
4 h	80	36	0.45	i	3	0.04	i	0	0.00	+	39	0.49	+
6 h	80	33	0.41	i	2	0.02	i	0	0.00	i	35	0.43	i

Fr., frequency D., statistical diagnosis; +, positive −, negative i, inconclusive; m = multiplication; probability levels = 0.05.

the exposed electrical field, the frequency, and the span between *D. melanogaster* and the antenna.

Studies have shown that EMR can induce various types of cancer and can cause DNA damage (Brainard et al. 1999; Hardell and Sage 2008; Phillips et al. 2009). In addition, there are experimental studies in the production of ROS which have shown no significant results in apoptosis (Hou et al. 2015). Although studies have shown that EMR has a genotoxic effect, the opposite results have also been observed (Liu et al. There are contradictions in the studies on the biological effects of EMR (Lonn et al. 2005; Cao et al. 2009). This suggests that various cell lines or different systems may have different results. To our knowledge, this is the first study in which the genotoxic effects of the 900 MHz, 1800 MHz, and 2100 MHz radiofrequencies have been studied in *D. melanogaster*. Today, extensive studies are needed to demonstrate the toxicity of RF-EMF (Vila et al. 2018).

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Disclosure of interest

The authors report no conflict of interest.

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