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Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow

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Abstract

In this study, the genotoxic and cytotoxic potential of extremely low frequency magnetic fields (ELF-MF) was investigated in Wistar rat tibial bone marrow cells, using the chromosomal aberration (CA) and micronucleus (MN) test systems. In addition to these test systems, we also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Wistar rats were exposed to acute (1 day for 4 h) and long-term (4 h/day for 45 days) to a horizontal 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Mitomycin C (MMC, 2 mg/kg BW) was used as positive control. Results obtained by chromosome analysis do not show any statistically significant differences between the negative control and both acute and long-term ELF-MF exposed samples. When comparing the group mean CA of long-term exposure with the negative control and acute exposure, the group mean of the long-term exposed group was higher, but this was not statistically significant. However, the mean micronucleus frequency of the longer-term exposed group was considerably higher than the negative control and acutely exposed groups. This difference was statistically significant ($p < 0.01$). The results of the MI in bone marrow showed that the averages of both A-MF and L-MF groups significantly decreased when compared to those in the negative control ($p < 0.001$ and $p < 0.01$, respectively). No significant differences were found between the group mean MI of A-MF exposure with L-MF. We found that the average of PCEs/NCEs ratios of A-MF exposed group was significantly lower than the negative control and L-MF exposed groups ($p < 0.001$ and $p < 0.01$, respectively). In addition, the group mean of the PCEs/NCEs ratios of L-MF was significantly lower than negative control ($p < 0.01$). We also found that the MMC treated group showed higher the number of CA and the frequency of MN formation when compared to those in all other each groups (p-values of all each groups <0.01) and also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to those in all other each groups (p-values of all groups <0.01). These observations indicate the *in vivo* suspectibility of mammals to the genotoxicity potential of ELF-MF.

Keywords: Extremely low frequency magnetic fields; Chromosomal aberration; Micronucleus formation; Mitotic index; The ratio of polychromatic erythrocytes to normochromatic erythrocytes; Wistar rat

1. Introduction

The use of electricity has been one of the most important contributions to the development of present day society. Environmental exposure to extremely low frequency (ELF) magnetic fields (MF) generated by power-lines and by domestic and industrial equipment

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is quite ubiquitous, especially for populations living in the developed world. In the last decades, human health hazard following exposure has become a subject of considerable interest by public health authorities and decision-making bodies.

Several epidemiological studies sought a relationship between ELF electromagnetic fields (EMF) exposure and cancer with both positive [1–5] and negative results [6–8] and a definitive answer cannot be given.

The cytogenetic effects of ELF-MF were investigated in humans and in various animal species using different endpoints such as micronucleus (MN) formation, induction of chromosome aberrations (CA), and sister chromatid exchange (SCE) [9–15]. The rodent bone marrow MN and CA tests are the most widely used short-term *in vivo* assays for identification of genotoxic effects such as chromosome damage and aneuploidy, associated with mutagenesis and carcinogenesis [16,17].

According to most reviews of genetic effects of ELF fields, it is concluded that they do not directly damage DNA or cause mutations [18–20]. Yet some laboratory investigations yielded positive results [21,22], and also biomonitoring studies on ELF-exposed subjects often showed an increased chromosome aberration frequency in exposed versus non-exposed individuals [9,11–15]. Other studies have also reported negative SCE findings in response to EMF in cultured bovine lymphocytes [10] and human peripheral blood lymphocytes [23].

With respect to genotoxic effects, there are critical reviews of genetic toxicology of electric and magnetic fields. Although most of the available evidence does not suggest that electric and/or magnetic fields cause DNA damage, the existence of some positive findings and the overall scarcity of published studies on this topic suggest the need for additional work.

The aim of this study is to investigate the potential genotoxic and cytotoxic effects of acute (4 h) and long-term exposure (45 days) to 50 Hz, 1 mT uniform magnetic fields on tibial bone marrow cells of Wistar rats. Two cytogenetic assays chromosome aberrations (CA) and micronucleus test (MN) were used. The two conventional cytogenetic tests (CA, MN) are able to detect the fixed unrepaired fraction of the induced DNA damage after exposure. Mitotic index reflects frequency of cell divisions and it is an important parameter to determine the rate of cytotoxicity. Mitotic index (MI) and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs) was also evaluated in order to determine the cytotoxic effects of ELF-MF in tibial bone marrow.

2. Materials and methods

2.1. Subjects

Sixteen healthy adult female Wistar rats (8 weeks of age, with average body weight of 178 ± 15 g) were used in this study. Rats were obtained from an experimental animal center in Mersin, Turkey. The rats were randomly selected and housed in polycarbonate cages (4 rats/cage) with free access to tap water and rat chow with a dark/light cycle of 12/12 h. The temperature was $22 \pm 2\,^{\circ}$C and relative humidity 50–70%. All procedures were performed in accordance with the guidelines of the National Institutes of Health for the care and use of laboratory animals, under an institutionally approved protocol.

2.2. Helmholtz coil apparatus and exposure procedure

The horizontal, uniform and homogenous EMF that the animals were exposed to during the experimental procedures was generated by a Helmholtz coil apparatus. The EMF generated by the coils is classified as horizontal field since the field lines were perpendicular to the bottom plane of the animal's cage. Circular coil pairs of Helmholtz configuration were used. Coils of 42.75 cm diameter and 21.375 cm clearance were constructed by insulated copper wire and made of 160 turns. The electrical parameters of each one were resistance, 2 Ω; and inductance, 19.6 mH. Sinusoidal current of 50 Hz generated EMF; frequency was generated at the output of the circuit which was driven by the specially designed variable transformer, 2.5 kVA in power to feed the coils. EMF was measured by the axial probe of Hall-Effect Gaussmeter (Sypris Test Measurement, F.W. Bell Model 6010) and the value of magnetic field was fixed at 1.0 ± 0.05 mT.

Two rats were placed in a 26 cm × 17 cm × 13 cm polycarbonate cage and housed in the center of the Helmholtz coils in every circulation. The ambient geomagnetic field was recorded as 0.5 G (50 μT). No significant temperature change was detected during the experiments (all measurements were $21 \pm 0.02\,^{\circ}$C) between two activated Helmholtz coils and all experimental conditions were executed at a room temperature of 20–22 °C. The polycarbonate cages were cleaned after every test session so as to avoid any biochemical effects.

2.3. Experimental design

Sixteen rats were divided into four groups and each group had the same number of rats (4 rats/group). Experiment groups were identified as the acute ELF MF-exposed group (A-MF) and long-term ELF MF-exposed group (L-MF). The long-term experimental group was exposed to a horizontal 1 mT, 50 Hz sinusoidal magnetic field for 4 h/day for 45 days [24], while the acute group was exposed to the same amount of magnetic field on the forty-fifth day just 1 day for 4 h. Two control groups were used for the treatment: a negative control group (NC) not exposed to any detectable MF or to any chemical mutagen and a positive control group treated with Mitomycin-C

(MMC, 2 mg/kg BW), a well-known mutagen. At the end of 45-day-period, the negative control rats were treated with equal volumes of normal saline (0.25 ml/kg) and the positive control rats were given MMC only once, a single dose [25] via intraperitoneal (i.p.) injection. The same dose regimes were used in chromosome aberration (CA), micronucleus formation (MN), mitotic index (MI) and the PCEs/NCEs ratio measurements. Just after the completion of the exposure, the rats were left in a fixation time period for 24 h and then were sacrificed under Ketalar (Eczacıbası, Turkey) anesthesia (35 mg/kg, intramuscularly) in the end of the fixation time (24 h). All animals were injected (i.p.) with colchicine (4 mg/kg) 1.5 h prior to be sacrificed at the end of the fixation time period. At termination, both tibia of each rat were harvested and cleaned of any adhering muscle. Bone marrow cells were collected from the both tibias of rats. Contents of bone marrow were utilized for the cytogenetic tests (CA, MN) by separating the same amount of the specimens. After the cytogenetic tests were measured, cytotoxicity tests (MI, PCE/NCE ratio) were performed by the concerned preparate.

2.4. Chromosome aberration assay

Cytogenetic analysis was performed on bone marrow cells according to the recommendations of Adler [26], with slight modifications. Bone marrow cells were collected from the tibias by flushing in KCl (0.075 M, at 37 °C) and incubated at 37 °C for 25 min. Material was centrifuged at $2000 \times g$ for 10 min, fixed in aceto-methanol (acetic-acid:methanol, 1:3, v/v). Centrifugation and fixation (in the cold) were repeated five times at an interval of 20 min. The material was resuspended in a small volume of the fixative, dropped onto chilled slides, flame-dried and stained the following day in 5% buffered Giemsa (pH 6.8). At least 100 well spread metaphases containing 42 ± 2 chromosomes were examined per animal to score for different types of aberrations.

2.5. Micronucleus test

The frequency of micronucleated erythrocytes in tibial bone marrow was evaluated according to the procedure of Schmid [27], using the modifications of Agarwal and Chauhan [28]. The bone marrow was flushed out from tibias using 1 ml of fetal calf serum (FCS) and centrifuged at $2000 \times g$ for 10 min. The supernatant was discarded. Evenly spread bone marrow smears were stained using the May-Grünwald and Giemsa protocol. Slides were scored at a magnification of 1000× using a light microscope.

2.6. Mitotic index determination

The mitotic index was used to determine the rate of cell division. The slides prepared for the assessment of chromosomal aberrations were also used for calculating the mitotic index. Randomly selected views on the slides were monitored to determine the number of dividing cells (metaphase stage) and the total number of cells. At least 1000 cells were examined in each rat. Mitotic index values are expressed as % in this study.

2.7. Scoring

For the analysis of the frequencies of MN formation, 2000 PCEs per animal were scored to calculate the MN frequencies. Two hundred erythrocytes (immature and mature cells) were examined to determine the ratio of PCEs to normochromatic erythrocytes (NCEs).

2.8. Statistical analysis

The Kruskal Wallis test was used for the determination of significant differences in frequencies of CA between groups and followed by the Dunn test. The frequencies of MN formation, MI values and PCEs/NCEs ratios between groups were compared using ANOVA followed by the Tukey post hoc test. Type I error rate was accepted as 0.05 and in all calculations the SPSS (v 11.5) program was used.

3. Results

The metaphase analysis of the bone marrow cells revealed various types of chromosomal aberrations, which consisted of chromatid and isochromatid types of gaps and breaks, double minute (included isochromatid breaks) exchanges, dicentric chromosomes and fragments (Table 1). Numerical aberrations were not scored in this study. The results of the chromosome aberration assay (CA), mitotic index (MI), PCEs/NCEs ratios and micronucleus formation (MN) in bone marrow cells after acute and long-term ELF-MF exposure are summarized in Tables 1 and 2.

Results obtained by chromosome aberration analyses do not show any statistically significant differences between negative control and both A-MF and L-MF exposed samples (Table 1 and Fig. 1). When comparing the group mean CA of L-MF exposure with negative control and A-MF exposure, the group mean of L-MF was higher than the others, but this increase was not statistically significant.

The results of the mitotic index (used on a measure of evaluation of cell cycle kinetics) are summarized in Table 1 and Fig. 2. The results have shown that the MI of bone marrow cells exposed to ELF-MF was significantly decreased in both A-MF and L-MF groups compared to those in the negative control ($p = 0.0001$ and $p = 0.001$, respectively). When comparing the group mean MI of A-MF exposure with L-MF, the group average of A-MF was lower than the other, but this difference was not statistically significant.

Table 1
Frequency of chromosome aberrations and the value of mitotic index in bone marrow cells of female Wistar rat induced by ELF-MF

Treatment groups	Fixation time (h)	Rat number	Metaphase per 1000 cells	Total number of examined metaphases (*n*)	Chromatid		Chromosome		Fragment	Dic	TSCA	Mean ± S.D. %SCA (excluding gaps)
					Gap	Break	Gap	Break				
Negative control (isotonic saline) (0.25 mg/kg BW)	24	1	4.03	400/4	5	3	4	4	1	−	17	2 ± 0.0
		2	3.81									
		3	4.06									
		4	4.00									
		Mean ± S.D.	3.97 ± 0.11									
Acute ELF-MF exposed	24	1	3.05	400/4	5	5	4	4	1	−	19	2.50 ± 0.57[c*]
		2	3.45									
		3	2.98									
		4	3.07									
		Mean ± S.D.	3.13 ± 0.21[a*,c*]									
Long-term ELF-MF exposed	24	1	3.05	400/4	4	6	6	5	3	1	25	3.75 ± 0.95[e*]
		2	3.21									
		3	3.32									
		4	3.42									
		Mean ± S.D.	3.25 ± 0.15[d*,e*]									
Positive control (MMC) (2 mg/kg BW)	24	1	2.08	400/4	20	18	18	20	18	−	94	13 ± 2.44[c*,e*,f*]
		2	2.67									
		3	2.53									
		4	2.45									
		Mean ± S.D.	2.43 ± 0.25[c*,e*,f*]									

n: number of animals; BW: body weight; TSCA: total structural aberrations; SCA: structural aberrations; Dic: dicentric chromosome; ELF-MF: extremely low frequency magnetic fields; MMC: mitomycin C. [*] $p < 0.001$; [a]acute group compared to negative control group; [c]acute group compared to MMC group; [d]long-term group compared to negative control group; [e]long-term group compared to MMC group; [f]MMC group compared to negative control group. The cases where statistically significant differences were found are given in the table.

Table 2
Micronucleus induction and the PCEs/NCEs ratios in bone marrow cells of female Wistar rat induced by ELF-MF

Treatment group	Rat number	Micronucleus per 2000 PCEs	Fixation time (h)	PCEs per 200 erythrocytes
Negative control (isotonic saline) (0.25 mg/kg BW)	1	2	24	100
	2	2		110
	3	2		108
	4	3		98
	Mean ± S.D.	2.25 ± 0.5		104.00 ± 5.88
Acute ELF-MF exposed	1	2	24	70
	2	3		74
	3	3		76
	4	3		78
	Mean ± S.D.	$2.75 \pm 0.5^{b\hat{},c*}$		$74.70 \pm 3.41^{a*,b\prime,c\prime}$
Long-term ELF-MF exposed	1	4	24	90
	2	5		95
	3	5		92
	4	6		85
	Mean ± S.D.	$5 \pm 0.8^{b\hat{},d\prime,e*}$		$90.50 \pm 4.20^{b\prime,d\prime,e*}$
Positive control (MMC) (2 mg/kg BW)	1	22	24	47
	2	21		45
	3	22		48
	4	24		46
	Mean ± S.D.	$22.25 \pm 1.25^{c*,e*,f*}$		$46.50 \pm 1.29^{c\prime,e*,f*}$

BW: body weight; PCEs: polychromatic erythrocytes; ELF-MF: extremely low frequency magnetic fields; MMC: mitomycin C. $\hat{}\ p < 0.05$; $\prime\ p < 0.01$; $*\ p < 0.001$; [a]acute group compared to negative control group; [b]acute group compared to long-term group; [c]acute group compared to MMC group; [d]long-term group compared to negative control group; [e]long-term group compared to MMC group; [f]MMC group compared to negative control group. The cases where statistically significant differences were found are given in the table.

The effects of ELF-MF exposure on the frequency of MN formation are shown in Table 2 and Fig. 3. No statistically significant difference was found between A-MF and negative control group. The average of L-MF exposed group was found considerably and statistically higher than the negative control and A-MF exposed groups ($p = 0.003$ and $p = 0.011$, respectively).

The effects of ELF-MF exposure on the PCEs/NCEs ratios (used as a measurement of cytotoxicity) are shown in Table 2 and Fig. 4. Results obtained by the PCEs/NCEs ratios have shown that the average of A-MF exposed group was considerably and statistically lower than the negative control and L-MF exposed groups ($p = 0.0001$ and $p = 0.001$, respectively). The group mean of the PCEs/NCEs ratios of L-MF was lower than negative control and this difference was statistically significant ($p = 0.002$).

The rats treated with MMC (positive control) showed higher CA and the frequency of MN formation when compared to negative control, as expected ($p = 0.0001$, respectively). However, MMC treated group showed higher CA and the frequency of MN formation when compared to both A-MF and L-MF groups ($p = 0.0001$, respectively). In addition to the results obtained by the treatment of MMC, the mean of the group showed lower MI and the PCEs/NCEs ratios when compared to negative control ($p = 0.0001$ and $p = 0.0001$, respectively). And also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to both A-MF and L-MF groups ($p = 0.001$ and $p = 0.0001$, respectively).

4. Discussion

Medical and biological effects of magnetic fields have been widely discussed during recent years. The question has been raised as to whether exposure to such fields causes genetic damage or other biological effects.

Over the past decade, the possible genotoxic effects of ELF-MF exposure have been investigated in variety of biological systems. Most of the data available in the literature regarding ELF-MF genotoxicity suggest a lack of effects [29–34]. However, there is some limited evidence that exposure to ELF-MF directly cause genetic changes in biological systems [35–42].

Several *in vitro* studies have addressed a possible association between exposure to 50–60 Hz magnetic

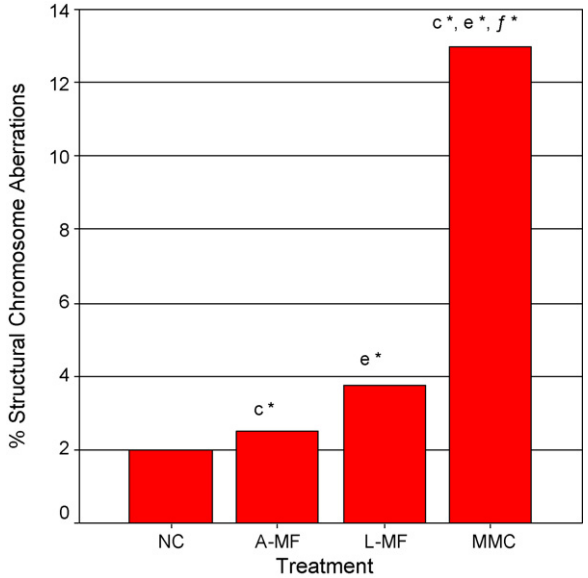

Fig. 1. Frequency of structural chromosomal aberrations (%) in relation to ELF-MF in rat bone marrow. NC: negative control (isotonic saline: 0.25 mg/kg BW); A-MF: acute ELF-MF exposed; L-MF: long-term ELF-MF exposed; MMC: positive control (mitomycin C: 2 mg/kg BW). $^{*}p < 0.001$; [c]A-MF compared to MMC; [e]L-MF compared to MMC; [f]MMC compared to NC. The cases where statistically significant differences were found are given in the figure.

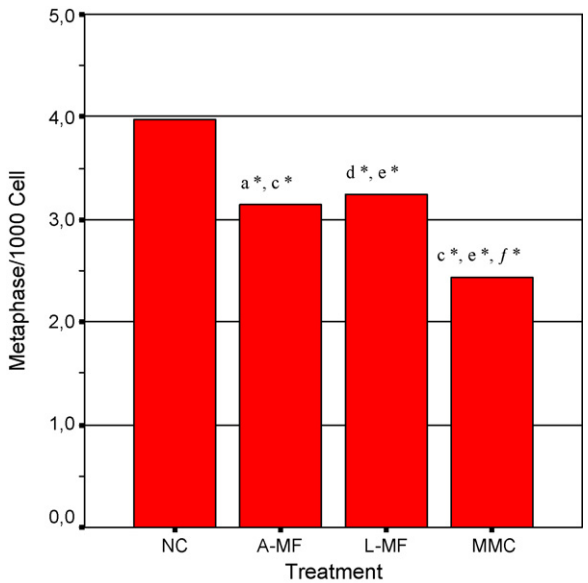

Fig. 2. The value of mitotic index of the groups in relation to ELF-MF in rat bone marrow. NC: negative control (isotonic saline: 0.25 mg/kg BW); A-MF: acute ELF-MF exposed; L-MF: long-term ELF-MF exposed; MMC: positive control (mitomycin C: 2 mg/kg BW). $^{*}p < 0.001$; [a]A-MF compared to NC; [c]A-MF compared to MMC, [d]L-MF compared to NC, [e]L-MF compared to MMC; [f]MMC compared to NC. The cases where statistically significant differences were found are given in the figure.

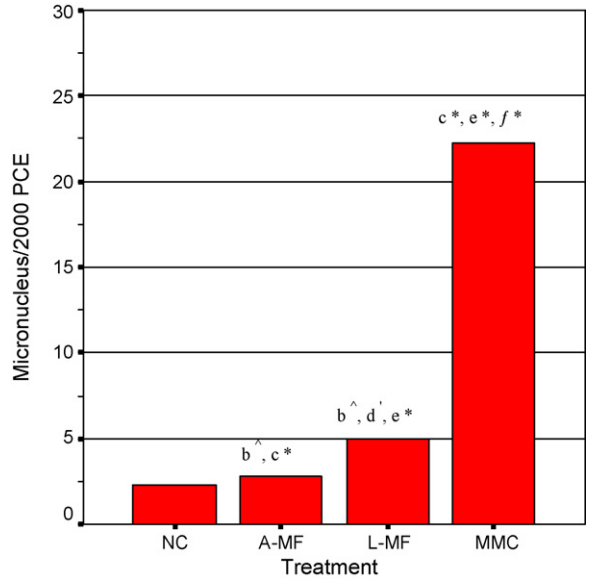

Fig. 3. Frequency of micronucleus formation in relation to ELF-MF on rat bone marrow. NC: negative control (isotonic saline: 0.25 mg/kg BW); A-MF: acute ELF-MF exposed; L-MF: long-term ELF-MF exposed; MMC: positive control (mitomycin C: 2 mg/kg BW). $^{\wedge}p < 0.05$; $'p < 0.01$; $^{*}p < 0.001$; [b]A-MF compared to L-MF; [c]A-MF compared to MMC; [d]L-MF compared to NC; [e]L-MF compared to MMC; [f]MMC compared to NC. The cases where statistically significant differences were found are given in the figure.

fields and the induction of genetic damage. Accumulating evidence suggests that this type of exposure is not able to induce mutations and initiate malignant cell transformation. Moreover, most studies reporting the induction of genotoxic effects have not been independently confirmed or have failed to replicate positive results. Nevertheless, the sporadic positive results must not be overlooked, but should stimulate further in depth laboratory investigations.

In view of conflicting results, we undertook the present study with the aim of obtaining a more exhaustive assessment of DNA damage induced by 1 mT of ELF short and long-term exposure by using cytogenetic tests (CA, MN, MI, PCEs/NCEs ratios), which detect biological effects resulting from unrepaired DNA damage.

In the present study, we have observed that acute ELF-MF *in vivo* exposure of Wistar rat bone marrow cells had no influence on MN and CA frequency. The data indicate that *in vivo* acute exposure of rat bone marrow cells to 50 Hz ELF-MF at a flux density of 1 mT is not able to induce genotoxic effects. This result is in good agreement with previous reports indicating that acute exposure to electric and magnetic fields is without genotoxic effects. Cohen et al. [29,30] found that *in vitro* exposure of human peripheral lymphocytes to

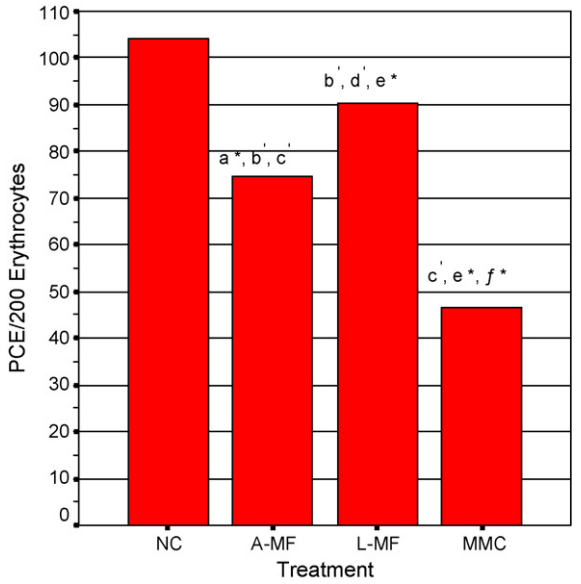

Fig. 4. The number of PCE/NCE ratios as a function of the ELF-MF in rat bone marrow. NC: negative control (isotonic saline: 0.25 mg/kg BW); A-MF: acute ELF-MF exposed; L-MF: long-term ELF-MF exposed; MMC: positive control (mitomycin C: 2 mg/kg BW); PCE: polychromatic erythrocyte. $'p < 0.01$; $^{*}p < 0.001$; [a]A-MF compared to NC; [b]A-MF compared to L-MF; [c]A-MF compared to MMC; [d]L-MF compared to NC; [e]L-MF compared to MMC; [f]MMC compared to NC. The cases where statistically significant differences were found are given in the figure.

60 Hz EMF did not affect the cell cycle progression, the frequency of chromosomal aberrations, or sister chromatid exchanges (SCE). Scarfi et al. [32] did not find any effect on micronucleus frequency in human lymphocytes exposed to 50 Hz sinusoidal MF, but they observed a slight influence on cell proliferation. Maes et al. [33] found that magnetic fields up to 2500 μT did not significantly influence the chromosome aberration and sister chromatid exchange frequency.

Heredia-Rojas et al. [43] found that incubation for 72 h of human peripheral blood cultures in the presence of 60 Hz sinusoidal magnetic fields at magnetic flux densities of 1.0, 1.5 and 2.0 mT led to stimulation of lymphocyte proliferation but had no influence on the frequency of sister chromatid exchanges. Stronati et al. [44] showed that a short exposure to ELF-MF at the intensity of 1 mT is not able to exert any genotoxic effect on human blood cells.

In the present study we found that although the increased frequency of CA was not statistically significant following treatment with long-term exposure to ELF-MF compared to negative control and acute exposure to ELF-MF, the frequency of MN in bone marrow cells of rats treated with long-term exposed to ELF-MF was statistically significantly higher than in bone marrow cells of rats treated with acute exposed to ELF-MF and negative control.

Nordenson et al. [9] performed *in vivo* and *in vitro* studies of the clastogenic effects of power frequency electric fields and transient electric currents. They screened peripheral lymphocytes from switchyard workers for chromosome anomalies. They found that the rates of chromatid and chromosome breaks were significantly increased compared to the rates in controls. Nordenson et al. [11] reported a statistically significant increase in the rate of chromosomal aberrations (CA) and cells with micronuclei (MN) in lymphocytes of 400 kV-substation workers. Simko et al. [38,39] showed that 50 Hz 1 mT continuous exposure to horizontal magnetic fields for 24, 48 and 72 h induced formation of MN in human amnion cells and in human squamous cell carcinoma cells. Testa et al. [45] investigated the possible genotoxic effects of 50 Hz 1 mT uniform MF by using the Comet assay plus sister-chromatid exchanges, CA and MN tests in human blood cells which were exposed *in vitro* for 48 h. The results obtained by this study indicate that the exposure to 50 Hz 1 mT MF for 48 h has no effects on DNA damage or cell proliferation. Fatigoni et al. [46] investigated the genotoxicity of ELF-MF by using the Tradescantia-micronucleus assay. They found that the exposure of Tradescantias to the ELF-MF at a flux density of 1 mT for 1, 6 and 24 h had a time-dependent increase in the frequency of MN formation and these results suggest that 50 Hz MF of 1 mT field strength is genotoxic. Winkler et al. [47] found that the intermittent (5 min field-on/10 min field-off, for 2–24 h) exposure to a 50 Hz sinusoidal 1 mT magnetic field caused a time-dependent increase in frequency of MN in cultured human fibroblasts.

Bone marrow cell toxicity (or depression) is normally indicated by a substantial and statistically significant decrease in the proportion of PCEs: a very large decrease in the ratio would be indicative of a cytostatic or cytotoxic effect. A decrease in the proportion of PCEs is used as an indication of mutagen-induced bone marrow cytotoxicity or changes in erythropoiesis [48]. In the present study we found that the decrease of the PCEs/NCEs ratios were statistically significant by treatment with acute and long-term exposure to ELF-MF compared to negative control. This data indicates that the 50 Hz sinusoidal ELF-MF at a flux density of 1 mT, is a possible potent inhibitor of mitosis. In addition, we also found that the MI of bone marrow cells exposed to ELF-MF was significantly decreased in both A-MF and L-MF groups compared to negative control. According to these results, L-MF and A-MF exposures lowered the

mitotic index. This observation indicates that ELF-MF is a potent inhibitor of mitosis.

In the present study, the observed inhibition of cell proliferation in rat bone marrow illustrates the cytotoxicity of ELF-MF. These data indicate the genotoxic and cytotoxic potential of ELF-MF. This is also in agreement with the previous reports on the genotoxic and cytotoxic potential of ELF-MF (50 Hz, 1 mT). Similar results were reported in studies performed with different ELF-MF [9,11,38,39,46,47].

On theoretical grounds, ELF MF do not have enough energy to break chemical bonds directly in DNA molecules. Genetic effects should therefore be induced by an indirect effect with ELF fields acting as a promoting or co-promoting agent rather than as an initiator. This positive results could be by increasing free radical life span and the concentration of free radicals or by affecting enzymatic processes involved in DNA repair in cells. As a result of these activations MI and PCE/NCE ratios may have decreased. The magnetic field can induce DNA–protein and DNA–DNA cross-links in a manner similar to the chemical mitomycin C and this activity could be the reason of the positive results observed. Similar insights were reported in studies performed with different ELF-MF [22,49–53].

In conclusion our *in vivo* study suggests that the 50 Hz, 1 mT ELF-MF used in the present investigation may be genotoxic and cytotoxic in view of these findings and contradictory reports in the literature, it is important to carry out more investigations using various cytogenetic tests under different experimental conditions to definitively resolve the controversy concerning the possible genotoxic and cytotoxic risk associated with magnetic fields.

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