# A 50 Hz 0.5 mT magnetic field induces cytogenetic effects and biological alterations in Wistar rat

Reem H. El-Gebaly, Nihal S. El-Bialy, Monira M. Rageh*

Department of Biophysics, Faculty of Science, Cairo University, Egypt
n_elbialy@hotmail.com

**Abstract:**The effects of continuous whole body exposure to extremely low frequency magnetic fields (ELF-MF) (50 Hz,0.5 mT for 30 days24 hrs) on cytogenecity, bone parameters and some hematological and biochemical parameters were evaluated.Male rats were exposed continuously to ELF-MF for a period of 30 days. The exposure effects were assessed by measurements of micronucleus formation, DNA fragmentation and bone parameters. Additionally the levels of some liver and blood parameters were calculated. Moreover, osmotic fragility of erythrocytes was also considered.Exposure to ELF-MF resulted in a 6.5 fold increase in the micronucleus formation and a decrease in polychromatic erythrocytes (PCE)/normochromatic erythrocytes (NCE) ratio, in addition to a significant increase in the level ofaspartate transaminase (AST), alanine aminotransferase(ALT), alkaline phosphatase(ALP), Magnesium (Mg)and uric acid in serum was observed. However, 0.5 mT ELF-MF was unable to cause either direct DNA primary damage or changes in bone parametersand erythrocytes lyses percent.The present results provide evidence that continuous exposure to ELF-MF causes micronucleus formation and some physiological disturbance but had no effect on DNA structure and bone parameters.Furthermore, the levels of glucose, creatinine, cholesterol and the percentage of erythrocytes lyses were not affected.
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**Keywords**: Extremely low frequency magnetic field (ELF-MF); Micronucleus test; DNA fragmentation; Bone mineral density; haematological and biochemical parameters.

## 1. Introduction

In modern society, the use of electricity is so widespread that it is impossible to avoid exposure to power frequency magnetic fields (MF). Many biological effects are observed upon exposure to different types of non-ionizing radiation[1].Such effects depend on many factors such as the mode of exposure, the type of cells studied, and the intensity and duration of exposure.

An extremely low frequency magnetic field (ELF-MF) can induce a number of changes in biological systems of living species. Epidemiological studies suggest a possible link between ELF-MF exposure and clinically recognized medical disorders such as leukaemia and many types of cancers [2- 3]. Following the two pooled analyses of childhood leukemia by Ahlbom*et al.*[4] and Greenland *et al.*[5], which reported a doubling of risk above 0.3/0.4 microtesla, IARC 2002 (International Agency for Research on cancer)[6] classified magnetic fields as a class II B possible carcinogen.The 2002 California Report[7] cited childhood and adult leukaemia, brain cancer, ALS (Amyotrophic lateral Disease) and miscarriage as associated with ELF-MF. SCENIHR, [8-9] and Davanipour and Sobel[10] introduced reviews that emphasized the risk of Alzheimer's disease upon exposure to magnetic field.

On the one hand some researchers have reported harmful effects arising from alternatingand static magnetic fields[11-12]. On the other handextremely low frequencyelectromagnetic fields (ELF-EMF) and magnetic fields have been used as a therapeutic tool, for example, low frequency sinusoidal magnetic fields are used for treatment of intractable bone fractures, and extremely low frequency for treating tumors [13- 14]. Other studies didn't show any effects on biological tissues[15].

Since low energy magnetic or electromagnetic fields are not known to transmit enough energy that affects chemical bonds in the molecules it is generally accepted that such fields are unable to damage the DNA directly [15]. However, exposure to ELF-EMF may lead to the production of free radicals that induce alterations in cellular processes directly responsible for DNA damage[16]. Numerous "*In vivo*" and "*In vitro*" studies have been investigated with the effort to determine a link, if any, between such fields and mutagenesis and to establish the possible mechanism of cancer risk. It was attempted to define whether 50/60 Hz ELF-EMF as generated by high voltage power lines or electrical appliances could give rise to *in vivo* primary DNA damage and cytogenetic effects such as chromosomal aberrations, sister-chromatid exchanges, or micronuclei formation.Some studies concerning with exposing human cells to magnetic fields, revealed significant increases in micronuclei formation and chromosomal aberrations. Moreover, a pronounced

effect on cell proliferation and apoptosis took place upon such exposure to ELF-EMF [17- 19].

The aim of the present work is to study the effects of continuous whole body exposure to ELF-MF (50 Hz,0.5 mT for 30 days) on cytogenecity, bone parameters in addition to some haematological and biochemical measurements as a step forward to spot light on the action of these fields on biological systems.

## 2. Material and Methods

### Animals

Adult male Wistar rats weighing 160- 180gm, 8 weeks old, were obtained from the Animal House of the National Research Center in Egypt. They were maintained for one week in the laboratory for adaptation. Rats (10 rats per cage) were housed in cages with free access to drinking water and standard chow diet.

All animals' procedures and care were performed using guidelines for the Care and Use of Laboratory Animals [20].

The rats were randomly divided into two groups of twentyeach: one control (sham) and one exposed. The latter group has been exposed to 0.5mT ELF-MF for 30 days24 hrs per day. The control (sham) group was treated like the exposed group with the sole difference that it was not exposed to ELF-MF. The two groups were treated equally considering light and food. The temperature and humidity were monitored continuously throughout the experimental period. This ensures that the control and the exposed animals were maintained in the same temperature. During the experimental period, all animal groups were maintained in clean first hand cages under standard condition in a separate laboratory which belongs to animal care unit. After 30 days of exposure, the two groups of rats were sacrificed by decapitation. Six rats from each group were used for micronucleus test and DNA analysis. Biochemical, hematological and osmotic fragility measurements were done by withdrawing blood samples from another six rats from each group. Lastly, other six rats from each group were dissected and their femora were isolated, cleaned from all soft tissues for bone parameters examination.

### Experimental Design

The animals were housed freely in a plastic cage in the center of a magnet with a fixed magnetic field value at 0.5 ± 0.025mT. The magnetic field was generated by a solenoid carrying current of 18 A (ampere) at 50 Hz from the main supply (220-230 Volt) via a variac (made in Yugoslavia). The magnet consisted of a coil with 320 turns made of electrically insulated 0.8 mm copper wire. The coil was wounded around a copper cylinder of 2mm thickness, 40cm diameter and 40cm length. The cylinder wall was earthed to eliminate the electric field. The magnetic field was measured at different locations to find out the most homogenous zone inside the solenoid core. This was done using the Gauss/ Tesla meter model 4048 with probe T-4048 manufactured by Bell Technologies Inc. (Orlando-Florida USA). The animals were exposed by placing the whole cage (made of plastic) inside the magnet core.Animals from control and exposed groups were kept under the same environmental conditions of temperature, lightening and feeding. Cleaning and changing water and food were done to all animals two times daily. The field was switched off during cleaning the cage.

### Micronucleus test

Bone marrow slides for micronucleus assay from 6 male rats of each group were prepared and stained according to the method described by Schmidt [21] using the modifications of Agarwal and Chauhan[22]. The bone marrow was flushed out from tibias using 1ml fetal calf serum and centrifuged at 2000xg for 10 min. The supernatant was discarded. Evenly spread bone marrow smears were stained using the May-Grunwald and Giemsa protocol. Slides were scored at a magnification of 1000x using a light microscopetype CX31 Olympus (Tokyo, Japan). 2000 polychromatic erythrocytes per animal were scored, and the number of micro nucleated polychromatic erythrocytes (MNPCE) was determined. In addition, the number of polychromatic erythrocytes (PCE) was counted in fields that contained 1000 cells (mature and immature) to determine the score of PCE andnormochromatic erythrocytes (NCE).

Statistical analysis was performed using the F-test with help of the software spss-version15 in order to compare the score of MNPCE, PCE and NCE for both the control and exposed samples.

### Analysis of DNA Fragmentation

Analysis of DNA fragmentation was measured using agarose gel electrophoresis, according to the protocol developed by Kasibhatla*et al.*[23]. 0.5 gm homogenized liver and spleen are transferred to 1.5 ml sterile micro centrifuge tubes. Centrifuge at 200xg in an Eppendorf table, top centrifuge for 5min at 4ºC and remove supernatant. 20 µl of TES (20mM EDTA (ethylene-diaminetetra-acetic acid), 100mM Tris (hydroxymethyl-aminomethane), pH8.0, 0.8% (w/v) Sodium dodecyl sulfate) lysis bufferwere added and mixed with cell pellet. 10 µl ofRNase Cocktail were added and mixed well. Incubate for 30-120min at 37ºC. 10 µl of proteinase K, were added and incubated at 50ºC for at least 90min. 5µl of 6x DNA loading buffer were added and load DNA samples into dry wells of a 1-1.5% agarose gel in TAE (242g Tris base, 57.1 ml Acetic acid, 100 ml of 0.5 M EDTA, pH 8.0**)** buffer containing 0.5 µg/ml

ethidium bromide. The gel was run at low voltage (i.e., 35V for 4 hours or until loading dye has run two-thirds of the way down the gel). DNA ladders are finally visualized by ultra-violet (UV) light source and documented by photography. The gels were analyzed using the software: Gel-Pro Analyzer 3.1.The used chemicals were purchased from Sigma chemical co. (St. Louis MO, USA)

**Bone Parameters**

Femora of 6 rats each from control and exposed group were used to measure bone minerals density (BMD), bone minerals content (BMC) and cross sectional area. Bones, after cleaning all of soft tissues, were immediately weighed with an automatic balance (Sartorius research, USA), to receive wet bone weight (WW). Their lengths were measured with a digital caliper (± 0.01mm). The BMD, BMC and cross sectional area of the whole right femora and their diaphysis were measured by dual-energy X-ray absorptionmeter (DEXA, Nortand XR.46 version 3.9.6/2.3.1 made in USA). The X-ray absorption meter is routinely calibrated daily. The reproducibility of these measurements was established by repeating the scans three times, for all samples. The data were treated statistically by analysis of variance (ANOVA) test.

**Biochemical and Hematological Measurements**

Six blood samples were withdrawn from each rat (n=6) for control and exposed groups. Blood was collected in tubes containing (EDTA) for hematological analysis while others was maintained without anticoagulant in order to obtain serum for further biochemical analysis. The methods of measuring AST (aspartate transaminase), ALT (alanine aminotransferase), Ca (calcium), ALP (alkaline phosphatase), glucose, uric acid, creatinine, cholesterol and hemoglobin were carried out as described by Henderson and Moss, [24] Eraslan*et al.*[25]; Tietz, [26] Allston, [27] Dacie and Lewis [28]. All the chemicals used for both hematological and biochemical measurements were purchased from ELI Tech (Paris, France).

**Determination of Osmotic Fragility of Erythrocytes**

For the determination of osmotic fragility of erythrocytes, 6 blood samples were collected from 6 rats of each group. Blood was collected in heparinized tubes and the test was carried out within 2h of collection at room temperature according to the method described in Dacie and Lewis [28]. Fourteen test tubes each containing 5ml saline solution with a concentration range of 0.0-9.0 gm Nacl/L were prepared. Fifty µl of well mixed blood were added to each tube and they were incubated for 30 min at room temperature. After incubation time, the suspensions were centrifuged for 5 min at 1200xg. Then, the absorbance of supernatant of each tube was measured at wavelength 540 nm using a spectrophotometer [6405UV/Vis (ultra-violet/visible) spectrophotometer JENWAY England UK)]. The lyses percentage was calculated by the relation % of lyses= $A_{samples}/A_{100\%lysis}$ x 100,where $A_{samples}$ and $A_{100\%lyses}$ are the absorbance of the hemoglobin released from erythrocytes incubated with different concentration of saline and that incubated with distilled water (100% lysis) respectively.

## 3. Results

Table 1 shows the formation of MNPCE, PCE and NCE in the bone marrow cells in both control and exposed groups. The results show a decrease in the formation of PCE and an increase in the number of NCE formed in the exposed samples compared to the control ones. These differences were statistically significant ($p<0.05$)*.

The results from DNA fragmentation using agarose gel electrophoresis technique are shown in figure (1). Figure(1) shows that, lane(1) represents marker of standard molecular weight, lane(2) represents DNA isolated from the spleen of the control group, lanes(3,4,5) represent DNA isolated from the spleen of the exposed group, lane(6) represents DNA isolated from the liver of the control group, lanes(7,8,9) represent DNA isolated from the liver of the exposed group. The results show that the DNA from viable cells stayed on the top of the gel as a high molecular weight band. The DNA from apoptotic cells formed as a distinct DNA ladder. The results do not show any difference between the DNA isolated from the spleen and liver of the control and exposed groups.

Table 2 shows the BMD, BMC and cross sectional area of the femora for both control and exposed groups. The data revealed no significant change in bone parameters of the femora for exposed group compared to the intact one.

There was no significant difference found between bone minerals density of control group compared to the exposed one (ANOVA $P$=0.15), see table 2. Also there was no significant change found in the bone minerals content gm/cm of the control group with reference to the exposed one (ANOVA, $P$=0.26), see table 2. There was no significant difference found between cross sectional area of control group compared with the exposed one (ANOVA, $P$=0.27), see table 2.

Table 3 shows changes in hepatic enzymes (AST),(ALT) and (ALP). The hepatic enzymes (AST and ALT) increased after being exposed to the magnetic field and table 4 shows some hematological data for magnesium (Mg),(Ca), uric acid, Glucose, Creatinine, Cholesterol and hemoglobin.The plasma

magnesium level was significantly higher in exposed group compared with control one. In addition serum uric acid level is significantly higher in exposed group compared with the control one.

Figure(2) shows the osmotic fragility curves for both control and exposed groups. The percentage of lyses in erythrocytes is slightly increased for the exposed group compared with the control one. The error bars represent standard deviation.

**Table 1: Effect of ELF- MF on NPCE, PCE and NCE formed in bone marrow cellsof six rats after 30 days of ELF-MF exposure.**

| **Samples** | NCE/1000 cells | PCE/1000 cells | MNPC/2000 cells |
|---|---|---|---|
| **Control** | 509 ± 6 | 491 ± 6 | 3.5 ± 0.5 |
| **Exposed** | 558 ± 11 | 442 ± 11 | 23 ± 4 |
| ***p*-value** | 0.05 | 0.05 | 0.05 |

Errors indicate the standard error of mean (SEM) for N = 6

**Table 2: Effect of ELF-MF on bone parameters in the right femora of six rats after 30 days of ELF-MF exposure.**

| **Samples** | BMD gm/cm$^2$ | BMC gm/cm | Area cm$^2$ |
|---|---|---|---|
| **Control** | 0.122 ± 0.002 | 0.22 ±0.05 | 1.93 ± 0.40 |
| **Exposed** | 0.121 ±0.007 | 0.22 ±0.05 | 1.93 ± 0.19 |
| ***p*-value** | 0.15 | 0.26 | 0.23 |

Errors indicate the standard error of mean (SEM) for N = 6

**Table 3: Effect of ELF- MF on the level of AST, ALT and ALP in six rats after 30 days of ELF-MF exposure.**

| **Samples** | AST U/L | ALT U/L | ALP U/L |
|---|---|---|---|
| **Control** | 12 ±14 | 38 ±1.2 | 749 ±83 |
| **Exposed** | 160 ±10 | 55 ±1.7 | 496 ±33 |
| ***p*-value** | 0.05 | 0.001 | 0.04 |

Errors indicate the standard error of mean (SEM) for N = 6

**Table 4: Effect of ELF-MF on the level of Mg, Ca, Glucose, Uric acid, Creatine, cholesterol and Hb in six rats after 30 days of ELF-MF exposure.**

| **Sample** | Mg mg/dl | Ca mg/dl | Glucose mg/dl | Uric acid mg/dl | Creatinine mg/dl | Cholestrol mg/dl | Hbgm/dl |
|---|---|---|---|---|---|---|---|
| **Control** | 1.5 ±0.06 | 7.6 ±0.2 | 116 ±7.12 | 0.93 ±0.09 | 0.5 ±0.06 | 73 ±4.041 | 10.93 ±0.03 |
| **Exposed** | 2.23 ±0.03 | 8.1 ±0.15 | 127 ±16 | 1.93 ±0.26 | 0.63 ±0.03 | 74.33 ±10.86 | 10.66 ±0.49 |
| ***p*-value** | 0.001 | 0.12 | 0.55 | 0.02 | 0.12 | 0.91 | 0.61 |

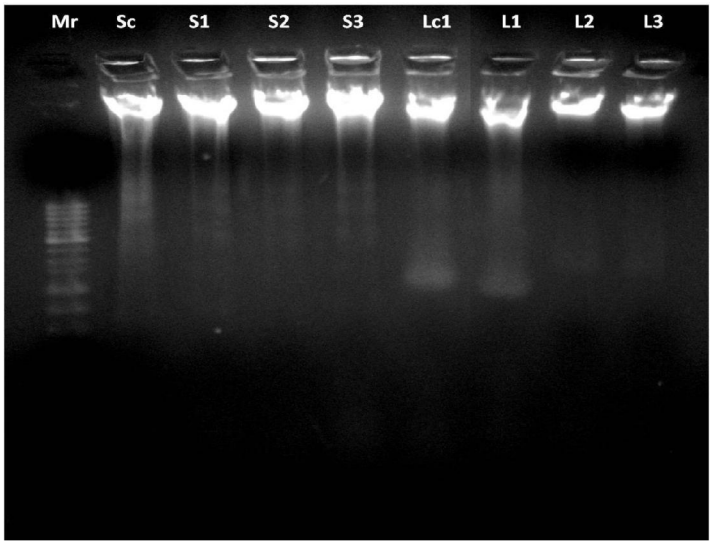


**Figure (1): Gel electrophoresis of DNA. Lane(1) marker of standard molecular weight, lane(2) DNA isolated from spleen of control group, lanes(3,4,5) DNA isolated from spleen of exposed group, lane(6) DNA isolated from liver of control group, lanes(7,8,9) DNA isolated from liver of exposed group**

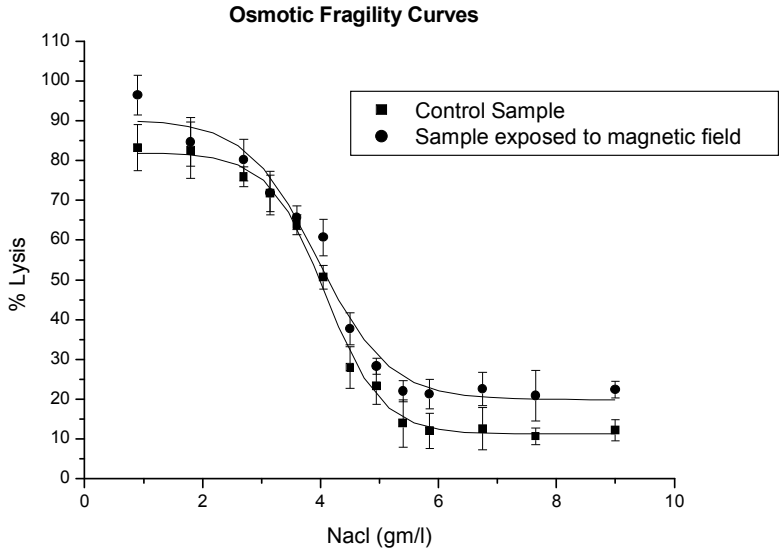


**Figure (2): Effect of 0.5 mT, 50 Hz magnetic field on the erythrocytes lyses for blood samples from control ■ and exposed ● groups. Values are means ± SD from n = 6 in each group.**

## 4. Discussion

The present study is concerned with the effects of ELF-MF on the formation ofmicronucleus, structure of DNA, density and content of bone minerals and some hematological parameters. The data indicated that continuous whole body exposure of rats to 50 Hz, ELF-MF at a flux density of 0.5 mT induced cell toxicity which was observed by a significant decrease in both PCE proportion and PCE/NCE ratio. In addition to a marked increase in the MNPC formation table 1, such effects are in agreement with previous study of Nurten *et al.* [29] who reported the occurrence of bone marrow cytotoxicity upon exposure to magnetic field. The above results are also considered as an evidence that 50 Hz ELF-MF 0.5 mT is a possible potent inhibitor of mitosis.

However, ELF-MF has no effect neither on DNA damage figure(1) nor on the bone examination made for rats femora table 2. The present data of DNA is in consistent with the studies of Milena *et al.*[30] and McNamee *et al.*[31] who reported that there was no significant effect on DNA strand breaks upon ELF-MF exposure. Moreover, the percentage lyses of erythrocytes for exposed group showed a slight increase compared to that of the control one (Fig.2). Such increments were considered within their normal range indicating that magnetic field exposure level in this study, had no marked effect on both structure and function of erythrocytes membrane **[**32**].**

Obvious changes were observed in clinical pathology measurements shown in tables 3 and 4. Exposing rats to 0.5 mT over a long period of time (30 days) produced an increase in Mg level and a non-significant increase in the glucose level. This might be due to changes in the metabolic rate [33]. In addition, there was significant increase in the hepatic enzymes of the exposed group. This finding supports the suggestion that the exposure to ELF-MF is associated with higher levels of oxidative stress and formation of free radicals [34, 35]. The lifetime of free radicals is very short and their high reactivity and the rapid rate of collisions among molecules cause them either to recombine or to interact with their surrounding within short time of their formation. Accordingly, such free radicals can affect some enzyme activities. The proportion of radicals reacting with biological molecules would increase leading to possible adverse effects on cell structure and organs function. This is supported by significant changes in ALP activity, creatinine and uric acid measurements which are considered as evidence on tissue damage in the exposed group.

Literatures on cellular effects of ELF electric and magnetic fields were reviewed bySantini*et al.*[36] who pointed out that the majority of the "*In vitro*" experimental results indicated that such fields induce numerous types of changes in cells. Such analysis concluded that this myriad of effects on biological systems should not be ignored in evaluating human health risk. Vijayalaxmi and Prihoda[37] published a meta-analysis of data from 87 publications concerning genericdamage in mammalian cells following EMF exposure. The authors main

conclusion was that differences between exposed and control cells were biologically small although statistically significant with very few exceptions.

## 5. Conclusion

It was concluded from the present study, that continuous exposure to ELF-MF (50Hz, 0.5mT) might cause micronucleus formation and some physiological disturbance in Wistar rats. The same exposure dose might have no effect on the DNA structure and bone parameters. 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.

**Declaration of interest**

The authors report no conflicts of interest. The authors alone are responsible for the content andwriting of the paper.

**Corresponding author**:
**Monira M. Rageh**
Biophysics Dept. Faculty of science
Cairo University-Egypt
e-mail:moniraageh@yahoo.com

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