

Investigation of Potential Genotoxic Effects of Magnetic Field Used in Imaging

Görüntüleme de Kullanılan Manyetik Alanın Genotoksik Etkileri

✉ Handan Kayhan¹, ✉ Serdar Koca², ✉ Melike Özdemir², ✉ Berk Dayanir³, ✉ Barış Söylemez³, ✉ Yelda Özsunar⁴

¹Gazi University Faculty of Medicine, Department of Adult Hematology, Ankara, Turkey

²Aydın Adnan Menderes University Faculty of Art and Sciences, Department of Biology, Aydın, Turkey

³İzmir Bahçeşehir Science College, İzmir, Turkey

⁴Aydın Adnan Menderes University Faculty of Medicine, Department of Radiology, Aydın, Turkey



Abstract

Objective: Although the high magnetic field is commonly accepted as harmless for biological tissues, there is no consensus about its biological effects. This study aims to investigate probable genetic damages of magnetic field on biological tissues using a simple and widely accepted method, Allium test.

Materials and Methods: The same sized healthy Allium cepa (onion) plants were exposed to 0.5 Tesla magnetic field for 0, 8, 24 and 72 hours as groups of four. Allium test was applied and at least 4.000 cells were counted for each group. Observed chromosomal aberrations were analyzed and photographed.

Results: Magnetic field application adversely affected the mitotic activity in the experiment group compared to the control. The chromosomal aberrations increased in proportion to increased magnetic field exposure times. The most encountered aberrations were C-metaphase, stickiness, lagging chromosome, anaphase bridge, micronucleus, irregular anaphase, and polar deviation. The group comparisons showed statistically significant differences between the control group and 8, 24 and 72 hour magnetic field exposure groups.

Conclusion: This study has shown potential genotoxic and mutagenic effects of high magnetic field on Allium cepa root tip cells using Allium test. Although there is a need for more studies, the data in the study show that the strong magnetic field leads to chromosomal disorders.

Keywords

Magnetic field, metaphase, mutagenic effects, Allium test, *Allium cepa*

Anahtar Kelimeler

Manyetik alan, metafaz, mutajenik etkiler, Allium testi, *Allium cepa*

Received/Geliş Tarihi : 29.03.2017

Accepted/Kabul Tarihi : 30.11.2017

doi:10.4274/meandros.galenos.2017.99608

Address for Correspondence/Yazışma Adresi:

Handan Kayhan MD,
Gazi University Faculty of Medicine, Department of Adult Hematology, Ankara, Turkey
Phone : +90 536 439 25 99
E-mail : kayhanhandan@gmail.com
ORCID ID: orcid.org/0000-0003-4967-2740

©Meandros Medical and Dental Journal, Published by Galenos Publishing House.
This is article distributed under the terms of the Creative Commons Attribution NonCommercial 4.0 International Licence (CC BY-NC 4.0).

Öz

Amaç: Yüksek manyetik alan yaygın olarak biyolojik dokular için zararsız kabul edilse de, biyolojik etkileri konusunda fikir birliği yoktur. Bu çalışma, basit ve yaygın olarak kabul gören Allium testini kullanarak biyolojik dokulardaki manyetik alanın muhtemel genetik hasarlarını araştırmayı amaçlamaktadır.

Gereç ve Yöntemler: Aynı büyüklükte sağlıklı Allium cepa (soğan) bitkileri 0, 8, 24, 72 saat 0,5 Tesla manyetik alana maruz bırakılan dört grupta incelendi. Maruziyet sonrası Allium testi yapılmış ve her grup için en az 4.000 hücre incelenmiştir. Bu hücrelerde gözlemlenen kromozom anormallikleri analiz edildi ve fotoğraflandı.

Bulgular: Manyetik alan uygulaması, mitotik aktiviteyi olumsuz etkiledi. Kromozomal sapmalar, manyetik alana maruz kalma sürelerine oranla arttı. En sık görülen sapmalar C-metafaz, yapışkanlık (stickiness), geri kalmış kromozom (lagging chromosome), anafaz köprüsü (anaphase bridge), mikronükleus (micronucleus), düzensiz anafaz (irregular anaphase) ve kutup sapması (polar deviation) oldu. Grup

karşılaştırmaları, kontrol grubu (0 saat) ile 8, 24 ve 72 saatlik manyetik alan maruziyet grupları arasında istatistiksel olarak anlamlı farklılıklar gösterdi.

Sonuç: Bu çalışma, Allium testini kullanarak güçlü manyetik alanın potansiyel genotoksik ve mutajenik etkilerini göstermiştir. Her ne kadar daha fazla çalışmaya ihtiyaç bulunsa da, bu çalışmadaki veriler göstermiştir ki, güçlü manyetik alan kromozomal bozukluklara yol açmaktadır.

Introduction

Living organisms are continuously exposed to magnetic fields at different doses in our modern world. The use of devices generating high magnetic fields in industrial processes, medical diagnostics, new vehicles for transportation and some research facilities is expected to expand significantly in the near future.

In the last years, there has been a substantial increase in human exposure to strong static magnetic fields, especially after the usage of magnetic resonance (MR) technology as a clinical method for imaging. With MR, a high static magnetic field has become a standard for imaging in the health sector. Today most of the MR systems are applied in 0.2 to 3.0 Tesla ranges and their use for imaging is becoming more and more widespread.

According to the U.S. Food and Drug Administration, static magnetic fields up to 8.0 Tesla which is used in clinical MR system, is considered harmless to humans. Accordingly, numerous MR procedures are being performed on all age groups, including pregnant women and newborns. However, many researchers found that the safety and the potential effects associated with MR systems and procedures are still under debate (1-3). Due to the frequent use of high magnetic field and the controversial reports about the effects of static magnetic field, this study aims to investigate the potential genotoxic effects that might be produced by static magnetic field, using Allium cepa test.

A magnetic field is generated by magnetic materials or electrical currents. The geomagnetic field (the magnetic field of Earth) is due to its solid iron core and it is not constant. It varies at the surface from 26 micro-tesla (μT) from the equator to about 60 μT to the poles. Magnetic fields can be also produced artificially by medical imaging systems, power lines, electromagnets and everything that carries electric current. Due to the wide use of artificial magnetic fields, the level of magnetic fields exposure to humans may be considerably increased over the last century.

In Magnetic Resonance Imaging (MRI) field, different types of electromagnetic fields are used: 1. The static magnetic field used in this study, which aligns the axes of the proton, and creates a magnetization vector in the physical body, 2. The radio-frequency electromagnetic wave, centered at the proton resonant frequency and 3. The gradient magnetic field, producing different resonant frequencies for aligned protons that means different slices of the body will resonate at different frequencies contingent on their spacial positions on the axes; these gradient magnetic fields permit spacial localization of bi-dimensional MRI slices and so the reconstruction of three-dimensional MRI images (1-3).

This study used the Allium cepa test to identify the biological effects of MR. This test is accepted as a practical and sensitive method to detect environmental genotoxic and mutagens (4). This test is also enables to demonstrate the effects on DNA of the exposed organisms for various chemicals or other tested agents on DNA. The Allium cepa test has good correlativity compared with other test systems, such as mammalian test systems and also it is very sensitive. Actually, it has been accepted as a standard test to determine chromosomal damages affected by environmental and chemical agents (4). One study showed 82% of correlation for Allium cepa test in relation to the carcinogenicity test in rodents showing that Allium cepa test was virtually the same as the one observed for mammalian test systems (5). The Allium cepa test was found to be more sensitive than the Ames and the Microscreen tests (4). In addition, another study reported that the Allium cepa test system is one of the best-constituted test systems to evaluate the potential of genotoxicity (6). Thus, it is suggested that Allium cepa test is a good alternative to screen genotoxic potential of environmental chemicals.

Materials and Methods

We did not use any sample belongs to humans or animals in our study. For this reason ethics committee approval or informed consent was not necessary.

Preparation of Allium Cepa Materials

Healthy bulbs of Allium cepa (onion) (4n=20) that were about the same size, were chosen. One control group (n=5) and 3 exposed groups (n=3x5) were placed on a plate. The onions were kept away from moisture and direct sunlight exposure. The outer shells of the bulbs were carefully peeled. Rooting was done in an incubator at 25±2 °C temperature without light. The control group was kept in the same conditions without any exposure to magnetic field.

Magnetic Field Exposure of Allium Cepa Materials

Allium cepa plants were placed at the back of the MR bore on a table in platters as is shown in Figure 1 with a red cross and then exposed to 0.5 tesla magnetic field (Philips Achieva 1.5 T). The strength of the magnetic field was measured with a specific device called Teslameter (Metrolab, precision NMR Teslametre PT 2025, Switzerland) (Figure 2). The intensity of magnetic field was confirmed to be 0.5 T at 112 mm of Z axis in the central bore (Figure 1). Then the three groups of onions were kept in that location for 8, 24, and 72 hours.

Rooting of Allium Cepa Materials

Following the magnetic exposure, onions were placed into small tubes filled with tap water for rooting in an incubator set at 25±2 °C temperature without light. The control group was rooted under the same conditions without any exposure to magnetic field. Each group consisted of five onions.

Chromosomal Analysis

The roots were cut when they reached a length of 1.5-2.0 cm and were fixed for 24 hours at +40 °C within freshly prepared ethyl alcohol and glacial acetic acid (3:1) mixture. The roots were then washed with 70% ethyl alcohol and kept in a refrigerator immersed in 70% alcohol. Roots were hydrolyzed with 1N HCl (2-3 minutes) before examination and then stained with acetoorcein for 3-4 hours. Examination slides were prepared according to the squash method (7) and chromosomes examined under the microscope (Olympus BX51). Allium test was applied and at least 4.000 cell nuclei were examined for each group. The scorings were done in a blind way. The chromosomal aberrations were counted in each nucleus and some of them photographed (Figure 3).

Statistical Analysis

The statistical analyses were performed by scoring the dividing cells. Both mitotic index and chromosomal aberrations were analyzed. To determine the significance among mean values, One-Way ANOVA was used. The variance was normal.

Results

Examination of slides has shown that magnetic field exposure adversely affected mitotic activity compared to the control group. However, this effect was not found to be statistically significant between groups (p>0.05) (Table 1).

Chromosomal aberrations during mitosis following exposure of Allium cepa root tip cells to magnetic field was given in Figure 3. Chromosomal aberrations increased in proportion to the in-creased time of magnetic field exposure. The most frequently encountered aberrations were C-metaphase stickiness, anaphase bridges, lagging chromosomes, fragments and polar deviations (Figure 3).

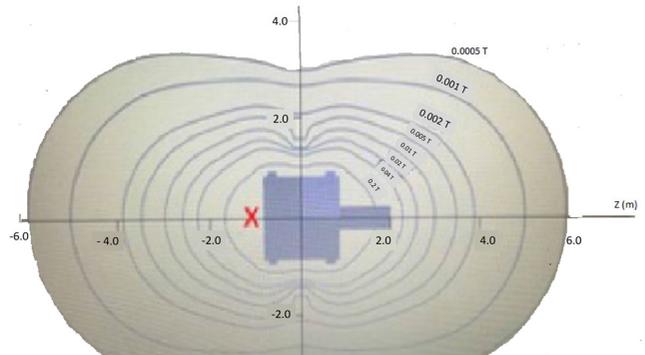


Figure 1. The placement of the plants within the magnetic room is marked as red x on a Gauss map. The magnet is shown in the center, the magnetic field strength decreased gradually with the square function of distance as indicated by Gauss lines



Figure 2. A Tesla-meter used for the measurement of strength of location shown in Figure 1

Table 1. The genetic analyses results of the control and 0.5 Tesla magnetic field exposed groups (8, 24 and 72 hours) of Allium cepa roots

Exposure Groups	ETC	Proph	Metaph	Anaph	Teloph	TM	MI	TA	TA(%)*
Control	4880	57	35	46	41	179	3.66	25	0.51*
8 hours	5920	26	23	48	48	145	2.44	80	1.35*
24 hours	4612	66	58	48	52	224	4.85	117	2.53*
72 hours	4560	54	41	43	43	181	3.96	117	2.56

ETC: Examined total cell, Proph: Prophase of cell cycle, Metaph: Metaphase of cell cycle, Anaph: Anaphase of cell cycle, Teloph: Telophase of cell cycle, TM: Total mitosis, MI: Mitotic index, TA: Total Aberrant Cells, *The differences between the ones marked with different letters were statistically significant (p< 0.05), The significance was determined by one-way ANOVA test

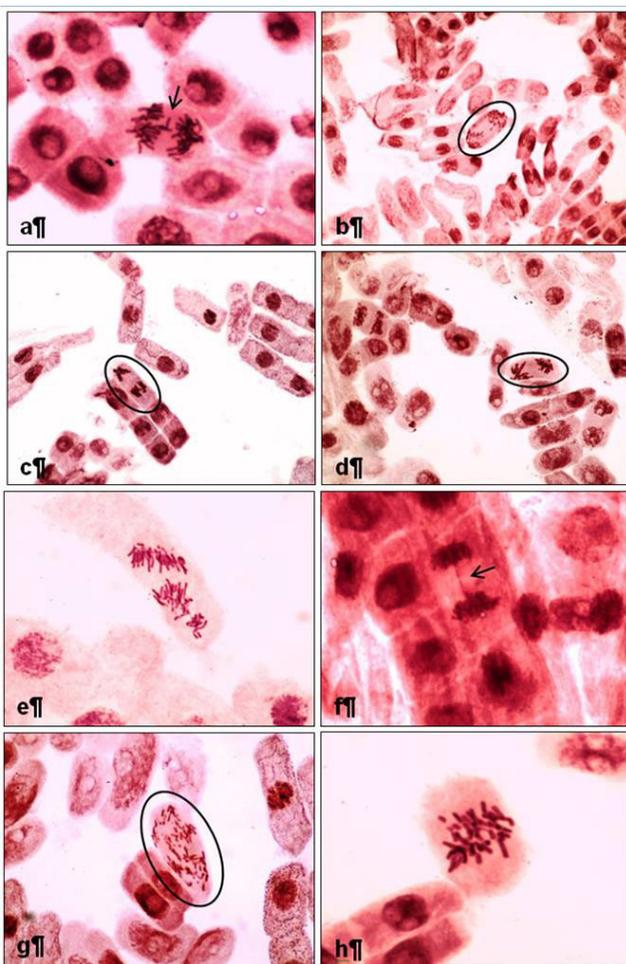


Figure 3. Different types of aberrations induced by static magnetic field in Allium cepa root-tips after 8, 24 and 72 hours static magnetic field exposures. The pictures were visualized with light microscopy at 1000x magnification. a) lagging chromosome, b) irregular anaphase, c) stickiness, d) polar deviation, e) multipolarity, f) anaphase bridge, g) fragment, h) C-mitosis

Table 2. Comparison of the total aberrant cells of exposure groups with each other and the resultant statistical significance levels

ANOVA statistical test results for exposure groups comparisons		
Groups		p
Control	8 th hour	0.003 *
	24 th hour	<0.001 *
	72 th hour	<0.001 *
8 th hour	24 th hour	0.044 *
	72 th hour	0.044 *
24 th hour	72 th hour	1.000

The significance was tested by one-way ANOVA test, *The difference was statistically significant (p<0.05)

Comparing groups that were exposed to magnetic field 24 and 72 hours to 8 hours have shown that as the time of magnetic field exposure increased, aberrations were encountered more frequently. The groups were compared using one-way ANOVA test. The percentages of total aberrations were significantly different between the control group and 8, 24 and 72 hours magnetic field exposure groups (p<0.05). However, there was no significant difference when 24 and 72 hours exposure groups were compared (p>0.05). The statistical significance values are presented in Table 2.

Discussion

Our study showed that static magnetic field brings about potential genotoxic effects on Allium cepa

plant. Many chromosomal aberrations pointing to the presence of chromosomal breakage were seen in common *Allium cepa* exposed to a magnetic field when compared to the control. All these findings reveal that static magnetic field especially within the first 24 hours, result in chromosomal breakage and mitotic anomalies.

Although it was shown that magnetic field causes root elongation (8), little is known about its genotoxic effect. These studies usually employed low electromagnetic fields (9). In these studies, low-intensity magnetic field found in high voltage electric lines was shown to increase mitotic activity and chromosomal anomalies.

There were studies showing static magnetic field interaction with free radicals causing genetic mutations, supporting our findings (10). However, these studies point out the difficulty in demonstrating the biological effect of static magnetic field (3). Because *Allium* test method is accepted as a practical, fast, simple and easy method (11), we preferred this valid method to show the effect of magnetic field on biological tissues. This method has been used to evaluate DNA damages, such as chromosome aberrations and disturbances in the mitotic cycle (12). The causes of chromosomal aberrations had collected in three groups (13). In the first group, we see mitotic chromosomal aberrations such as C-mitosis, multipolarity, polyploidy and lagging chromosome due to the interaction of chemical substances with mitotic apparatus as spindle and aster fibers. The second group comprises chromosomal stickiness as the result of a physiological effect on chromosomes during division. Chromosomal breakage, chromosomal bridges, fragments, and micronucleus can be mentioned in the third group. C-mitosis was one of the aberrations observed in all three magnetic exposure times. C-mitosis is the cell phase where chromosomes are irregularly distributed in the metaphase. The employed magnetic field might have caused a disturbance in spindle fibers similar to colchicine. As a result, centromere division lags and chromosomes are paired (replicated) but could not separate from one another and are irregularly distributed within the cell. Such abnormalities in the metaphase could result in a decrease of mitotic index. C-mitosis has been encountered in many tests where physical and chemical agents were used (14,15,16). These aberrations in metaphase may lead

to a decrease in mitotic index. C-mitosis has been observed in many test materials exposed to various physical and chemical stresses. Another aberration we encountered was anaphase bridges. Chromosomal bridges are often the result of clastogenic effect from use of environmental and chemical agents or sticking of chromosomes in metaphase causing chromosomal breaking and fusion of chromosome and chromatids (17). Irregular inversion and translocation of chromosomal segments may also lead to chromosomal bridges (18). Chromosome bridges or inter-chromatid connections, are fibers that hold sister chromatids together until late anaphase or telophase. In other words, chromosomes could not be separated easily at anaphase because of these bridges. This abnormal state causes genomic instability. Sometimes, when connections become too stretched, chromatids might break at or near the centromeres (19). This breakage may occur at different points in both sister chromatids resulting in chromosome like bodies called fragments.

It is accepted that static magnetic fields smaller than 1 T are not genotoxic (20,21). However, Suzuki et al. (22) found that micronucleus (a chromosome or a fragment of a chromosome that is not integrated by one of the daughter cell nuclei during cell division) frequency in mice were increased significantly in a dose dependent manner, when the mice exposed 2, 3, or 4.7 T static magnetic fields for 24, 48, or 72 h. Micronucleus frequency was significantly increased after 4.7 T and 3 T but there was no significant effect after 2 T exposure. Therefore, the study showed that higher magnetic fields may have induced directly or indirectly chromosome separation during cell division.

The mitotic index of the exposure groups differed from that of the control group according to the exposed magnetic field. Mitotic index was observed decrease to 2.44 in 8 hour exposure group compared to the control group, which had a mitotic index of 3.66. Although mitotic index in 24 and 72 hours exposure groups increased to 4.85 and 3.96 respectively, the difference was not statistically significant (Table 1).

The decrease observed in the mitotic index could be attributed to mitotic inhibition. Various physical and chemical factors could lead to disturbances in cellular loops. Fitzgerald and Brehaut (23) have proposed that the decrease in the mitotic index could be a result of inhibition of DNA synthesis. Van't Hof has pointed out

that blockage or prolongation of the G2 phase of cell cycle delays or prevents the cell to enter mitosis (24).

Although further studies are needed in cell cultures, mammals, and human tissue, the present study shows the effects of static magnetic field on cell division and chromosomes in *Allium cepa* plants. Revealing the relationship between magnetic field and plant responses may lead to further studies on mammals to explain how and why these chromosomal aberrations may occur. Despite numerous investigations, the connection between a magnetic field and cancer or any other diseases is not clear. However, from the past and present studies, it seems that exposure to prolonged static magnetic field may be capable of producing chromosome aberration. The reasons of increased number of chromosome aberrations may be thought to results from the damages in DNA in interphase caused by free radicals to defects in the processing of the signals because of the magnetic field.

Study Limitation

The biochemical differences between plants and mammals.

Conclusion

It seems that, in this study and the literature, magnetic field may affect living organisms negatively. Therefore, long exposure time of magnetic field must be avoided. There are studies reporting the systemic effects of the static magnetic field. For example, Ghodbane and colleagues (25) showed that the static magnetic field of 128mT, 1 hour/day induced apoptosis in rat liver and increased liver catalase activity. Abdelmelek et al. (26) showed that noradrenergic systems in rat's gastrocnemius muscles were affected and HSP72 levels were increased. Another study showed that static magnetic field had a possible effect on blood through enzymes release and the blood cell proliferation (27). Observing adverse effects of high magnetic field on chromosomes makes one think whether this physical effect has any adverse effects on other living organisms, besides the target tissue. Therefore, more research is required to adequately understand the mechanisms associated with the static magnetic field used for imaging purposes.

Acknowledgement

We would like to thank Prof. Dr. Serdar Koca who helped and mentored this study and master

student Melike Özdemir from Aydın Adnan Menderes University Faculty of Science, Department of Biology; Prof. Dr. Mevlüt Türe from Biostatistics; Prof. Dr. Yelda Özsunar Dayanır from Aydın Adnan Menderes University Faculty of Medicine, Department of Radiology; and our consultant teacher Timothy Timur.

Ethics

Ethics Committee Approval: It was not necessary.

Informed Consent: It was not necessary.

Peer-review: Externally and internally peer-reviewed.

Authorship Contributions

Concept: Y.Ö., S.K., Design: S.K., Y.Ö., Data Collection or Processing: S.K., M.Ö., B.D., B.S., Analysis or Interpretation: S.K., M.Ö., Literature Search: H.K., Y.Ö., Writing: H.K., Y.Ö.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

References

1. Margonato V, Nicolini P, Conti R, Zecca L, Veicsteinas A, Cerretelli P. Biologic effects of prolonged exposure to ELF electromagnetic fields in rats: II. 50 Hz magnetic fields. *Bioelectromagnetics* 1995; 16: 343-55.
2. Formica D, Silvestri S. Biological effects of exposure to magnetic resonance imaging: an overview. *Biomed Eng Online* 2004; 3: 11.
3. Schenck John F. Safety of Strong, Static Magnetic Fields. *J Magn Reson Imaging* 2000;12;2-19.
4. Leme DM, Marin-Morales MA. *Allium cepa* test in environmental monitoring: a review on its application. *Mutat Res* 2009; 682: 71-81.
5. Rank J. The Method of *Allium* anaphase-telophase Chromosome Aberration Assay. *Ekologija* 2003; 1; 38-42.
6. Chauhan LKS, Saxena PN. Cytogenetic Effects of Cypermethrin and Fenvalerate on the root Meristems Cells of *Allium cepa*. *Environmental Experimental Botany* 1999; 42: 181-9.
7. Dubrovsky JG, Contreras-Burciaga L. A squash preparation method for root meristem field studies. *Biotech Histochem* 1998; 73: 92-6.
8. Maffei ME. Magnetic Field Effects on Plant Growth, Development, and Evolution. *Front Plant Sci* 2014; 5: 445.
9. Aksoy H, Unal F, Ozcan SJ. Genotoxic Effects of Electromagnetic Fields from High Voltage Power Lines on Some Plants. *Int Res* 2010; 4: 595-606.
10. Ghodbane S, Lahbib A, Sakly M, Abdelmelek H. Bioeffects of Static Magnetic Fields: Oxidative and Cancer Studies. *Biomed Res Int* 2013; 2013: 602987.
11. Rank J, Nielsen MH. Evaluation of the *Allium* anaphase-telophase test in relation to genotoxicity screening of industrial wastewater. *Mutat Res* 1994; 12: 17-24.

12. Eleftheriou EP, Adamakis ID, Melissa P. Effects of hexavalent chromium on microtubule organization, ER distribution and callose deposition in root tip cells of *Allium cepa* L. *Protoplasma* 2012; 249: 401-16.
13. Badr A. Effects of the S-triazine Herbicide Turbutryn on Mitosis, Chromosomes and Nucleic acids in root tips of *Vicia faba* root meristems. *Cytologia* 1986; 51: 571-8.
14. Dönbak L, Rencüzoğulları E, Topaktaş M. The Cytogenetic Effects of the Food Additive Boric acid in *Allium cepa* L. *Cytologia* 2002; 153-7.
15. El-Ghamery AA, El-Kholy MA, Abou El-Yousser MA. Evaluation of cytological effects of Zn²⁺ in relation to germination and root growth of *Nigella sativa* L. and *Triticum aestivum* L. *Mutat Res* 2003; 537: 29-41.
16. Rencüzoğulları E, İla HB, Kayraldız A. Chromosome Aberrations and Sister Chromatid Exchanges in Cultured Human Lymphocytes Treated with Sodium Meta bisulfit a Food Preservative. *Mutat Res* 2001; 490: 107-12.
17. Türkoğlu S. Genotoxicity of Five Food Preservatives Tested on Root Tips of *Allium cepa* L. *Mutat Res* 2007; 626: 4-14.
18. Gömürgeç AN. Cytological effect of the potassium metabisulphite and potassium nitrate food preservative on root tips of *Allium cepa* L. *Cytologia* 2005;70:119-128.
19. Türkoğlu S. Evaluation of genotoxic effects of sodium propionate, calcium propionate, and potassium propionate on the root meristem cells of *Allium cepa*. *Food Chem Toxicol* 2008; 46: 2035-41.
20. Matthes JH, Bernhardt PFA. ICNIRP, "Exposure to static and low frequency electromagnetic fields," in *Biological Effects and Health Consequences (kHz)*. R.Vecchia, and B.Veyret, Eds 2003; vol. M^{Druckun}chen, Germany. 13,0-100.
21. McCann J, Dietrich F, Rafferty C, Martin AO. Martin A critical review of the genotoxic potential of electric and magnetic fields. *Mutat Res* 1993; 297: 61-95.
22. Suzuki Y, Ikehata M, Nakamura K, Nishioka M, Asanuma K, Koana T, et al. Induction of micronuclei in mice exposed to static magnetic fields,". *Mutagenesis* 2001; 16: 499-501.
23. Fitzgerald PH, Brehaut LA. Depression of DNA synthesis and mitotic index by colchicine in cultured human lymphocytes. *Exp Cell Res* 1970; 59: 27-31.
24. Van't Hof J. Control of cell progression through the mitotic cycle by carbohydrate provision. I. Regulation of cell division in excised plant tissue. *J Cell Biol* 1968; 37: 773-80.
25. Ghodbane S, Ammari M, Lahbib A, Sakly M, Abdelmelek H. Static magnetic field exposure-induced oxidative response and caspase-independent apoptosis in rat liver: effect of selenium and vitamin E supplementations. *Environ Sci Pollut Res Int* 2015; 22: 16060-6.
26. Abdelmelek H, Molnar A, Servais S, Cottet-Emard JM, Pequignot JM, Favier R, et al. Skeletal muscle HSP72 and norepinephrine response to static magnetic field in rat. *Journal of Neural Transmission* 2006; 113: 821-7.
27. Amara S, Abdelmelek H, Salem MB, Rached Abidi R, Sakly M. Effects of static magnetic field exposure on hematological and biochemical parameters in rats. *Brazilian Archives of Biology and Technology* 2006; 49: 889-95.