

Research Article

# Anticancer Effects of Moderate Static Magnetic Field on Cancer Cells In Vitro

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## Abstract

**Background:** Expansion of the use of magnetic fields in metals, mining, transport, research, and medicine industries has led to a discussion about the effects of magnetic fields and whether their strength is negligible.

The aim of this study was to investigate the effects of magnetic field on the viability and proliferation rate of HeLa cells.

**Materials and Methods:** We studied the effects of magnetic field on the viability, proliferation rate and membrane lipid peroxidation of cells, thus, HeLa cells (cancer cells) and human fibroblast cells (normal cells) were used. Initially, the cells were cultured in DMEM and to determine the impact of the magnetic field, the cells were treated with magnetic field at 4 specific intensity levels (0, 7, 14 and 21 mT) and 2 exposure times (24 h and 48 h). The viability percentage and inhibition of cell proliferation were calculated by MTT assay and Trypan blue staining, respectively.

**Results:** Lipid peroxidation of the cell membrane was examined by malondialdehyde (MDA) method. As the intensity and exposure time of the static magnetic field (SMF) increased, the viability percentage and proliferation rate decreased and the lipid peroxidation levels increased in the HeLa cells.

**Conclusion:** In this study, we have shown the anticancer effects of static magnetic field and propose a suitable intensity range that can be effective for the treatment of cancer.

**Keywords:** Fibroblast cells, Human cervical cancer (HeLa) cell line, Lipid peroxidation, Proliferation rate, Viability percent

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## 1. Introduction

In modern society, humans, animals as well as plants are exposed to magnetic fields that are usually produced by high voltage transmission lines and many electrical devices. Today, one of the major questions related to magnetic field exposure is whether they can

affect biological systems (1, 2). It has been seen that magnetic fields affect cell survival (3), cell differentiation (4), reproduction (5), apoptosis (6), and gene expression (7), cause phenotypic abnormalities in mice (8) and imbalance in the concentration of sodium ions (9) and calcium ions (10, 11) on both sides of the membrane (12) and enzymatic activity (13). A few reports have shown that magnetic fields can be used to treat and relieve pain (14, 15). In addition, recent advances in the field of medical devices such as magnetic resonance imaging (MRI) and trans cranial magnetic stimulation (TMS) have raised concerns about the effects of strong magnetic fields on human health. In living organisms, apoptosis is one of the processes that is influenced by magnetic fields. Apoptosis is a physiological process that occurs in normal as well as abnormal cells. Morphological changes observed at the time of apoptosis include rapid compression of cytoplasm and chromatin, DNA fragmentation and creation of bubbles in the cell membrane and the subsequent fragmentation of cells and formation of apoptotic bodies. These apoptotic bodies contain a portion of the cytoplasm and organelles along with nuclear material and are surrounded by a cell membrane (16). Apoptosis could be due to the effect of magnetic fields on the cell membrane. In recent decades, the incidence of cancer has increased in most modern societies (17) and it is the second most common cause of mortality in the world. Although chemotherapy is common in cancer treatment (18), chemotherapy drugs have harmful effects, including depression, severe weakness, loss of appetite, body weight loss and infertility (19). Due to these side-effects of chemotherapy, the use of physical factors such as magnetic fields can be helpful. In this study, different intensities and exposure times of magnetic fields were used and their impact was investigated on the viability percentage, proliferation rate and membrane lipid peroxidation in cells.

## 2. Materials and Methods

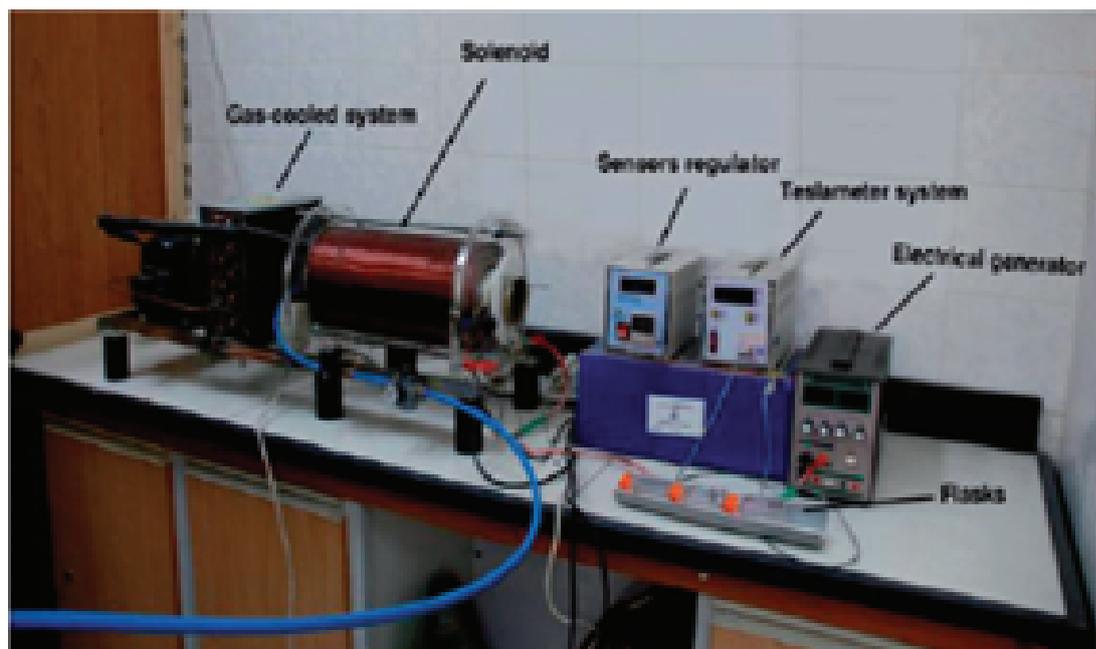
### 2.1. Cell culture

The human cervical cancer (HeLa) cell line and fibroblast (mesenchymal) cells were purchased from National Cell Bank of Iran (NCBI) and cultured at 37°C, 5% CO<sub>2</sub> in DMEM supplemented with 10% fetal bovine serum, 100 U/mL of penicillin and 100 µg/mL of streptomycin. The cells were grown until they reached a confluency of 70 to 80%. The confluent cells were passaged and plated at 1:2 or 1:3 dilutions every 3 to 4 days using 0.25% trypsin and 1 mM EDTA (Invitrogen LT, Merelbeke, Belgium). The cells were frozen in DMEM with 93% FBS and 7% DMSO (Merck, Germany) in liquid nitrogen.

### 2.2. Magnetic field application

Exposure to SMF was performed using a locally designed SMF generator (Figure 1). The electrical power was provided using a power supply working at a range of 0-50 V and 0-20 A with a maximum power of 1 kW. This system consisted of a 40-cm-long solenoid (1800 loops of 2.5 mm coated copper wire) equipped with an incubator included inside the solenoid (a copper container of 40 cm length and 8 cm diameter). Using three different sensors, the controller system was able to control the temperature, humidity

and CO<sub>2</sub> level. Heat was efficiently removed by a gas-cooled system. The circulation system consisted of a condenser, refrigerator engine and heat-exchanging pipe network of copper with 8-mm thickness which covered both the inner and the outer sides of the solenoid. This system was designed to generate SMF in the range of 0.5  $\mu$ T to 90 mT under stable conditions. A stabilizer board was used to stabilize the system so that a uniform SMF could be generated inside the exposure unit. Calibration of the system as well as tests for the accuracy and uniformity of the SMFs were performed using a teslameter (13610.93, PHYWE, Gottingen, Germany) with a Hall Effect sensor, which is a transducer that varies its output voltage in response to the magnetic field. The accuracy of the teslameter was  $\pm 0.1\%$  for SMF and the range of measurements taken were between 3  $\mu$ T to 30 mT. The presence of any pulsation in the efferent current was tested by an oscilloscope (40 MHz, model 8040, Leader, Japan). Temperature was routinely checked before and after all fields and control exposures. The intensities selected for the experiments were 0, 7, 14 and 21 mT. Three flasks were placed at the center of the incubator (10 cm distance from the center on each side) within the solenoid, which was generating a homogenous magnetic field, for each exposure. The durations of exposure were selected as multiples of the cell cycle duration and were equal to 24 h and 48 h. Before each exposure, the SMF was set to an appropriate intensity using a teslameter. The value of the intensity of the geomagnetic field in our lab was calculated to be 47 mT based on the measurement performed by Tehran geomagnetic observatory, Institute of Geophysics, University of Tehran.



**Figure 1:** Photograph showing the apparatus used to generate the static magnetic field.

### 2.3. MTT assay

The cell suspensions were seeded into a 96-well-plate and incubated for 24 h (10,000 HeLa cells and 4000 fibroblasts per well). The cells were allowed to reach exponential

growth by resting for least 24 h which is equal to the duration of one cell cycle for the selected cells. After removing the used medium, 100  $\mu\text{L}$  of fresh medium was added into each well. Five wells were selected for evaluating the effect of different intensities (0, 7, 14 and 21 mT) and exposure times (24 h and 48 h). The cells were incubated with 10  $\mu\text{L}$  of 5 mg/ml 3-(4,5-dimethylthiazol-2-yl)2,5 diphenyltetrazolium bromide (MTT), which was dissolved in 90  $\mu\text{L}$  of medium, for 4 h after each treatment. The viable cells cleaved the yellow tetrazolium salt to an insoluble purple precipitate (formazan). Hence, decrease in the percentage of living cells is correlated with the reduction in the amount of formazan precipitate crystals. Then the supernatant was discarded, the formazan precipitate was saved and 100  $\mu\text{L}$  of DMSO was added into the wells. The absorbance of the specimen was measured at 460 nm using the BioTek ELx808 microplate reader. The cell viability results were shown as percentages in comparison with that of the control group. To quantify the sensitivity of the selected cell types, the half maximal inhibitory concentration ( $\text{IC}_{50}$ ) which is the intensity of stocktickerSMF required for a 50% inhibition of cell growth was also measured.

## 2.4. Trypan blue staining

After each treatment, a cell suspension was prepared from each flask by washing the cells with PBS and trypsinizing them. To investigate the proliferation rates of cells, staining with Trypan blue was done followed by cell counting. We measured the proliferation rates of cells by dividing the number of cells per milliliter before and after treatment. Since it is a ratio of two numbers with the same dimension, the result, which is the proliferation rate, will be without dimension.

## 2.5. Estimation of lipid peroxidation

The cell membrane damage was determined by measuring malondialdehyde (MDA) as the end product of membrane lipid peroxidation. In brief,  $5 \times 10^5$  cells/mL was homogenized in 10% (v/v) trichloroacetic acid (TCA). After sonication on ice, 1 mL of 0.5% (v/v) thiobarbituric acid (TBA) solution was added to 1 mL of the supernatant. The final solution was incubated for 30 min in a 100°C water bath and then transferred to an ice-cold water bath. The absorbance of MDA was read at 532 nm followed by correlation for the nonspecific absorbance at 600 nm. The amount of MDA-TBA complex was calculated using an extinction coefficient of  $155 \text{ mM}^{-1} \text{ cm}^{-1}$  (20).

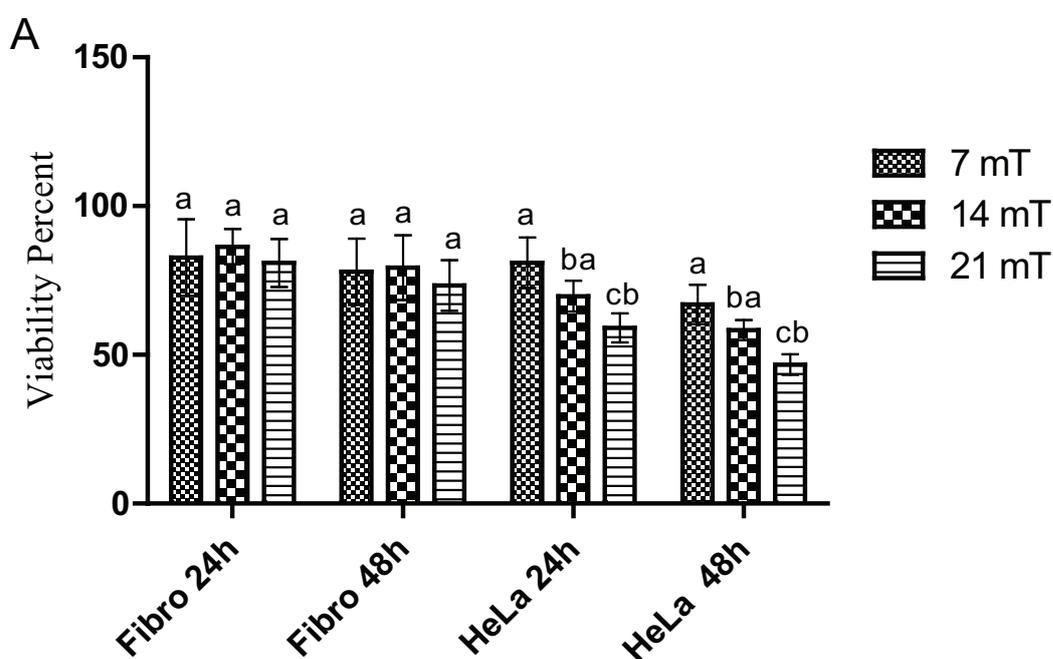
## 3. Statistics

Data are represented as mean  $\pm$  SD (standard deviation) of at least three independent replicates were compared by analysis of variance (ANOVA) and the Post test (Tukey-Kramer multiple comprehension test).  $P < 0.05$  was considered the criteria for significant differences.  $\text{IC}_{50}$  results were calculated by the program in Graphpad prism 5.

## 4. Results

### 4.1. Viability percentage

In order to determine the effect of the intensities and exposure times of the magnetic field, we selected 4 intensity levels (0, 7, 14 and 21 mT) and 2 exposure times (24 h and 48 h). These time durations were selected based on the doubling time of HeLa cells and fibroblasts following a 24-h cycle. The results showed that increasing the time of exposure and intensity of the static magnetic field reduced the viability percentage of the treated cells. Whereas this reduction was significant in HeLa cells, it was not pronounced in the normal cells. The greatest reduction in viability percentage was observed at an intensity of 21 mT for a duration of 48 h (Figure 2A).



**Figure 2:** The mean viability percentage (A) and proliferation rate (B) of fibroblast and HeLa cells after exposure to different intensities (7, 14 and 21 mT) and exposure times (24 h and 48 h). Data is represented as mean  $\pm$  SD,  $n = 3$ . Different letters denote significant differences according to the Tukey test ( $P < 0.05$ ).

### 4.2. Proliferation rate

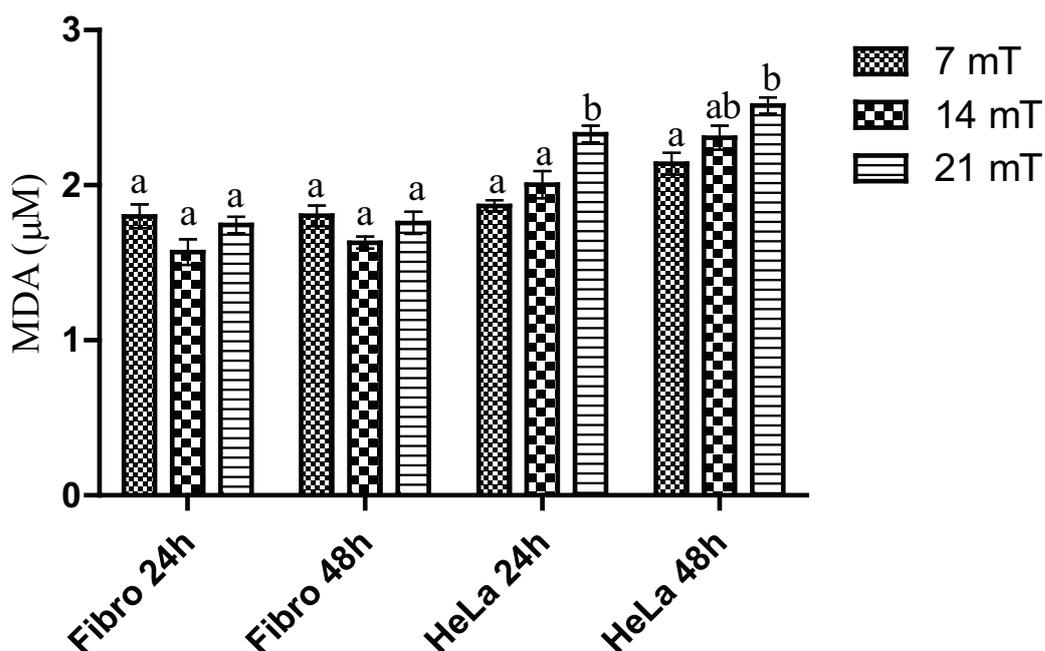
Similarly, the results show that by increasing the exposure time and intensity of the static magnetic field, the rate of cell proliferation in HeLa cancer cells decreased more drastically compared with the fibroblast cells. Figures 2B showed the calculated proliferation rate of HeLa and fibroblast cells after subjecting them to different intensities of SMF and exposure times. The result exhibited that by increasing the intensity as well as exposure time, the proliferation rate was decreased. However, this decrease was less obvious in the fibroblast group than the HeLa group. In addition, the most significant result was also observed in the group which was exposed for 48 h to 21 mT of SMF magnetic field.

### 4.3. Half maximal inhibitory concentration (IC<sub>50</sub>)

The intensity of SMF required for 50% inhibition of cell growth (IC<sub>50</sub>) was obtained by extrapolation from an inhibition curve. In the presence of SMF, the IC<sub>50</sub> values of fibroblast cells were  $75.90 \pm 30.79$  and  $48.51 \pm 16.47$  mT at 24 h and 48 h, respectively. The IC<sub>50</sub> values of HeLa cells were  $30.9 \pm 4.53$  and  $17.57 \pm 2.05$  mT at 24 h and 48 h, respectively. The obtained IC<sub>50</sub> values for fibroblast cells were greater than that of the HeLa cells. This result suggested that HeLa cells were more sensitive to SMF.

### 4.4. Lipid peroxidation

The level of the peroxidation of the membrane lipids in SMF-treated HeLa cells were significantly higher than that seen in the fibroblast cells at both the exposure time periods (Figure 3). Interestingly, there was an obvious increase in the cell membrane lipid peroxidation after 48 h of exposure and thus, it seems that long term exposure to SMF has more pronounced effect on the cells compared to short term exposure. The results exhibited that by increasing the intensity as well as exposure time, the levels of lipid peroxidation increased.



**Figure 3:** Rate of lipid peroxidation in HeLa cell line and fibroblast cell in treated groups. Data is represented as mean  $\pm$  SD of at least 3 independent experiments. Different letters denote significant differences according to Tukey test ( $P < 0.05$ ).

## 4.5. Morphological changes

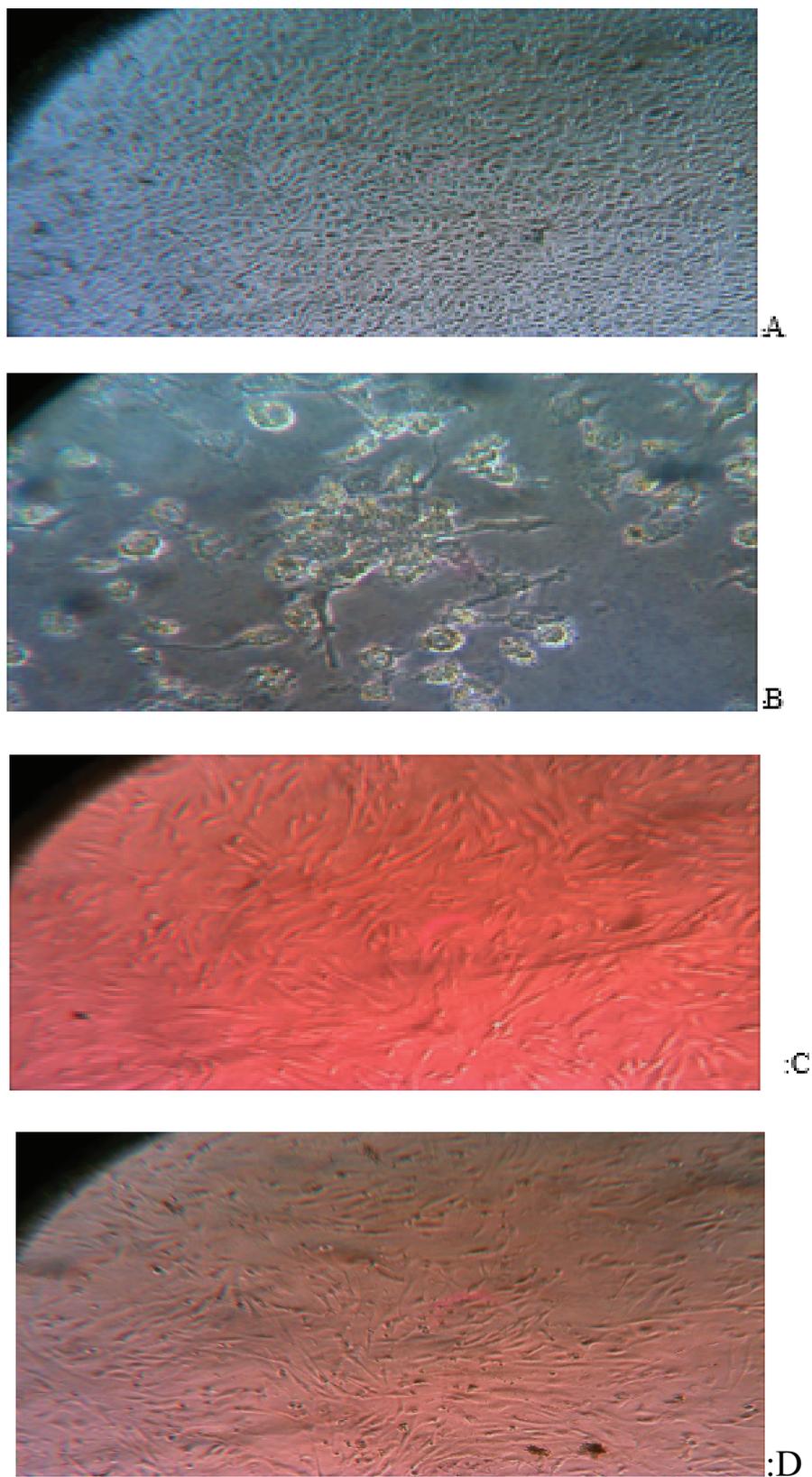
During the early process of apoptosis, we observed cell shrinkage and pyknosis using light microscopy (21). Due to cell shrinkage, the cells became smaller in size, the cytoplasm became denser and the organelles were more tightly packed. Pyknosis is the result of chromatin condensation and is the most characteristic feature of apoptosis. Apoptosis involves single cells or small clusters of cells and an apoptotic cell appears as a round or oval mass (22). Morphological changes and apoptosis were observed in the HeLa and fibroblast cells that were exposed to SMF (Figure 4).

## 5. Discussion

Electromagnetic fields (EMFs) are widely present in the environment and their effects have increased significantly due to the increased use of electric and magnetic devices in industries, medicine as well as in people's daily lives. However, the effects of EMF on biological systems are contradictory and controversial (23). One of the first areas of the cell to be affected by magnetic field is the cell membrane. Some reports indicate that the magnetic field is likely to alter the action of the calcium ion ATPase pump via calcium ion channels or calcium ion-binding proteins that cause a significant reduction in the cellular ion concentration and this leads to a reduction in proliferation and increased apoptosis in these cells (24). In this study, we evaluated the influence of SMF up to 21 mT on the viability percentage, proliferation rate and  $IC_{50}$  of HeLa cells as representative of cancer cells and fibroblasts as representatives of normal cells. As shown in Figures 2, the static magnetic field was more effective on the cancer cells than on fibroblasts and it was seen that by increasing the duration and intensity of the static magnetic field, the cell viability and proliferation rate were decreased. These changes may be due to increased apoptosis and necrosis in these cells. If SMF can indeed affect the proliferation rate, then it is obvious that tumor cells growing uncontrollably should be affected to a greater extent than normal cells. This difference was markedly observed in the cells exposed to 21 mT for 48 h.

When we measured  $IC_{50}$  for both the groups of cells during SMF treatment, we found that the  $IC_{50}$  decreased with increase in exposure time. The obtained  $IC_{50}$  value for HeLa cells was less than that obtained for fibroblast cells at different exposure times (24 h and 48 h). The maximum difference in the obtained  $IC_{50}$  was seen at 24 h of exposure to SMF which changed from  $75.90 \pm 30.79$  mT for fibroblast cells to  $30.9 \pm 4.53$  mT for HeLa cells. This indicated that the HeLa cells were more sensitive than fibroblast cells. It can be concluded that the cell type, which determines the sensitivity or complexity of cells, is a crucial factor for evaluating the proliferation rate. The higher sensitivity of HeLa cells can be attributed to its higher proliferation rate and higher metabolism and also its poorer differentiated than fibroblast cells.

Our findings showing that exposure to magnetic field can increase apoptosis are consistent with those in the reports by Javani Jouni et al (24), Sarvestani et al (25), Zafari et al (26), Singh and Lai (27), Kim et al (28). Some other reports have also shown increased DNA damage in lymphocytes of patients treated with both iron chloride and 7 mT static



**Figure 4:** A: HeLa cells (x10) in the control group (0 mT SMF). B: HeLa cells (x40) in treated group (21 mT SMF for 48 h). C: Fibroblast cells (x10) in control group (0 mT SMF). D: Fibroblast cells (x10) in treated group (21 mT SMF for 48 h).

magnetic field, which led to cell death through apoptosis or necrosis (29). In a study investigating the simultaneous effects of static magnetic fields and iron ions, the effects of the iron ions alone were also tested. The results showed that while in the presence of static magnetic field of 15 mT for 5 h, iron ions could increase the apoptosis of the cells; they did not have an additive effect on apoptosis when used alone. This phenomenon can be due to the role of iron in the production of free radicals in cells (30). Some reports also point out that magnetic fields may affect the homeostasis of iron ions inside certain cells and increase the concentration of free iron ions in the cytoplasm as well as the nucleus. Iron levels can also be affected through the Fenton reaction ( $\text{Fe}^{2+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH} + \text{OH}^-$ ) which leads to an increase in the concentration of hydroxyl free radicals (30). The fact that cancer cells have a more aggressive metabolism than normal cells and due to their intracellular iron storage, there is an increased production of oxygen free radicals in cancer cells (31). The measurement of membrane lipid peroxidation in this study showed that when cells were treated with magnetic field, the membrane lipid peroxidation increased. This enhancement was caused by an increase in the levels of reactive oxygen species. Several studies have indicated that SMF can affect oxidative stress through the processes associated with the production of ROS, which cause DNA damage and finally, apoptosis/necrosis (32). SMF increases the half-life of the intracellular free radicals. Free radicals can influence cells and damage macromolecules such as DNA, proteins and lipids in the membrane (33). Increased reactive oxygen species levels cause membrane lipid peroxidation and any antioxidant defense mechanism is powerless against the damage caused by free radicals. After treatment with 21 mT of SMF for 48 h, HeLa cells showed the lowest cell viability and proliferation rate. Since the HeLa cells were found to be more sensitive to static magnetic field, the application of this approach should be considered in cancer treatment.

In conclusion, the evidence shown in this research supports the hypothesis that SMF dramatically decreases the viability and proliferation rate of cancer cells compared to that of normal cells such as fibroblasts. These results highlight that a combination of SMF and other therapeutic approaches can be potentially used in the treatment of different forms of cancer such as cervical cancer. However, further investigations should be performed to elucidate the exact mechanisms of these effects.

## Acknowledgements

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## Conflict of Interest

The authors declare no conflict of interest.

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