

*Original Research*

# Protective Effect of Static Magnetic Field on the Antioxidant Response in Fibroblast Exposed to Oxidative Stress

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## Abstract

The effect of influence of static magnetic field which is widespread in environment is the aim of many studies. Static magnetic field is present in our everyday life and can cause functional disorders in cells, tissues or biological systems. The study's results do not give the answer about negative or positive effect of the static magnetic field on human body. On the one hand, the magnetic field has been successfully used in medicine for many years, in the diagnosis and treatment of diseases of the musculoskeletal system, nervous system, eyesight, upper respiratory tract, circulatory system, gastrointestinal tract, skin and soft tissues. To induce oxidative stress, fibroblasts had been treated with hydrogen peroxide at a concentration of 1 mM and 0.5 mM for 4 hours, then the medium was changed to medium without of the hydrogen peroxide and cells were placed in permanent magnets with 0.7 T induction. The cultures were maintained in the test chambers or placebo at 37°C in a 5% CO<sub>2</sub> incubator (Heraeus) for 24 hours. In cells lysates SOD, GPx, CAT activities were measured and also total antioxidant status. There was statistically significant decrease in the SOD and GPx activity in cultures with hydrogen peroxide addition. In the cultures to which hydrogen peroxide had been added, there was a statistically significant increase in the CAT activity. The simultaneous exposure of the fibroblasts to oxidative stress and the SMF caused a statistically significant increase in the SOD and GPx activity and decrease CAT activities when compare to the cultures exposed to hydrogen peroxide. The results showed that hydrogen peroxide causes oxidative stress in fibroblasts. On the other hand, the static magnetic field protects the cells and removes the harmful effects of hydrogen peroxide on the fibroblasts.

**Keywords:** static magnetic fields, oxidative stress, redox homeostasis, fibroblasts, cell cultures

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## Introduction

The widespread use of a static magnetic field (SMF), both in everyday devices, medical devices and in industry, means that more and more research is being carried out on the assessment of its impact on living organisms. Most of them are based on *in vivo* experiments in an animal model, while not many of them assess the direct effects of SMF on cells. Redox homeostasis is very important for the proper functioning of cells. A static magnetic field seems to be a factor that may modulate the cellular oxidative status. However, current studies do not give a definitive answer regarding the influence of SMFs on cells [1-3]. The source of the static magnetic field are magnets with a permanent magnetic value. The value of the magnetic induction vector  $B$ , its spatial distribution and polarization are the quantities characterizing the static magnetic field. This field can be obtained by using permanent magnets or by inductors powered by direct electric current. Static magnetic fields are classified as weak (<1 mT), moderate (1 mT-1 T), strong (1-5 T) or ultra-strong (>5 T). Currently, neodymium iron boron magnets (NdFeB) are one of the most commonly used types of permanent magnets [4].

Static magnetic field and electromagnetic field are generated by devices accompanying human in everyday life. These are not only industrial devices, diagnostic equipment, but also household appliances for everyday use. The applicable health and safety standards for people professionally exposed to the magnetic field divide the work zone due to the magnetic field operating there into a safe zone in which the employee can stay without time limits, a protection zone, in which the employee may stay, provided that the exposure time is shortened or protective clothing is used. This zone is divided depending on the field induction to the intermediate zone (0.825-2.5 mT), in which the employee may stay for 12 hours, the danger zone (2.5-25 mT), in which the employee may stay maximum 8 hours and the danger zone (over 25 mT) in which the employee is not allowed to stay [5, 6]. In recent years, there has been a growing interest in using the magnetic resonance (MRI) in the diagnosis of many diseases. Most used in Poland, MRI machines generate a static magnetic field with an induction ranging from 0.2 to 3 T, and in other European Union countries, devices generating SMF up to 8 T are used. The use of these devices may pose a threat to the safety and health of both patients and employees working with scanners. It is still unknown what long-term health effects may be among workers exposed to a magnetic field. The International Agency for Research on Cancer has classified magnetic fields in group 2B, potentially carcinogenic factors for humans. When performing tests with the use of MRI scanners, pharmacological agents are very often used – contrasting agents, the application of which takes place most often during the break in diagnosis. Then the electromagnetic field is turned off, while it is impossible

to turn off the magnetic field generated by permanent magnets [7, 8].

In a living organism, free radicals have long been considered harmful. The free radical theory of aging suggests that free radicals are an important factor leading to aging. Free radicals can be generated from either endogenous or exogenous sources. Exogenous radicals can assign a variety of external stimuli such as ultraviolet irritation, air or water pollution, toxic chemicals, smoking, alcohol, drugs and stress. Free radicals are responsible for aging, tissue damage and various diseases such as Parkinson's disease, Alzheimer's disease, diabetes and cardiovascular disease. In particular, free radicals can increase the risk of cancer by activating the original cancer gene transcription [9-12].

One of the conditions for the proper functioning of cells is to maintain redox homeostasis, the balance between the amount of free radicals and the biological ability to inactivate them quickly. The static magnetic field may be one of the factors that will modulate the maintenance of this balance, and the results obtained so far do not give an unambiguous answer to the question whether SMF has an anti- or pro-oxidative effect. Therefore, the aim of the study was to assess the influence of the static magnetic field on the oxidation-reduction parameters of fibroblasts exposed to oxidative stress.

## Material and Methods

### Reagent and Chemicals

FBM (Fibroblast Basal Medium) – Lonza, Fibroblast growth factor (hFGF-B), insulin, gentamicin (FGM™ SingleQuots™) – Lonza, Trypsin/EDTA, TNS, Hepes: ReagentPackSubculture – Lonza, 0.4% Trypan Blue – Invitrogen, DMSO (dimethyl sulfoxide) – Sigma Aldrich, PBS (buffered saline solution) – Lonza, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) – Sigma Aldrich, Hydrogenperoxide – Sigma Aldrich, ABTS2,2'-azynobis (3-etylobenzotiazolino-6-sulfonian) – Sigma Aldrich, Potassium peroxodisulphate – Sigma Aldrich.

### Cell Culture Conditions

Normal human dermal fibroblasts (NHDF cell line) were obtained from the Clonetics (CC-2511; San Diego, CA, USA) and routinely maintained in a FBM medium, with a human fibroblast growth factor-basic (hFGF-B), insulin, and gentamicin (FGM™ SingleQuots™) at 37°C in a 5% CO<sub>2</sub> incubator (Heraeus).

Both the number of cells and their viability were monitored by cell counting in a Countess TM Automated Cell Counter (Invitrogen, USA) after

staining with 0.4% trypan blue. The experiment was performed on cells that were in the logarithmic phase of growth under conditions of  $\geq 98\%$  viability as assessed by the trypan blue exclusion. For the experiments, the fibroblasts cells were used at three to five passages.

#### Exposure of Fibroblast to Oxidative Stress

To induce oxidative stress fibroblasts had been treated with hydrogen peroxide at a concentration of 1 mM (P 1mM) and 0.5 mM (P 0.5 mM) for 4 hours, then the medium was changed to medium without of the hydrogen peroxide and cells were placed in permanent magnets with 0.7 T induction (P 1 mM + SMF 0.7 T, P 0.5 mM + SMF 0.7 T) or in placebo (P 1 mM, P 0.5 mM). The cultures were maintained in the test chambers or placebo at 37°C in a 5% CO<sub>2</sub> incubator (Heraeus) for 24 hours. Next, the cells were washed with PBS and the cell numbers were determined by cell counting in a Countess TM Automated Cell Counter (Invitrogen, USA) after staining with 0.4% trypan blue. The concentration 1mM and 0.5 mM of hydrogen peroxide was selected for the experiment because it was cytotoxic effect for fibroblasts. The hydrogen peroxide concentration was selected with 0.25 mM-50 mM in pilot study.

#### Cytotoxicity of the Hydrogen Peroxide

Method of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) conversion was used to determine whether hydrogen peroxide at concentrations between 0.25 mM-50 mM was toxic to the fibroblast cell cultures. Hydrogen peroxide was prepared as stock solution in water and then diluted in culture medium. Viability of cells was evaluated after 24 of exposure to hydrogen peroxide. The effect of hydrogen peroxide on cell viability was evaluated in two independent experiments. In the MTT assay, the ability of the cells to convert MTT indicates mitochondrial activity and in consequence cell viability. Normal human dermal fibroblasts were seeded into 96-well culture plate sat a density of 5000 cells/well and were treated with hydrogen peroxide for 24 h. MTT (1 mg/ml) was added to the medium for 3 h (37°C) before the end of the experiment. After cells were lysed in 100  $\mu$ l of dimethyl sulfoxide which enabled the release of the blue reaction product-formazan. Absorbance at the wavelength of 540 nm was read on a microplate reader Wallac 1420 VICTOR (Perkin Elmer, Waltham, MA, USA).

#### Exposure of Fibroblasts to Static Magnetic Fields

To study the fibroblasts in a static magnetic field, magnetic chambers composed of permanent magnets and a ferromagnetic yoke were used (patent P-396639). The ferromagnetic yoke is the bottom and cover

of the chamber and there is a window in the front wall of the chamber that is matched to the dimensions of the cell culture flask [13]. A homogeneous distribution of the magnetic induction over the surface of the flask is conditioned by the structure of the test chamber. The static magnetic field is generated by neodymium magnets and the magnetic field intensity is proportional to the magnetic field strength. The chambers are constructed with the following materials: N42SH magnets, Br = 1.28-1.34 T, HcB $\geq$ 955 kA/m, HcJ $\geq$ 1512 kA/m, (BH)<sub>max</sub> = 310-342 kJ/m<sup>3</sup>, S235JR steel and a diamagnetic material. The maximum operating temperature of the chambers is 150°C. A chamber with a field induction of 0.7 T was used for the tests, which was checked with a gaussmeter before each experiment. The placebo chamber is made of steel instead of permanent magnets and the field induction in this chamber is 0 T.

#### Preparing the Cell Lysates

After 24 hours, the cells were detached from the surface of the culture vessel using a trypsin/EDTA solution. After trypsin neutralization, the cells were centrifuged for 10 minutes at 2,000 RPM, the supernatant was removed, and the cell pellets were washed with a PBS solution and used to prepare the cell lysates. The composition of the lysis buffer was a protease inhibitor (1.4 mg) and a phosphatase inhibitor (10  $\mu$ l) that had been dissolved in 1 ml of PBS. The tubes containing the suspension of studied material in a lysis buffer were placed in liquid nitrogen for 30 minutes and stored at -80°C until further analysis. All of the studied biochemical parameters were recalculated for 10<sup>6</sup> cells.

#### Determining the Superoxide Dismutase Activity (SOD)

SOD activity was determined using a Cayman Chemical's Superoxide Dismutase Assay Kit (Cayman Chemical, USA) and were performed according to the manufacturer's protocol. Previously prepared cell lysates were used as the test material, centrifuged after thawing and the collected supernatant was used for further studies.

The SOD activity was determined spectrophotometrically at 440-460 nm. Xanthine and hypoxanthine generate superoxide radicals which, when bound with a tetrazolium salt, transform it into red formazan. One unit of SOD activity is defined as the amount of the enzyme that is needed to convert 50% of the superoxide radicals.

#### Determining the Glutathione Peroxidase Activity (GPx)

GPx activity was determined using a Cayman Chemical's Glutathione Peroxidase Assay Kit (Cayman

Chemical, USA) and were performed according to the manufacturer's protocol.

The GPx activity was also evaluated based on the spectrophotometric method. The GPx activity was indirectly measured using a coupled reaction with glutathione reductase (GR (glutathione reductase). GPx catalyzes the reduction reaction of cumenehydroperoxide and as a result, an oxidized form of glutathione is formed, which was then reduced in the presence of GR and NADPH oxidation to NADP + (accompanied by a decrease in absorbance). The decrease in A340 absorbance is directly proportional to the GPx activity in the sample.

#### Determining the Catalase Activity (CAT)

The CAT activity was measured using a Catalase Assay Kit (Cayman Chemical, USA). The method is based on the reaction of CAT with methanol in the presence of an optimal concentration of H<sub>2</sub>O<sub>2</sub>. The formaldehyde that was produced was measured spectrophotometrically using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole as the chromogen. Purpald specifically forms with aldehydes, which upon oxidation changes from colorless to purple.

#### Determining the Total Antioxidant Status (ABTS)

The total antioxidant status was measured using the ABTS<sup>+</sup> radical cation. The technique for the generation of ABTS<sup>+</sup> involves the direct production of the blue/green ABTS<sup>+</sup> chromophore through a reaction between ABTS and potassium persulfate. The addition of antioxidants to the pre-formed radical cation reduces it to ABTS, to an extent and on a time-scale that depends on the antioxidant activity, the concentration of the antioxidant and the duration of the reaction. The extent of decolorization as percentage inhibition

of the ABTS<sup>+</sup> radical cation is determined as a function of concentration and time and is calculated relative to the reactivity of Trolox. The decrease in the absorbance at 734 nm was measured [14].

#### Statistical Analyses

All data are expressed as the mean±the standard deviation. An ANOVA and Tukey's *post hoc* test were used to evaluate the results of the experiments. The statistical calculations were performed using STATISTICA 12.0 and the statistical significance was defined at  $p < 0.05$ .

## Results and Discussion

### Effect of Hydrogen Peroxide on Fibroblast Viability

According to the results of a cell viability test, hydrogen peroxide was cytotoxic to the normal human dermal fibroblasts in concentrations between 0.5-50 mM (Fig. 1).

### Effect of Oxidative Stress on the Activity of the Antioxidant Enzymes and Total Antioxidant Status

There was statistically significant decrease in the SOD activity in cultures with hydrogen peroxide addition (P 1 mM  $p = 0.02$ ; P 0.5 mM  $p = 0.022$ ) (Fig. 2). In the cultures to which hydrogen peroxide had been added, there was a statistically significant increase in the CAT activity (P 1 mM  $p = 0.002$ ; P 0.5 mM  $p = 0.007$ ) (Fig. 3). The GPx activity was statistical significant decrease in cultures with both cultures with hydrogen peroxide (P 1 mM  $p = 0.004$ ; P 0.5 mM  $p = 0.007$ ) (Fig. 4). The addition

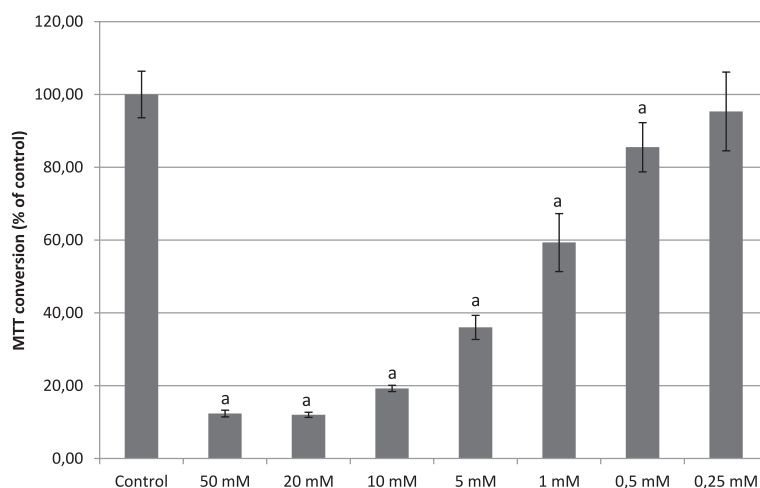


Fig. 1. Cell viability in normal human dermal fibroblast cultures exposed to hydrogen peroxide (between 0.25-50 mM for 24 h). Each bar represents the mean±SD of two independent experiments. Statistical significance, \* $p < 0.05$  vs. control.

of hydrogen peroxide to fibroblasts in both concentrations significantly decreased the antioxidant potential by 50% for P 1 mM and about 30% for P 0.5 mM (P 1 mM  $p = 0.001$ ; P 0.5 mM  $p = 0.004$ ) (Fig. 5).

#### Effect of Oxidative Stress and Static Magnetic Field on the Activity of the Antioxidant Enzymes and Total Antioxidant Status

The simultaneous exposure of the fibroblasts to oxidative stress and the SMF caused a statistically significant increase in the SOD and GPx activity in compare to the cultures exposed to hydrogen peroxide (SOD: P 0.5 mM + SMF  $p = 0.02$ ; GPx: P 1 mM + SMF  $p = 0.004$ ; P 0.5 mM + SMF  $p = 0.007$ ) (Fig. 2, Fig. 4). There was no statistical significance in cultures

co-exposure of hydrogen peroxide with concentration of 1mM and SMF compared to the cells exposed only to the oxidative stress. In cultures exposed to oxidative stress and SMF the CAT activity was decreased compared to the fibroblast exposed only to oxidative stress (P 1 mM + SMF  $p = 0.002$ ; P 0.5 mM + SMF  $p = 0.003$ ) (Fig. 3). Simultaneous exposure of cells to oxidative stress and static magnetic field resulted in partial normalization of the antioxidant potential of fibroblasts in comparison to the control cultures (P 1 mM + SMF  $p = 0.03$ ; P 0.5 mM + SMF  $p = 0.01$ ) (Fig. 5).

The effect of the influence of static magnetic field which is widespread in environment are the aim of many studies. Static magnetic field is present in our everyday life and can cause the functional disorders in cells, tissues or biological systems. The study's results do not give answer about negative or positive effect

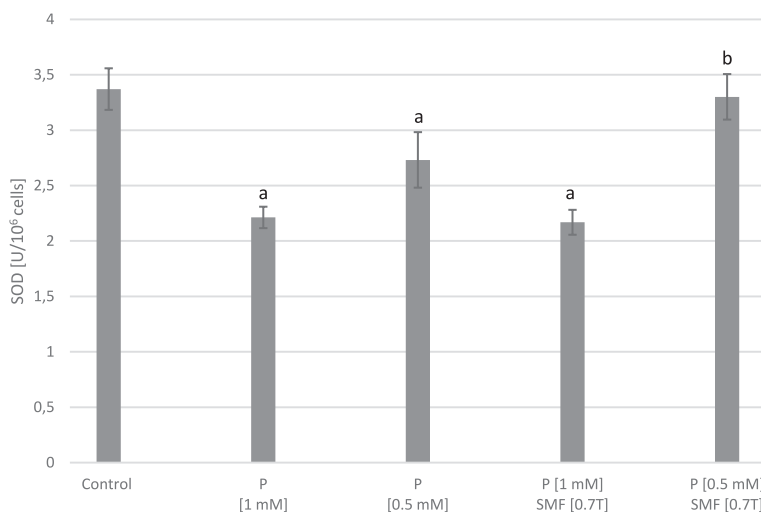


Fig. 2. SOD activity in normal human dermal fibroblast cultures exposed only to oxidative stress (P 1 mM, P 0.5 mM) and both static magnetic field and oxidative stress (P 1 mM + SMF 0.7T; P 0.5 mM + SMF 0.7 T). Each bar represents the mean $\pm$ SD. Statistical significance, <sup>a</sup> $p < 0.05$  vs. control, <sup>b</sup> $p < 0.05$  vs. P 1 mM or P 0.5 mM.

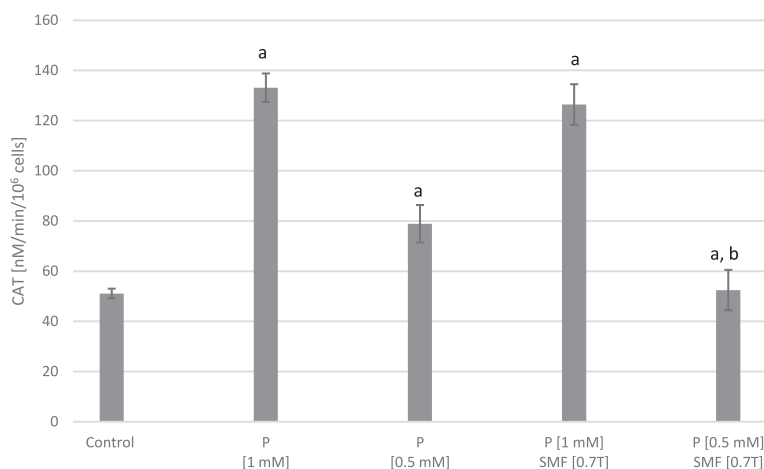


Fig. 3. CAT activity in normal human dermal fibroblast cultures exposed only to oxidative stress (P 1 mM, P 0.5 mM) and both static magnetic field and oxidative stress (P 1 mM + SMF 0.7T; P 0.5 mM + SMF 0.7 T). Each bar represents the mean $\pm$ SD. Statistical significance, <sup>a</sup> $p < 0.05$  vs. control, <sup>b</sup> $p < 0.05$  vs. P 1 mM or P 0.5 mM.

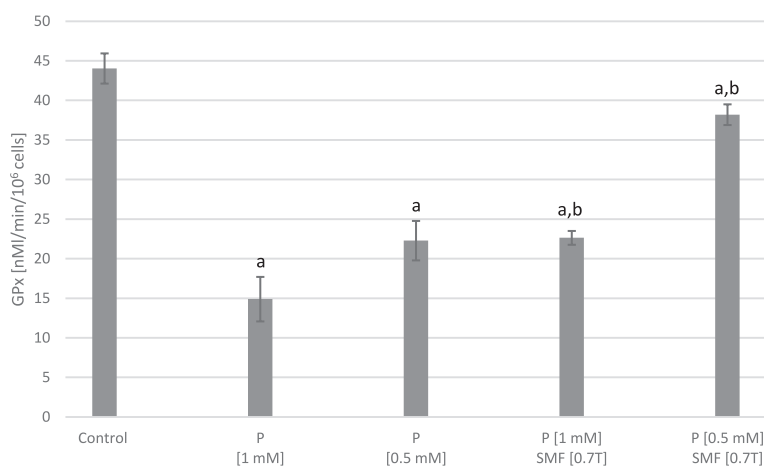


Fig. 4. GPx activity in normal human dermal fibroblast cultures exposed only to oxidative stress (P 1 mM, P 0.5 mM) and both static magnetic field and oxidative stress (P 1 mM + SMF 0.7T; P 0.5 mM + SMF 0.7 T). Each bar represents the mean±SD. Statistical significance, <sup>a</sup>p<0.05 vs. control, <sup>b</sup>p<0.05 vs. P 1 mM or P 0.5 mM.

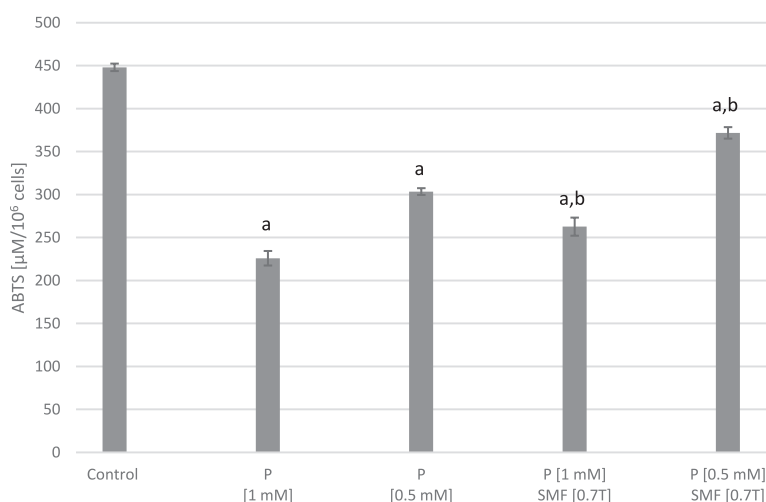


Fig. 5. Total antioxidant status (ABTS) in normal human dermal fibroblast cultures exposed only to oxidative stress (P 1 mM, P 0.5 mM) and both static magnetic field and oxidative stress (P 1 mM + SMF 0.7 T; P 0.5 mM + SMF 0.7T). Each bar represents the mean±SD. Statistical significance, <sup>a</sup>p<0.05 vs. control, <sup>b</sup>p<0.05 vs. P 1 mM or P 0.5 mM.

of the static magnetic field on human body. On the one hand, the magnetic field has been successfully used in medicine for many years, in the diagnosis and treatment of diseases of the musculoskeletal system, nervous system, eyesight, upper respiratory tract, circulatory system, gastrointestinal tract, skin and soft tissues. The basis for the use of the magnetic field in medicine is its participation in the regeneration processes of soft tissues, as well as anti-inflammatory and anti-swelling properties. However, on the other hand, there are reports that the magnetic field may contribute to the generation of free radicals and, as a result, induce oxidative stress, which results in the disturbance of cellular redox homeostasis [15, 16].

Hydrogen peroxide is often used as an oxidative stress factor in *in vitro* studies. In this study the cytotoxicity of hydrogen peroxide was assessed in the

concentration range of 0.25-50mM. Concentrations of 0.5 and 1mM were selected for further stages of the experiment, which in the MTT test decreased the cell viability by 17 and 40%, respectively. In studies by Cao et al. [17] also induced oxidative stress with hydrogen peroxide in the concentration of 100 to 400 μM in HK2 cell cultures. Their results showed that cell viability decreased with increasing concentrations of hydrogen peroxide in a manner suggestive of concentration dependence which was also confirmed in our research. On the other hand, Peng et al. [18] Human lung cancer cells (A549) were exposed to hydrogen peroxide in the concentration of 50-2000 μM and observed a decrease in viability at about 60% at a concentration of 800 μM.

In order to counteract the effects of production of free radicals and their reactions with cell components,

human organism have developed the defense mechanisms, both enzymatic and non-enzymatic. The first line of defense is the antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase. Superoxide dismutase catalyzes the dismutation of the superoxide radical anion to water and hydrogen peroxide, which in turn is a substrate for catalase and glutathione peroxidase. These enzymes decompose hydrogen peroxide, but glutathione peroxidase has a greater affinity for  $H_2O_2$  than catalase [19]. This study, found the disturbance of fibroblasts oxidation-reduction balance in cell cultures exposed to hydrogen peroxide in both concentrations. Being administrated with 0.5 mM and 1 mM of hydrogen peroxide for 4 h, caused oxidative stress in fibroblasts the excess ROS depleted endogenous antioxidant enzymes. That is why the activities of SOD and GPx significantly decreased upon  $H_2O_2$  stimulation. At the same time, despite the reduced activity of SOD and GPx, an increase in the activity of catalase was observed, as catalase becomes involved in the reaction of hydrogen peroxide decomposition when its concentration in the cell is high. The cooperation of CAT and GPx ensures the removal of excess hydrogen peroxide, thanks to which there is no formation of other, more reactive radicals, and thus these enzymes protect the cell against the toxic effects of  $H_2O_2$  on proteins, lipids or DNA. Few reports indicate that if the amount of free radicals is small, we observe an increase in the activity of antioxidant enzymes, in particular SOD and GPx, while a high level of ROS leads to the failure of enzymatic defense mechanisms, which results in a decrease in the activity of antioxidant enzymes [20-22]. Yang et al. [23] observed that gene expression of several antioxidant genes were downregulated under after exposed HeLa cells to hydrogen peroxide. Also Jin et al. [24], inducing oxidative stress in osteoblasts with hydrogen peroxide, noted a decrease of the activity of antioxidant enzymes SOD and GPx. In this study we also assessed the antioxidant potential of fibroblasts. The exposure of cells to the hydrogen peroxide caused a significant decrease in the antioxidant activity of cells, which was proportional to the  $H_2O_2$  concentration.

Few results of *in vitro* studies show that the sensitivity of cells to a static magnetic field depends on many factors, namely the SMF induction, the duration of exposure to it and the type of cells used for the tests. And so, 24-hour exposure to SMF inducing 120  $\mu$ T of endothelial cells increased their number [25]. On the other hand, in cultures of stem cells exposed to SMF with 0.5 T induction for 7 days, their proliferation rate was inhibited [26]. Difficulty in comparing test results from the use of different sources emitting a static magnetic field. This field can be generated both by magnetic coils or magnetic neodymium disks placed in Petri dishes, and by permanent magnets. In our study, it was found that a static magnetic field with an induction of 0.7 T protects fibroblasts exposed to oxidative stress and reduces oxidative stress in cells, as evidenced by

the normalization of the activity of SOD, GPx and CAT antioxidant enzymes in relation to control cultures. The protective effect of SMF is more evident with a lower concentration of hydrogen peroxide. The protective effect may possibly result from the influence of SMF on the orientation of molecules influenced by SMF. It has been reported that exposure to strong static magnetic fields on the order of 1 T can result in the orientation of macromolecules such as collagen and of animal cells *in vitro*. Human foreskin fibroblasts were also oriented using the magnetic orientation of collagen with static magnetic fields of 4.0 and 4.7 T. Furthermore, osteoblast cells have been shown to be oriented under exposure to a strong static magnetic field of 8 T in the absence of collagen [27-29]. Kimsa-Dudek et al. [30] in their studies confirmed that the static magnetic field generated by permanent magnets reduces the toxicity of fluoride in cell cultures. Yu et al. [31] discovered that the SMF could partially prevent the development of HFD-induced diabetes. The SMF-treated mice have reduced blood cholesterol and glucose levels, as well as reduced body weight gain and lipid accumulation in liver. These beneficial effects are correlated with improved gut microbiota, reduced labile iron, and reduced ROS levels in pancreatic cells, which have protective effects on the pancreas and increase insulin secretion.

## Conclusion

In conclusion, we showed that hydrogen peroxide causes oxidative stress in fibroblasts. On the other hand, the static magnetic field protects the cells and removes the harmful effects of hydrogen peroxide on the fibroblasts.

## Conflict of Interest

The authors declare that they have no conflict of interest.

## Acknowledgments

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