

# SUB-ACUTE EXPOSURE TO MAGNETIC FIELD INDUCED APOPTOSIS IN THYMUS FEMALE RATS

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## ABSTRACT

**Objective:** Apoptosis induced by magnetic field in female rats was investigated by using the Tdt-mediated dUTP nick-end labeling (TUNEL) assay in thymus, liver and kidney.

**Design:** Female's rats were exposure to 128 mT 1hour/day for 10 consecutive days. Small pieces of thymus, liver and kidney were fixed overnight at room temperature by direct immersion in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene and embedded in paraffin wax. Serial sections (5 µm thick) were mounted on gelatin-coated glass slides and stained with hematoxylin and eosin or treated with TUNEL (Tdt-mediated dUTP-digoxigenin Nick and Labeling) method.

**Setting:** Female Wistar rats were housed in a cage, with free access to food and water (Faculté des Sciences de Bizerte, Tunisia). Rats were cared for under the Tunisian Code of Practice for the Care and Use of Animals for Scientific purpose and the Experimental Protocols were approved by the Ethics Committee.

**Subjects:** Treated and control groups (n=12) weighing 100-150g at the time of experiments were housed in the same condition three weeks before the beginning of the experiments.

**Main outcome measures:** MF-induced apoptosis in thymus was demonstrated by the terminal deoxynucleotidyl transferase (Tdt) mediated dUTP-Biotin Nick End Labeling (TUNEL) assay.

**Results:** Following sub-acute exposure to MF, morphological examinations revealed numerous apoptotic cells in thymus characterized by nuclear fragmentation and condensation (figure 2a). Interestingly, no labeling was found in control thymus. The density of the apoptotic cells was significant in cortical zone compared to control (2.95±0.34 % vs 0.74±0.10 %, p<0.05), than in the medullar zone (2.105±0.356% vs 0.634±0.038%, p<0.05) (figure 2a, b). By contrast, as shown in figures (3c, d), no labeling was found in liver and kidney following MF exposure (figure 4e, f).

**Conclusions:** Thus, it may be concluded that static magnetic field induced apoptosis in thymic cell death but not in the liver and kidney.

**KEY WORDS:** Magnetic field, apoptosis, thymus, liver, kidney, rat.

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## INTRODUCTION

The effects of magnetic field (MF) on the developing mammalian have been discussed for many years. Many macro- and microscopic analyses have reported that exposure to MF induced in Brain, many dead cells, ectopic gray matter, and reduction of cortex thickness, abnormal neuronal migration, and disturbance of synaptogenesis between neurons.<sup>1,2</sup> Moreover,<sup>3</sup> Hisamitsu et al. (1997) showed that MF can induce apoptosis in human leukemic cells *in vitro*, assessed by a DNA fragmentation method as one of the biochemical markers of cell death.

Apoptosis is a mode of cell death with morphological features quite distinct from those of necrosis.<sup>4</sup> Injury to cells by chemicals, such as metal or other noxious agents, lead to a complex series of events that can culminate in the death of the cell. Two fundamentally different forms of the cell death are possible: necrosis and apoptosis. Necrosis is caused by physical, chemical, or osmotic damage and is associated with the disruption of internal and external membranes, whereas the process of apoptosis requires the target cell to be active in its own death.<sup>5</sup> Morphologically, the apoptotic process includes features such as cell shrinkage, loss of normal cell contacts, dense chromatin condensation, fragmentation of DNA into oligonucleosome-sized fragments, and cellular budding.<sup>6</sup> An important function of apoptosis is the removal of genetically damaged cells. This function serves to protect the host from disease, including carcinogenesis.<sup>7</sup>

Hence it was for interest to examine whether MF exposure induces apoptosis in thymus, liver and kidney of female rats.

## MATERIALS AND METHODS

### **Animals and surgery:**

Female Wistar rats (Pasteur Institute, Tunisia) weighing 100-150g at the time of experiments were housed at 25°C in a cage under a 12-12 h light/dark cycle, with free access to food and water. Treated rats (n=6) were exposed to magnetic field (128 mT; 1h/day) for 10 consecutive days. Control and treated animals were sacrificed under light anesthesia (halothane 2.5%, in air).

Rats were Cared for under the Tunisia Code of Practice for The Care and Use of Animals for Scientific Purposes. The Experimental Protocols were approved by The Ethics Committee (Faculté des Sciences de Bizerte, Tunisia).

### **Exposure system:**

Lake Shore Electromagnets (Lake Shore Cryotronic, Inc, Westerville Ohio, USA) are compact electromagnets suited for many applications such as magnetic resonance demon-

strations. For the present experiment, we used an air gap of 15 cm. Water-cooled coils provide an excellent field for stability and uniformity when high power is required to achieve the maximum field capability for the electromagnet. We have an accurate pole alignment by precise construction of the air gap adjustment mechanism.

### **Histological analysis:**

Small pieces of thymus, liver and kidney were fixed overnight at room temperature by direct immersion in 4% paraformaldehyde in 0.1M phosphate buffer, pH 7.4. The samples were dehydrated with ethanol and toluene and embedded in paraffin wax. Serial sections (5 µm thick) were mounted on gelatin-coated glass slides and stained with hematoxylin and eosin or treated with TUNEL (TdT-mediated dUTP-digoxigenin Nick and Labeling) method.<sup>8</sup>

### **TUNEL Assay:**

After deparaffinization and rehydration, tissues sections were incubated with 0.1% (v/v) Triton X-100 for 2min on ice, followed by washing of the slides twice in PBS (CaCl<sub>2</sub> 2H<sub>2</sub>O 0.8mM, KCl 2.6mM, KH<sub>2</sub>PO<sub>4</sub> 1.4mM, MgCl<sub>2</sub> 6H<sub>2</sub>O 0.4mM, NaCl 136mM, Na<sub>2</sub> HPO<sub>4</sub> 8 mM, pH 7.2). The specimens were then incubated one hour at 37°C in a solution consisting of 1mM cobalt chloride, 140mM sodium cacodylate and terminal deoxyribonucleotidyl transferase (TdT) at a final concentration of 0.1U/µl to insert biotin-16-dUTP at the 3' – ends of DNA fragments. A straptavidin-peroxydase complex and 3-amino-9-ethylcarbazole served as the detection system for biotin. Sections were lightly counterstained with hematoxylin and mounted in glycerin jelly. Negative control included omission of TdT from the labeling mixture.

### **Statistical analysis:**

Data were analyzed using Stat View 512+ software (Abacus Concept, Inc). Means were given with standard error and were subjected to the unpaired Student's *t*-test.

## RESULTS

As shown in Figure 1, a significant decrease in thymus relative weight was observed in MF rats. This decrease reached 30% compared to control ( $0.198 \pm 0.010$  g/100g vs  $0.281 \pm 0.028$  g/100g,  $p < 0.05$ ). The sub-acute exposure of rats to MF failed to alter relative liver and kidney weight respectively ( $0.4132 \pm 0.276$  g/100g vs  $3.755 \pm 0.294$  g/100g,  $p > 0.05$ ;  $0.415 \pm 0.010$  g/100g vs  $0.448 \pm 0.030$  g/100g,  $p > 0.05$ ) (Figure 1).

Immunocytochemical detection of apoptosis following sub-acute exposure to MF, morphological examinations revealed numerous apoptotic cells in thymus characterized by nuclear fragmentation and condensation (figure 2a). Interestingly, no labeling was found in control thymus. The density of the apoptotic cells was significant in cortical zone compared to control ( $2.95 \pm 0.34$  % vs  $0.74 \pm 0.10$  %,  $p < 0.05$ ), than in the medullar zone ( $2.105 \pm 0.356$ % vs  $0.634 \pm 0.038$ %,  $p < 0.05$ ) (figure 2a, b). By contrast, as shown in figures (3c, d), no labeling was found in liver and kidney following MF exposure (figure 4e, f).

## DISCUSSION

The major finding of this study is that sub-acute exposure to MF induced apoptosis in thymus but not in liver and kidney in female rats.

Our results demonstrated that sub-cute exposure to MF produced a marked decrease of thymus relative weight whereas previous

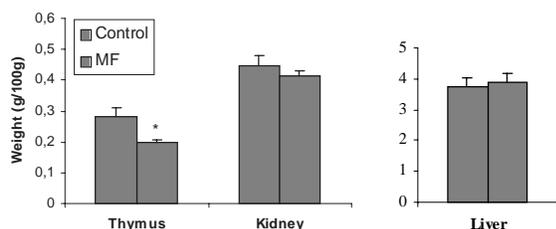


Figure 1: The effect of sub-acute exposure to magnetic field (MF) on relative weight of thymus, liver and kidney in female rats.

Rats treated are exposure to MF 128 mT (1h/ day) for 10 days [Each value is the mean  $\pm$  SE of 12 determinations]. \*  $p < 0.05$  compared to control (Student's *t*-test)

investigations reported opposite data<sup>(9)</sup>. However, the weight of liver and kidney not change after ten days of exposure.

MF-induced apoptosis in thymus was demonstrated by the terminal deoxynucleotidyl transferase (TdT) mediated dUTP-Biotin Nick End Labeling (TUNEL) assay. This technique is based upon TdT catalyzing the addition of nucleotides at a free 3' OH end of DNA, including 3' ends produced by endonuclease action during programmed cell death. The TUNEL assay enabled us to simultaneously examine cell morphology and staining intensity as well as to determine the percentage of the total cell population actually undergoing apoptosis at a give time. We recognize that the extreme sensitivity of this assay may also

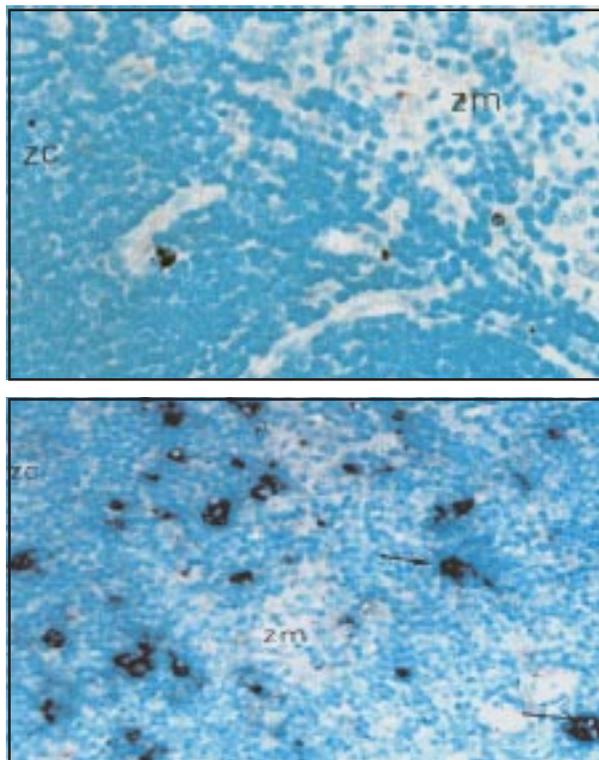


Figure 2: Photomicrographs of sections of (a) thymus, from control female rats and (b) thymus from MF-exposure rats stained by TUNEL technique. (Magnification x400). Thymus was fixed by direct immersion in a 4% paraformaldehyde in 0.1M phosphate buffer. Serial sections (5  $\mu$ m) were fixed mounted on gelatin-coated glass slides cut and stained with TUNEL technique (see Materials and methods). Rats are exposed to MF 128 mT (1h/ day) for 10 days.

T: Thymocyte, ZC: Cortical Zone, ZM: Medullar Zone.

allow for the detection of nicks in DNA that are not necessarily associated with programmed cell death, such as those resulting from the DNA repair process or necrosis.<sup>10</sup> Several additional assays are required to confirm that thymus cells were truly undergoing MF-induced programmed cell death.

Nick end-labeling revealed a high percentage of TUNEL-positive cells in the thymus, 10 days after MF-exposure compared to control. The density of the cells is more significant in cortical than in the medullar zone. The results of the present study suggest that exposure during 10 days at 128 mT, MF enhances thymocytes death. Similar findings have been re-

ported by<sup>11</sup> Flipo et al. (1998), stated that exposure to SMF produced markedly increased apoptosis of murine thymic cells, as determined by flow cytometry. In mammals, the thymus is the primary central organ of the lymphoid system; where lymphocytes, produced by bone marrow, enter and differentiate into antigen-recognizing cells.<sup>12,13</sup>

Previous report showed that in the rat model a prolonged exposure to 50 Hz electric and magnetic fields, independently of field strength, seems to affect thymic cell death and possibly thymic physiology, since alterations in the balance of cell death and other parameters such as mitoses might interfere with the positive and

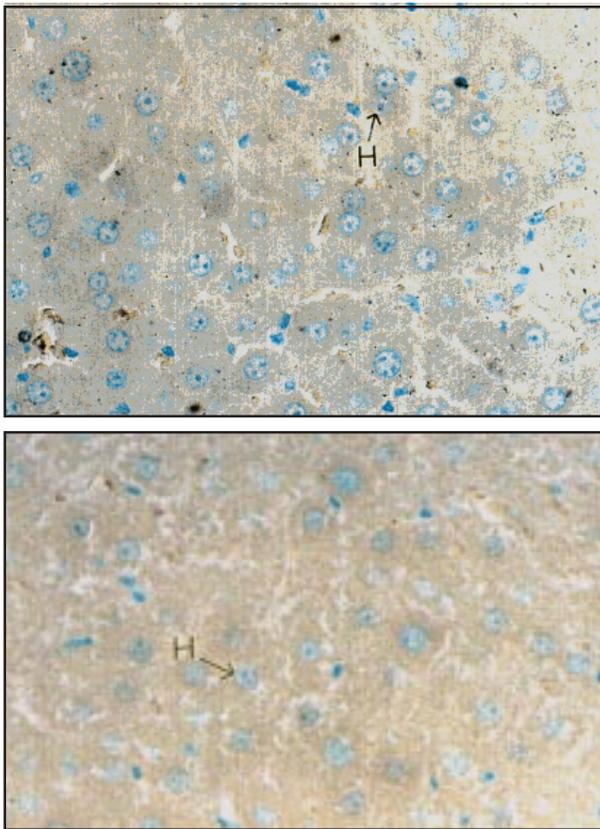


Figure 3: Photomicrographs of sections of (c) liver from control female rats and (d) liver from MF-exposure rats stained by TUNEL technique. (Magnification x400).

Liver was fixed by direct immersion in a 4% paraformaldehyde in 0.1M phosphate buffer. Serial sections (5  $\mu$ m) were fixed mounted on gelatin-coated glass slides cut and stained with TUNEL technique (see Materials and methods). Rats are exposed to MF 128 mT (1h/ day) for 10 days.

H: Hepatocyte.

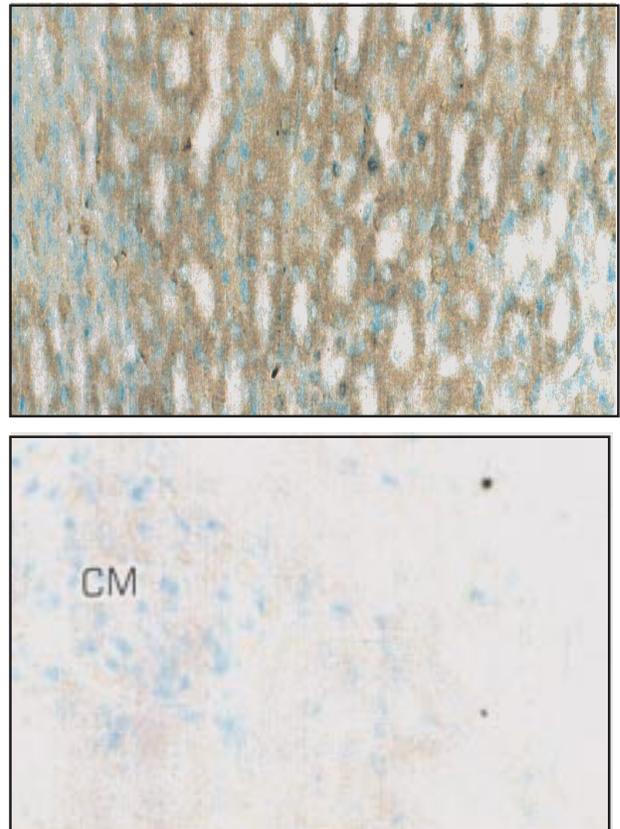


Figure 4: Photomicrographs of sections of (c) kidney from control female rats and (d) kidney from MF-exposure rats stained by TUNEL technique. (Magnification x400).

Kidney was fixed by direct immersion in a 4% paraformaldehyde in 0.1M phosphate buffer. Serial sections (5  $\mu$ m) were fixed mounted on gelatin-coated glass slides cut and stained with TUNEL technique (see Materials and methods). Rats are exposed to MF 128 mT (1h/ day) for 10 days.

CM: corpuscle of Malpighi.

negative selection of thymocytes and with the immunosurveillance properties of the thymus.<sup>14</sup> We did not find any statistically significant differences between control and MF rats in liver and kidney. Previous investigation reported that power frequency MFs induce apoptosis in human leukemic cells *in vitro*, assessed by DNA fragmentation method as one of biomarkers of cells death (apoptosis)<sup>3</sup>. By contrast, other researchers reported that an extremely low frequency MF can induce apoptosis mainly in transformed cells, *in vitro*, but the mechanism of this effect remains unknown.<sup>15,16</sup> Several studies from different laboratories have indicated that reactive oxygen species (ROS) may directly or indirectly participate in the initiation of apoptotic or necrotic cell death.<sup>17,18</sup> Chemical or physical, ROS production may interfere with the mechanism which controls apoptosis and can thereby stimulate or prevent cell death.<sup>19,20</sup> Similarly, the results of our recent studies have indicated that exposure to static magnetic field (7 mT), may increase the oxidative DNA damage due to iron compounds, by stimulating free radical reactions involving reactive oxygen species, and thus influence cell death.<sup>21</sup>

#### **Role for gender and species:**

In our investigation the animal model used was the rat Wistar. As far as we know in majority of physiological studies dealing with the bioeffects of magnetic field (MF), the animal model used is Sprague Dawley or Wistar. No sexual differences were noted in the response of thymus to MF.

#### **Triggers and rate of apoptosis in MF:**

Apoptosis is an active process fundamental to development and homeostasis of multicellular organisms. It is characterized by dramatic morphological alterations, particularly membrane blebbing, cell shrinkage and chromatin condensation, and is accompanied by fragmentation of the nuclear DNA, ensuring genetic death. Apoptosis can be triggered by a wide variety of cellular stresses, including DNA damage, UV radiation, ionizing radiation, heat

shock and oxidative stress as well as by extracellular stimuli acting through cell-surface receptors.<sup>22,23</sup> In the present investigation, the density of apoptotic cells was significant in cortical ( $2.95 \pm 0.34$  % vs  $0.74 \pm 0.10$  %,  $p < 0.05$ ) and medullar zone ( $2.105 \pm 0.356$  % vs  $0.634 \pm 0.038$  %,  $p < 0.05$ ) of thymus.

Moreover, it is well known that the oxygen free radicals induce lipid peroxidation, and produce DNA, and protein damage.<sup>24-26</sup> Recent data in our laboratory as previous studies demonstrated that MF exerts its influence on the oxidative mechanisms of cellular systems and may considerably enhance these processes in the presence of substances which exhibit a potential to produce reactive oxygen species.<sup>27</sup> Interestingly, The MF increases the availability of iron which in turn, through a chemical process known as the Fenton Reaction, increases the availability of free radicals. The free radicals cause localized damage to lipids and proteins involved in the cell's structural integrity.

#### **Difference in cell cycle between organs investigated:**

Previous reports demonstrated that liver and kidney cells divide only rarely but can increase their proliferation in order to regenerate damage partially. By contrast, in mammals, the thymus is primary central organ of the lymphoid system, where lymphocytes, produced by bone marrow, enter and differentiate into antigen-recognizing cells.<sup>28,29</sup> After birth, the thymus progressively diminishes in size, undergoing gradual atrophy.<sup>30-32</sup>

These results suggest that, in female rats, sub acute exposure to static MF produced markedly increased apoptosis of thymic cells, as determined by TUNEL. Although the mechanisms by which MF initiates apoptosis in these cells are presently not known, reactive oxygen species are likely to play a role.

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