

## Protective Effect of Vitamin E Supplement in Electromagnetic Field Induced Damages in Spleen: An Ultrastructural and Light Microscopic Studies

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**Abstract:** Considerable attention is focused on effects of electromagnetic field (EMF) and its increasing use in everyday life. Appliances and various equipments are sources of electromagnetic fields with a wide-range of technical characteristics. Twenty four male Wistar rats were selected and divided into three groups (Control, test group 1, test group 2). The test group 1 was exposed to EMF (50 Hz, 3 mT) 8 hours a day, 6 days per week for two months. Test group 2 was exposed to EMF (50 Hz, 3 mT) 8 hours a day, 6 days per week for two months but received 30 mg Vitamin E/day in their food. The rats in control group neither were exposed to electromagnetic field nor received Vitamin E. At the end of 2 months, the rats in all groups were sacrificed, dissected and samples from spleen in all groups were taken and processed for light and electron microscopic studies. 40 microscopic fields from each group were randomly selected and studied. The data showed that in the spleen of test group1 the diameter of white pulps and the number of megakaryocytes were reduced, while the number of macrophages was increased and also EM study showed that totally cellular nuclei were heterochromatic in comparison to control group. Test group 2 was similar to control group. These findings indicated that immune system is weakened by electromagnetic field. However, Vitamin E supplementation prevented above alteration.

**Key words:** Spleen • Electromagnetic fields • Vitamin E • Free radicals • Macrophages

### INTRODUCTION

The wide-spread application of electromagnetic field (EMF) in everyday life has produced many concerns on health effects of daily environmental exposure to electromagnetic radiation. There are many types of equipments emitting such radiation, among them, some could have therapeutic application [1]. EMF can affect cells via different mechanisms. For instance, electromagnetic radiations effect on cytoplasmic membrane could cause a change in functional potential due to biochemical changes followed by a change in concentration of ions trafficking within the membrane [2]. In addition, the physical reaction between EMF and

chemical bonds among atoms could lead to formation of free radicals in the body of living creatures [3, 4]. There are many data that show long time exposure to EMF has side effect on living organism [5-11]. However, there are also studies that show electromagnetic fields has not side effect on living organism [12]. There are a few studies about the EMF effects on lymphatic organs in literature and all studies have been focused on serum parameters like different enzymes. For instance, the study by Koyu [13] demonstrated that when rats were exposed to EMF (900 MHz), activities of CAT, SOD, GSH - Px, XO were changed. Regarding the role of lymphatic organs in immune system and the increased application of EMF-generating equipment, the present study was aimed

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at targeting the long-term effects of such radiations on the lymphatic organs. On the other hand, there are data that show EMF exert their effect via formation of free radicals [14] and Since Vitamin E (V.E.) is a well known antioxidant [15] we have studied the protective role of V.E in EMF - exposed rats.

**MATERIALS AND METHODS**

In the present study four months old wistar rats were used as laboratory models. The animals were kept in a humid atmosphere (65-70%) at 25°C, a time interval of 12 hours of access to light and darkness during each day for a total of one week was provided for acclimatization. In order to generate electromagnetic field (EMF), a generator that was made in histology department of Medical School was used. This generator uses 220 V and 50 Hz electricity and made based on Helmholtz coil which generates a field of 3 mT.

The apparatus is so designed that could accommodate 8 rats and a water circulator system prevents increasing of internal temperature. The power system, frequency producer were checked by Teslometer for an accurate and homogenous EMF performance. Also, the intensity of EMF generating apparatus was examined before and during the study using the apparatus-associated Tesla meter. Initially, the animals were randomly divided into 3 groups, two test groups and one control group with eight rats in each group. The rats in control group, received standard food without being exposed to EMF. Test group 1 received their food like the control group, but was exposed to EMF (3 mT, 50 Hz) for 8 hours a day and a total period of 2 months. The test group 2, received 30 mg V.E supplements every day in their food and was exposed to EMF (3 mT, 50 Hz) for 8 hours a day and a total period of 2 months. After the termination of experimental period, all rats were desensitized and sampling was done from spleen. Samples were fixed in 10% formalin for 48 hours and processed in tissue processing device. The samples were embedded in paraffin and 5 µm thick sections were produced by rotator microtome (Shandon AS 325). Out of many sections from each sample, the sections no. 2, 4, 6, 8 and 10 were chosen and stained with H and E technique. Finally, 40 microscopic fields from a similar number of slides in each group were randomly selected for cell count, photography and light microscopic study. At the same time, some samples were fixed in 2% glutaraldehyde and prepared for EM studies. The thin sections were studied with LEO 906 TEM.

**Statistical Analysis:** Data obtained by statistical analysis are expressed as mean±SEM. Statistical analysis was performed by using T-test. Differences at a probability value (P) of 0.05 or less were considered to be significant.

**RESULTS**

In the present study, the effects of electromagnetic fields on spleen were investigated and possible inhibitory effects of V.E were assessed. The results were as follows: the mean diameter of white pulps in the control group was 191.16±14.15 µm (10 fields were counted in each slide), in test group 1 was 132.52±6.01 µm and in test group 2 it was 181.78±2.01 µm (Table 1). This data showed significant difference between control and test group 1 (p<0.05), but not between control and test group 2 (p>0.05). Higher magnification revealed that red pulps in addition to constitutive cells of splenic cords, contain macrophages and megakaryocytes (Fig. 1-A). The mean number of macrophages and megakaryocytes in the control group was 26.2±2.41 and 0.8±1.33, respectively, in each microscopic field. In the test group 1, that was exposed to EMF, the mean number of macrophages was increased to 40.94±9.53 and the mean number of megakaryocytes was decreased to 0.12±0.52 whereas both parameter were significantly differ from control values (p<0.05) (Fig. 1-B) (Table 1). The average numbers of macrophages and megakaryocytes in the test group 2 that along with EMF exposure received 30 mg/day V.E were 28.36±7.65 and 0.6±1.55, respectively, which were similar to control group (Fig. 1-C) (Table 1).

Regarding to the presence of different types of cells in spleen which was not possible to study all of them with EM, we focused on the status of nuclei and organelles (Such as Mitochondria and Endoplasmic Reticulum) in reticular cells, eosinophilic cells and macrophages. The organelles in the control and test group 1 were not different. (Fig. 2-A), but EM study showed that totally cellular nuclei were heterochromatic in the test group 1 (Fig. 2-B) in comparison to the control group (Fig. 2-C). The test group 2 was similar to the control group.

Table 1: Effect of EMF on number of megakaryocytes, macrophages and Diameter of white pulps (µm)

Group	NO.	Megakaryocytes	Macrophages	Diameter of white pulp
Control	40	0.8±1.33	26.2±2.41	191.16±14.15
Test group 1	40	0.12±0.52	40.94±9.53	132.52±6.01
Test group 2	40	0.6±1.55	28.36±7.65	181.78±2.01

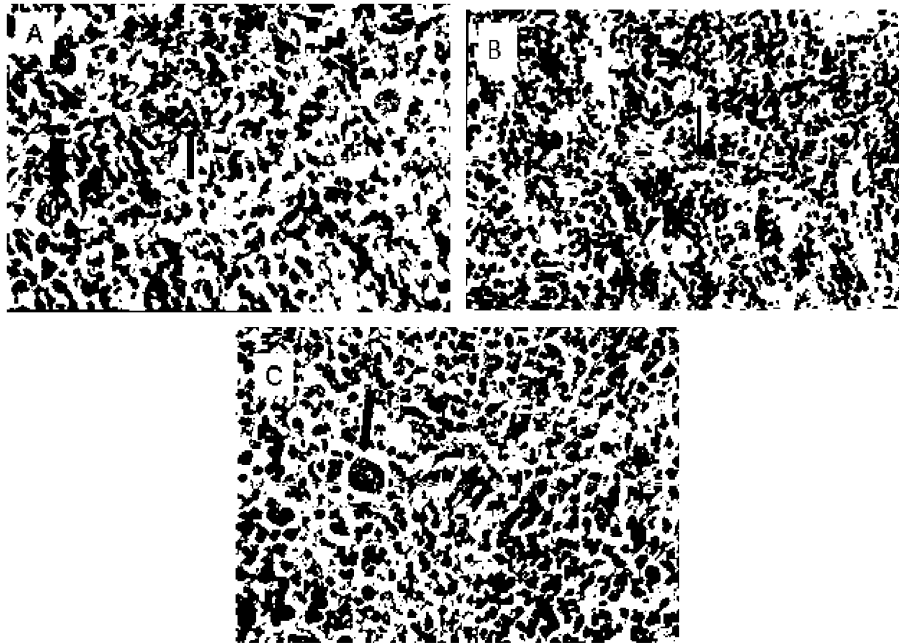


Fig. 1: A photomicrograph from rat spleen

A, red pulp containing macrophages (narrow arrow) and megakaryocytes (Thick arrow).

B, red pulp from EMF - exposed spleen having numerous macrophages (Arrow).

C, red pulp from a rat that were exposed to EMF and received vitamin E at the same time, which is similar to control and megakaryocytes were present. 660 X

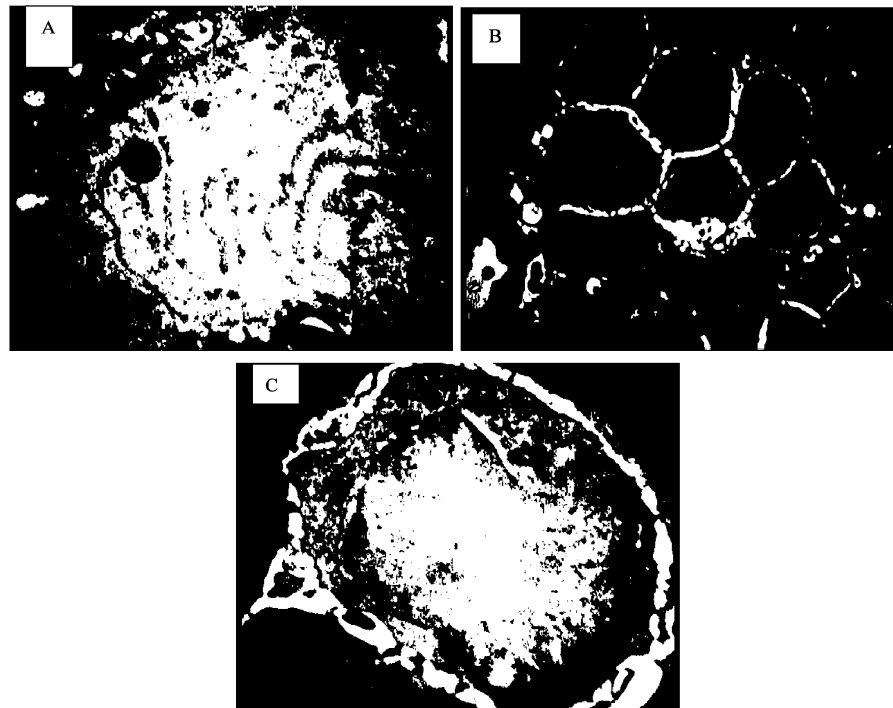


Fig. 2: An electronmicrograph from rat spleen

A, rough endoplasmic reticulum in splenic cell. 10000 X

B, lymphocytes from spleen of a rat exposed to EMF. Note the condensed nuclei. 3000 X

C, an splenic lymphocyte with higher magnification with euchromatic nucleus. 6000 X

## DISCUSSION

In spite of the enormous studies on the effect of EMF on living organisms [8, 9, 16], studies on the effect of EMF on immune system is limited on lymphatic cells in the circulating blood or factors released into the blood by cells involved in immunity [17, 18]. In the present study, the effect of EMF on one of the main lymphatic organ (Spleen) and protective role of V.E is investigated. Our finding was consistent in condensation of cell nuclei in experimental the test group 1, indicating that EMF has a nuclear effect and could at least suppress cellular metabolism.

This finding was well supported by previous studies showing that EMF has a nuclear effect for instance Zhang [19], Yokus[20] and Focke [21] showed that EMF causes DNA damage. It was believed that EMF, by producing free radicals, could attack organic bases in the nucleotides and break hydrogen bonds between purine and pyrimidine bases that would result in chromosomal breakage, point mutations and or changes in nucleotide sequences [22]. On the other hand, addition of V.E supplement prior to EMF exposure prevented the nuclear effect of EMF. Accordingly, in our experiment in the test group 2, that received V.E along with exposure to EMF, nuclear condensation was not occurred. This finding is also in support of previous studies that EMF induces damages via formation of free radicals [3, 4] and V.E as an antioxidant could neutralize detrimental effects of EMF [15]. In another study, Valentina[23] demonstrated that V.E reduced chromosomal damage caused by increased reactive oxygen species (ROS).

It is also shown that melatonin as an antioxidant, promotes DNA repair produced by ionizing radiation [24].

Our studies also showed that in the EMF exposed group, mean diameter of white pulps were reduced. It is clear that splenic white pulps are an active site of lymphatic cell proliferation and differentiation so it is appear that these changes are due to the decreased cellular population caused by detrimental effect of EMF on proliferating cells in those areas.

This finding is well supported by previous studies, because it is established that actively proliferating cells are more sensitive to environmental factors including EMF and Kaszuba *et al.*[18] showed that EMF - induced cellular mortality in cultured cells, was increased by addition of phytohaemagglutinin as a mitogen and also Onodera [25] showed that EMF has a detrimental effect on proliferating B and T lymphocytes rather than nonproliferating cells.

However, the changes were not appeared in those that received V.E. Another finding that the number of megakaryocytes was significantly decreased in test group 1 but not in test group 2 has a similar explanation.

Regarding the increase in the number of macrophages, it was clear that they are defensive and resistant cells, there were several reports showing that under stimulatory condition and tissue damage macrophages become more activate [26-28] and their number increase under any stimulatory conditions [29]. On the other hand, Simko[30] have shown that EMF resulted in significantly increased phagocytic activity of macrophages. So it is probable that macrophages were increased and got more activate due to the increasing of cellular damages. In test group 2 that received V.E supplement simultaneous to being exposed to electromagnetic fields, any increase in the number of macrophages was not seen.

It was concluded that in general, EMF by affecting the population of lymphatic cells has a suppressive effect on immune system and EMF-induced detrimental effects on lymphatic organ could be prevented by V.E supplementation.

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