Histological Study of Thyroid Gland after Experimental Exposure to Low Frequency Electromagnetic Fields in Adult Male Albino Rat and Possible Protective Role of Vitamin E

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Abstract

Introduction: Thyroid gland is one of the most important endocrine organs and almost all cells of the body are target sites for its hormones. Electromagnetic field became progressively more common constituent of the general and workplace environments.

Aim of work: To evaluate the effect of low frequency electromagnetic fields (LF-EMF) on the structure and function of the thyroid gland and to evaluate their reversibility and role of vitamin E.

Materials and Methods: Forty five adult male albino rats were equally divided into four groups: group I (control group), group II: rats were exposed to 50 Hz EMF for 1 month, group III: rats were received vitamin E just before EMF exposure for the same period and group IV (recovery group). At the time of sacrifice, blood samples were collected to estimate TSH, T3 and T4 levels. Thyroid gland specimens were processed for microscopic examination. The heights of follicular epithelium, collagen fiber area % were morphomertically estimated and statistically analyzed.

Results: Exposed group showed significant decline T3 and T4 levels together with significant increase in TSH level as compared to other groups. Histologically, this group showed congestion and dilatation of blood capillaries, cellular infiltration, follicular disintegration and vacuolar degeneration of some follicular cells. Other apoptotic follicular cells appeared with condensed chromatin or desquamated. The epithelial height and connective tissue area also affected. Vitamin E improves the biochemical and histological picture of thyroid gland (group III). Recovery group (IV) had variable alteration as compared to other groups.

Conclusion: LF-EMF has very serious effect on thyroid gland and vitamin E could have a protective role against this tissue damage.

Keywords: EMF; Thyroid gland; Ultrastructure; Vitamin E

Introduction

During the last decades of the 20th century, the constant enhancement of electrical power use led to increased exposure of living organisms, especially the human population, to a variety of artificially produced electromagnetic fields (EMFs). These fields originate from a number of different sources and are characterized by various frequencies and intensities [1].

Electric and magnetic fields are present around electrical equipment and wiring all through industry. The EMFs can be classified into static, extremely low frequency, intermediate frequency and radiofrequency fields. Among the previous types; the low frequency (LF) electromagnetic field (EMF) is wide spread in human environment and has a considerable attention among scientific community. This field can originate from sources as trains for public transport, any device involved in the generation, distribution or use of electric power [1]. Also, it can be produced by power lines and many kinds of electric appliances [2-4].

For many years, ELF-EMFs were considered as an absolutely neutral form of radiation [5]. Epidemiological data and results of many experimental studies have suggested that their consequences on human health become the subject of immense public debate [6] and one of the major concerns [7]. There have been many studies of the possible relations between residential exposure to ELF-EMFs and some kinds of cancer, including leukemia, lymphomas, brain, lung and breast tumors [8].

International Agency for Research on Cancer has evaluated the ELF-EMF as possibly carcinogenic to humans (Group 2B) [9].

Among the characteristic biological effects of EMF are the functional changes in CNS, endocrine and immune systems [Ahlbom 2001 Additionally, EMF can influence the learning and memory, cardiovascular system and reproductive system [10,11].

Concerning the endocrine system; the sensitivity of pineal, pituitary, adrenal and thyroid glands as well as of the islet of pancreas, testicles and ovaries to EMFs has been reported [12]. Thyroid gland is considered as body's primary regulator of metabolism through the action of thyroid hormones (T3 and T4). High concentration of these hormones may change the metabolism of oxygen in many cells and stimulate the production of free radicals in them [13].

The thyroid is considered as one of the most exposed vital organs and may be a target for EM radiation [12]. However, only few papers report the biological effect of low frequency EMF on the function and histological structure of thyroid gland. So, objective of the present study

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was to study the EMF-induced alterations in the histological structure and function of thyroid gland. It was also aimed to evaluate the reversibility of such changes with or without vitamin E supplementation.

Materials and Methods

Study protocol

Forty five healthy adult (2 month-old) male albino rats weighing 180-200 gram were used in this study. The rats were obtained from the Laboratory Animals Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. They were housed in plastic cages to avoid any metallic interaction under environmental laboratory condition at 20 ± 2°C. The animals were subjected to a controlled photoperiod (14 h: 10 h light:dark), were allowed water ad-libitum and fed standard diet. The experiment was performed in accordance with the "Guide for the Care and Use of Laboratory Animals" [14]. The experimental protocol was approved by the Ethical Committee of Zagazig University.

After an acclimatization period of 1 week, rats were randomly divided into four equal groups: I, II, III and IV

Group I (control group): was subdivided equally into two subgroups (five each):

Subgroup Ia: Rats were maintained in an environment without the presence of artificially produced EMF.

Subgroup Ib: Rats stayed in the experimental setup with the same conditions as the exposure group but without radiation exposure (exposure device off) and considered as sham-exposed group.

Subgroup Ic: Rats were given corn oils orally by modified gastric syringe (5 ml/kg/day).

Group II (EMF-exposed): included ten rats that were exposed individually to the influence of electric magnetic field 4 hours/day for 1 month [15].

Group III (EMF-exposed-vitamin E): included ten rats that were exposed to EMF as group II and received vitamin E 150 mg/kg/day by IM injection just before EMF exposure all over the experimental period (1 month).

Group IV (recovery group): included ten rats that were kept alive for another 1 month after exposure without treatment.

At the time of sacrifice, rats were anesthetized with intraperitoneal injection of sodium pentobarbital (Nembutal, 30 mg/kg body weight). The blood samples were collected through a cardiac puncture [6] from each rat. After opening the thorax, all animals were euthanized by releasing the trachea and securing the chest to prevent any movements. The blood samples were collected into the glass tubes (without anticoagulant) to clot. It was centrifuged to obtain serum and then stored at-20°C until the assay. TSH, Total T3 and T4 hormone levels in serum were measured using RIA Kit (Diagnostic Products Corporation, LA, USA).

Hormonal assay

The blood samples were collected into the glass tubes (without anticoagulant) to clot. It was centrifuged to obtain serum and then stored at-20°C until the assay. TSH, Total T3 and T4 hormone levels were measured using RIA Kit (Diagnostic Products Corporation, LA, USA).

Statistical analysis

Data for all groups were expressed as mean ± standard deviation (mean ± SD). The data obtained from the image analyzer and the biochemical data were subjected to SPSS program version 15. Statistical analysis using one way analysis of variance (ANOVA) test is used for comparison between different groups. The results were considered statistically significant when the probability (P) value <0.05.

Results

Histological results

Group I (control group): Histological examination of all control subgroups; Ia, Ib and Ic showed nearly similar structure. Figures for subgroup Ia were used to differentiate with other groups. The thyroid gland of control animals showed well formed, closely impacted follicles separated by thin connective tissue septa. The thyroid follicles had variable sizes and shapes. Their lumina contained homogenous acidophilic structure less colloid. Groups of interfollicular cells were observed. The central follicles were lined by single layer of cuboidal cells with rounded nuclei while the peripheral follicles were lined

blue for light microscope examination [18]. Ultra-thin sections were stained with uranyl acetate and lead citrate [18] and were examined and photographed with JEOI JEM 1010 electron microscope in Electron Microscope Research Laboratory (EMRL) of Histology and Cell Biology Department, Faculty of Medicine, Zagazig University.
by flattened or low cuboidal cells. The colloid showed conspicuous peripheral vacuoles (Figure 1A). Little amount of collagen fiber was seen in connective tissue septa (Figure 1B). Additionally, parafollicular cells (C-cells) were observed in some follicles resting on the basement membrane and had large pale nuclei. Vascular connective tissue septa were seen between the follicles (Figure 2A).

**Group II (EMF exposed group):** The gland in the exposed group showed disintegration and disorganization of thyroid follicles. Some follicles appeared with interrupted follicular wall. Congested blood vessels were seen in C.T septa (Figure 1C). Many collagen fibers were detected in the connective tissue septa (Figure 1D). Additionally, some follicular cells had darkly stained nuclei and interfollicular cellular infiltration. Desquamated epithelial follicular cells in the lumen (Figures 2B and 2C) are also seen.

**Group III (EMF exposed- vitamin E treated group):** Some thyroid follicles in group III that treated with vitamin E during EMF exposure retained the appearance of controlled follicles. They were lined by cuboidal cells that had rounded nuclei. Some follicles were distended with colloid that showed peripheral vacuolation (Figure 1E). Minimal collagen fibers were seen in interfollicular connective tissue septa (Figure 1F). Semithin sections of the same group revealed nearly normal thyroid follicles that lined by cuboidal cells with rounded vesicular nuclei as well s distended with homogenous colloid (Figure 2D).

**Group IV (recovery group):** The thyroid gland of recovery group showed preserved secretory activity. It was mainly characterized by nearly normal thyroid follicles that lined with flattened or low cuboidal epithelium. There are some involated follicles (Figure 1G). Moderate amount of collagen fibers could be seen in the interfollicular spaces (Figure 1H). Semithin sections of this group showed variable shaped thyroid follicles, some of them were involuted. Few follicles had darkly stained nuclei, others had normal cuboidal cells. Wide interfollicular spaces contained many blood vessels and cellular infiltration (Figure 2E).

**Electron microscopic results:**

Ulstructurally, the the normal histological structure of thyroid gland were found in all subgroups of control rats. Thyroid gland of this group showed rounded follicles that formed of cuboidal cells. The follicles were bounded with thin basement membranes that surrounded with capillaries. The follicular cells had euchromatic rounded nuclei with clumps of heterochromatin also their apical surfaces showed microvilli (Figure 3A). Their cytoplasm contained rough endoplasmic reticulum with dilated cisternae, intracytoplasmic vacuoles. The apex of the cells, adjacent to the follicular lumen, showed apical cytoplasmic vacuolization with the presence of many lysosomes (Figure 3B). While parafollicular (C)cells appeared with rounded euchromatic nuclei, homogenus cytoplasm containing mitochondria, few tubular cisternae of rough endoplasmic reticulum (RER) and fine electron dense granules (Figure 3C).

**Group II (EMF exposed group):** In rats exposed to EMF, Some
of the follicles were lined by high cuboidal cells with rounded nuclei and clumps of peripheral heterochromatin. Their cytoplasm contained several vacuoles, electron dense lysosomes. Dilated cisternae of RER lost their lamellar arrangement were also observed. Their apical border showed microvilli. In some cells, nuclei appeared often irregular in shape, shrunken with condensed heterochromatin (Figures 3D and 3E). The interstitium contained dilated congested blood capillaries, large amount of collagen fibers, fibroblast (Figure 3F) and different cells are also seen (Figure 4A). Parafollicular cells had oval nuclei. Their cytoplasm contained abnormal mitochondria with ruptured cristae and few granules (Figure 4B).

Group III (EMF exposed- vitamin E treated group): In vitamin E-treated group, the thyroid follicles were lined by cuboidal cells with ovoid euchromatine nuclei. Their cytoplasm contained dilated cisternae of RER and lysosomes and few colloid vacuoles. Notice microvilli at apex of follicular cells (Figure 4C). Parafollicular cell with indented nucleus and peripheral condensation of heterochromatin, few electron dense granules and lysosomes were demonstrated (Figure 4D).

Group IV (recovery group): Electron microscopic examination of thyroid follicles in recovery rats showed that the follicles were lined by follicular cells of variable shaped nuclei. One has irregular nucleus with peripheral condensation of heterochromatin while, other cell has rounded nucleus. Intracytoplasmic electron lucent vacuoles were more prominent. In addition to that large fibroblast with large amount of collagen fibers were seen in interfollicular spaces (Figure 4E). Some follicular cells have electron dense shrunken nuclei and disorganized diluted heterochromatin while, other cells have electron dense shrunken nuclei (n). Its cytoplasm contains disorganized diluted RER (Rr), lysosomes (L) and variable sized vacuoles (V) (Figure 4F).

Table 1: Shows relatively significant difference in T3, T4 and TSH levels in comparison to exposed and control groups.

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>EMF exposed group</th>
<th>EMF-vitamin E</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>T3 ng/dL</td>
<td>54.00 ± 2.29</td>
<td>38.57 ± 2.14</td>
<td>50.00 ± 4.79</td>
<td>40.75 ± 2.1</td>
</tr>
<tr>
<td>T4 ng/dL</td>
<td>66.02 ± 1.32</td>
<td>50.59 ± 1.46</td>
<td>60.43 ± 3.61</td>
<td>51.58 ± 1.46</td>
</tr>
<tr>
<td>TSH microU/dL</td>
<td>33.96 ± 1.86</td>
<td>43.16 ± 1.46</td>
<td>34.01 ± 3.2</td>
<td>40.16 ± 1.4</td>
</tr>
</tbody>
</table>

The differences are significant at p<.05 highly significant p<0.001, compared to Group 1 (control).

Biochemical results

EMF exposed rats showed a significant decrease in serum T4 and a highly significant decrease in serum T3, as compared with the controls. However, there was a highly significant increase in serum TSH in the same group.

In vitamin E and recovery groups, there were regressions in these results as these groups showed relatively significant difference in T3, T4 and TSH levels in comparison to exposed and control groups (Table 1 and Figure 5).

Morphometrical results

In exposed group, the epithelial height was significantly decreased while; the collagen fiber content was significantly increased in comparison to control group.
However, little knowledge about its effect on both histological and functional structure of thyroid gland is available. So, this study was designed to study both alterations and their reversibility.

The biochemical assay of rats exposed to EMF (Group II) showed significant (p<0.05) and highly significant decrease (p<0.001) in the level of serum T3 and T4, respectively in comparison to control rats. These results could be attributed to irradiation or blood protein degeneration [22,23]. Other possible reason for low T3 and T4 levels is the impaired exocytotic and endocytotic processes [24].

On the other hand, the level of TSH of same rats showed a highly significant increase (p<0.001) as compared with control rats. This finding might result from acute effects of radiation. Serum levels of T3, T4, and TSH were generally used as reliable indicators of the thyroid function in both humans and experimental animals. All reactions necessary for the formation of T3 and T4 are influenced and controlled by TSH level [25]. The elevation in TSH levels following irradiation could be explained by stimulation of the hypophysis [26] and/or tissue heating induced by EMF [6]. Moreover, exposure to EMF might influence the iodine uptake in the thyroid gland, and also prejudiced by increased temperature effect on pituitary gland [27].

However, Larsen et al. and Sert et al. [28,29] suggested that elevated serum level of TSH occurred due to active feedback mechanism. When the serum levels of T3 and T4 were decreased due to the radiation effects on the thyroid gland, the anterior pituitary gland released more TSH in the serum to stimulate the thyroid gland to produce more T3 and T4.

In the present work, alterations in thyroid gland function in the exposed group were further confirmed by the histological examination of the thyroid follicles which showed apparent light microscopic and ultrastructural changes. Many of these follicles underwent disintegration and disorganization. Some follicles appeared invovled and the others had interrupted follicular wall. The follicular cells had vacuolated cytoplasm and others had shedding of their epithelial lining in the lumina. Additionally, some follicular cells had darkly stained nuclei and cellular infiltration in interfollicular septa. The histological alterations of thyroid follicles could be explained by deficiency of stimulatory effects of TSH on the gland and hypoactivity [1]. The alterations in epithelial height were confirmed by morphometrical results.

It is well known that TSH is main factor influences structure and function of thyroid gland. Its simulative effect on the thyroid follicular cell is modulated by the action of a variety of molecules such as neuropeptides, peptides that derived from parafollicular cells and growth factors [15,30].

Ultrastructurally, the glands of exposed rats showed that some follicles were lined by high cuboidal cells with clumping of peripheral heterochromatin. Their cytoplasm contained several vacuoles and electron dense lysosomes. Dilated cisternae of RER contained amorphous electron-lucent contents and lost their lamellar arrangement were also observed. Their apical border s showed a huge number of aggregated microvilli. In some cells, nuclei appeared often irregular in shape, shrunken with condensed heterochromatin. Parafollicular cells had abnormal mitochondria with ruptured cristae and few granules. These results accepted and explained by some researcher Somozy [31]. It was reported that the cellular responses to different form of radiation or exposure to electromagnetic fields is manifested as irreversible and reversible structural and functional changes to cells and their organelles. Moreover, beside the morphological signs related to cell death, there are numerous reversible alterations in the structure of different cellular

**Discussion**

Over the past few years, considerable attention has been given to the potential bio-effects of electromagnetic field (EMF) because it is a common constituent of many fields in our environments. Some lifestyles and occupations are associated with more than the average amount of exposure to electromagnetic fields [19].

As a consequence, the possible effects of EMF on biological systems were broadly investigated to offer optimistic data for a correlation between exposure to EMF and increased incidence of health hazards [20].

In the last few decades, various and intense investigations have been carried out in order to explore the possible biological effects of electromagnetic fields (EMF) on many human systems. Among several investigated systems; the endocrine gland has drawn the most considerable attention especially the pineal gland [21].

However, vitamin E- treated group, there was marked increase in epithelial height in follicles as compared to the other two groups (II, IV). Additionally, The Collagen content in this group appeared more or less similar to the control group (Table 2 and Figure 6).

**Table 2:** Shows marked increase in epithelial height in follicles as compared to the other two groups.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>EMF exposed</th>
<th>EMF-Vitamin E treated</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial height</td>
<td>9.05 ± 0.83</td>
<td>6.577 ± 0.9*</td>
<td>8.306 ± 0.83*</td>
<td>7.577 ± 0.9*</td>
</tr>
<tr>
<td>Connective tissue area %</td>
<td>1.365 ± 1.24#</td>
<td>4.727 ± 0.43*</td>
<td>1.534 ± 0.19</td>
<td>3.632 ± 0.40*</td>
</tr>
</tbody>
</table>

**Figure 5:** Shows relatively significant difference in T3, T4 and TSH levels in comparison to exposed and control groups

**Figure 6:** Shows marked increase in epithelial height in follicles as compared to the other two groups.

**Mean ± SD**
organelles. The radiation-induced changes in the supramolecular organization of membranes, including plasma membrane and membranes of different cell organelle those play a significant role in the development of acute radiation injury [16].

The biological effects of EMF remain a matter of debate; some reporters Wolf et al. [20] suggested that ELF-EMF induce free radical production through oxidative stress. On the other hand, LF-EMF could be induced lipid peroxidation that leads to alteration of the protein/lipid ratio within the cell or its membrane. This peroxidation causes distraction of thiol groups existing in cell membrane and produce initiation of reactions leading to cell death [32-34].

Congested blood vessels and cellular infiltration were seen in thickened C.T septa that contained increased collagen fibers as morphometrically confirmed. These findings were recorded in other researches Rajkovc [15]. These changes explained by the direct effect of radiation [35,36]. On the other hand the shedding of epithelium lining of some follicles explained by indirect mechanisms of this vascular damage [37]. The perivascular lymphocytic infiltration could be attributed to immune-mediated reaction [38]. Also, Cutili et al. [39] reported that increase lymphocytes after irradiation may act as defense mechanism against the effect of radiation and help in removal of apoptotic bodies.

Congestion observed in the vasculature of the thyroid gland was in agreement with Jubb et al. [40] who attributed it to the excess demand for blood to nourish the follicular cells. Moreover, Burton and Foster [41] explained these vascular changes by the effect of free radicals production and lipid peroxidation induced by radiation.

Also, the vascular effect of EMF might be exerted by the action of mediators released from mast cells that located around blood vessels and/or nerve fibers. These mediators are known to increase the thyroid blood flow, and additionally increase the capillary permeability [42]. Theoretically, the exposure to EMF enable the enhancement of blood flow through capillaries and concomitantly facilitate different molecules, such as TSH, to be driven to the thyroid by the bloodstream [15].

Some investigators Jereczek –Fossa et al. [43] and Madanat et al. [44] concluded that the reduction in blood level of thyroid hormone leads to activation of anterior pituitary to secrete more TSH to avoid the occurrence of hypothyroidism.

In the current work, examination of group III that received vitamin E during EMF exposure showed marked improvement in the histological structure of the gland as well as its biochemical parameters. Some thyroid follicles restored the appearance of controlled follicles. They were lined by cuboidal cells that had rounded nuclei. Other follicles were distended with colloid and lined by flattened cells. The peripheral vacuolation of colloid was returned indicating increase the activity of the gland. Minimal collagen fibers were seen in interfollicular connective tissue septa.

Vitamin E improves the biochemical and histological picture of thyroid gland. Combined EMF- exposed-vitamin E treated group showed variable sized follicles nearly similar to control with considerable improvement. El-Habit et al. [45] attributed this improvement to the radioprotective effect of vitamin E. Some reports [46] referred the radioprotective ability of antioxidant compounds to their ability to scavenge free radicals and/or their inhibition. Moreover, it was mentioned that vitamin E had the ability to intensify the proliferative activity of survived stem cells for replacement of the lost cells and the migration of viable cells from areas adjacent to the affected area to site of injured cells [47]. However, the partial recovery detected may be due to the short time left for the cell to regenerate. This had been demonstrated by Green et al. [48] who reported regeneration of follicular cells immediately and may be more effective with Vit.E for two weeks after exposure.

The levels of ROS in mammals are partially controlled by endogenous antioxidants; mainly glutathione (GSH) [49,50]. GSH concentrations in the plasma and tissue are inversely related to the level of oxidative stress [51]. A suppression of tissue GSH signifies that ROS and oxidative stress are increasing and the tissues became unprotected against the oxidative stress [26].

Some studies on mice liver had shown that exposure to EMF had a marked oxidant effect, suggesting that ROS were generated during the exposure period and may be a sign of the pathophysiological process of the EMF exposure. So, it is concluded that vitamin E could be improved the exposed rats by compensating the defect in endogenous antioxidant defense system [4].

In animals of recovery evaluation of thyroid gland, the secretory activity of their glands was preserved. The apparently normal follicles were lined by flattened or low cuboidal epithelium and contained vacuolated colloid in their lumina. There were still some distorted and involuted follicles. Some disorganized follicles were devoid of colloid. The connective tissue components around the follicles appeared more prominent than in vitamin E-treated group and contained dilated blood vessels and moderate amount of collagen fibers. However, few follicles had darkly stained nuclei and cellular infiltration was also observed in wide interfolicular spaces. This morphological recovery cannot be supported with the physiological status of exposed animal because the decreased concentrations of T3 and T4 observed from the beginning to the end of the investigated recovery period. It is well known that TSH stimulates thyroglobulin exocytosis, endocytosis and proteolysis of the colloid droplets under normal physiological conditions. Additionally, TSH causes cytoskeletal protein phosphorylation and it is essential for pseudopod formation that leads to colloid endocytosis and, ultimately, thyroid hormone release [24]. So, The promising reasons for low T3 and T4 levels after recovery period, might be the impaired processes of exocytosis and endocytosis at the apical membranes of follicular cells [12].

Some experimental investigations established the EMF effect on enzymes [52] and EMF-induced alterations in the structure and function of cellular membranes [53,54] which considered as primary targets of EMF action in biological systems [55,56]. Serum T4 and T3 remained at considerably lower concentrations to the end of the recovery period. This record indicates that a longer period of time is required for the re-establishment of thyroid physiological homeostasis after EMF exposure.

The current work revealed histological changes in parafollicular (C) cell that evident at the ultrastructural level. This cell showed abnormal mitochondria with ruptured cristae and few granules in exposed group. While in vitamin E-treated group, C-cell had nearly normal appearance. In recovery group, the cell had indented nucleus and peripheral condensation of heterochromatin, few electron dense granules and lysosomes. These observations indicate the possibility of a relationship between the functional state of the thyroid gland and the activity of C cells. It was suggested that the possible mechanisms involved in the changes in C cell with the thyroid status were in line with the alterations in the follicular cells. Considering that; the TSH level is increased in rats with hypofunctioning thyroid, three possible explanations (a) TSH indirectly regulates C cells, (b) follicular cells regulate the C cells, and (c) C cells regulate follicular cells. However; the first hypothesis is supported by different reports [57,58].
In conclusion, the thyroid gland is sensitive to EMF exposure. This exposure induced morphological changes with drop off in serum T4 and T3. These changes remained to the end of the experiment indicating that a longer period of time is required for the return of normal thyroid activity after EMF exposure. Also, the results revealed considerable improvement by administration of vitamin E during the exposure period. Therefore, vitamin E is considered as a therapeutic hope for those exposed to EMF. Further studies are recommended using combination of vitamin E and other antioxidants either before or for long duration after EMF exposure.

References


