


Antioxidant effects of astaxanthin on electric field stimulated skin and sciatic nerve tissue

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Abstract

Aim: In this study, we have investigated the oxidative effects of long-term electric field (EF) exposure on the skin and sciatic nerve tissue. It is seen that astaxanthin (AST) can have protective effects on the skin and sciatic nerve tissue with its powerful antioxidant effect.

Materials and Methods: Rats are divided into 3 groups as control, EF, and EF + AST, with 10 animals in each group. 0.1 ml 0.9% sodium chloride for 30 days in the control group, 10 kV/m (50 Hz) EF 23 hours a day for 30 days in the EF group, 10 kV/m (50 Hz) EF 23 hours a day for 30 days and 100 mg/kg/day AST in 0.1 ml solution for 30 days in EF + AST group is given by gavage. Skin and sciatic nerve tissue are removed bilaterally and homogenized for biochemical analysis. Malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) enzyme activities are studied in the skin and sciatic nerve.

Results: The applied EF increases the MDA levels in the skin and sciatic nerve compared to the control group ($p = 0.013$, $p = 0.011$, respectively). While AST treatment decreased MDA levels in the skin and sciatic nerve compared to the EF group (respectively; $p = 0.046$, $p = 0.039$), SOD (respectively; $p = 0.001$, $p = 0.009$) and CAT (respectively; $p = 0.004$, $p = 0.008$) enzyme activities were increased.

Conclusion: The results show that AST can be used to treat oxidative stress caused by the electric field due to its antioxidant properties.

Keywords: Astaxanthin; electric field; oxidative stress; sciatic nerve; skin

INTRODUCTION

Electric and magnetic fields are both components of an electromagnetic field. Due to the widespread use of high voltage facilities, safety concerns about the impact of exposure to electric fields (EF) on human health are increasing. The magnetic field cannot usually be shielded by common materials, thus increasing the risk of human exposure. The magnetic field (MF) at 50/60 Hz generated from power lines and electrical devices is associated with biological effects on public health (1). Therefore, the biological effects of the low-frequency electric field have become a topic of interest to researchers today (2). A significant number of studies in recent years have focused on the biological effects of EF and MF produced with electrical and communication technologies. It is generally accepted that electric and magnetic fields can trigger biological effects that raise concerns about health

risks. However, it is widely debated whether EF and MF exposure will cause adverse health consequences (3). Previous studies show little evidence between cancer and non-cancer health problems with exposure to EF and MF. Relevant epidemiological studies have been limited by exposure measuring errors and the lack of a clear dose-response relationship in studies suggesting possible health risks. More research is needed to clarify the uncertain results obtained from existing studies and to determine whether exposure to EF and MF includes health risks (4).

Since very low-frequency electrical and magnetic fields do not have enough energy to break molecular bonds, they cannot directly damage DNA (5). Even though very low-frequency EF and MF have non-thermal effects on cells, it has caused the publication of protective measures by the World Health Organization because

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of the increasing scientific interest in this issue (4). Researchers investigating the negative effects of EF and MF have focused more on nervous system diseases such as cancer and Alzheimer's, but fewer have studied non-cancer diseases. Oxidative stress is thought to be involved in the physiopathology of these diseases (6,7). Reactive superoxide molecules play a role in the development and maintenance of neuropathic pain. Studies have shown that endoneurial lipid peroxidation is increased as a result of chronic constriction damage in the sciatic nerve. Superoxide levels have been found to increase in the dorsal horn and dorsal root ganglia of the spinal cord following sciatic nerve narrowing. Increased reactive oxygen species (ROS) initiates and maintains both peripheral and central sensitivity resulting in hyperalgesic symptoms in neuropathic pain (8). The skin acts as a barrier to absorb serious hazardous substances in the environment. Healthy skin is dependent on the existence of an intact cell. Absorption of EF and MF begins in the skin long before internal organs (9). Oxidative stress plays a crucial role in human skin aging and skin damage. The mechanisms of internal and external aging include ROS formation through oxidative metabolism. ROS formation is a very important mechanism that leads to skin aging. The oxidant events of skin aging include DNA damage, inflammatory response, reduced antioxidant production, and matrix production that disrupt collagen and elastin in the dermal skin layer (10).

Many exogenous sources act as antioxidants including polyphenols and carotenoids. Astaxanthin (AST) has recently attracted the interest of researchers because of its powerful antioxidant activity, unique molecular and biochemical messenger properties that have effects in treating and preventing skin disease (11). AST has greater antioxidant capacity in human dermal fibroblasts than α -tocopherol and β -carotene. Particularly, AST inhibits ROS formation and regulates the expression of enzymes sensitive to oxidative stress. Thus, it acts as a regulatory mechanism involved in cell adaptation against oxidative damage (12). Also, AST is recommended as an alternative therapy to reduce the risk of major neurodegenerative diseases such as Alzheimer's, Parkinson and amyotrophic lateral sclerosis caused by oxidative stress (13). There are several studies on the effects of long-term EF exposure on the sciatic nerve and skin. Hence, the oxidative effects of long-term exposure to low-frequency EF on the skin and sciatic nerve are investigated in this study. Besides, it is planned to investigate the healing role of AST against possible oxidative effects of EF on the skin and sciatica.

MATERIALS and METHODS

Test Animals

In the study, 30 Wistar Albino (250-300 g) female rats were used. Procedures on rats were reviewed and approved by Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee (Ethics No: 683, 2020). All animals were fed standard food as required, drank the same water, and kept under standard laboratory conditions

(temperature, 21-23 °C, 55% - 60% humidity, and 12 hours light/dark). The animals were placed in cages made entirely of plastic.

Collection of Skin and Sciatic Nerve Samples

The skin sample was excised from rat dorsal thoracolumbar area in 2 cm diameter. The sciatic nerve samples were dissected and excised in 2 cm length from rat dorsal gluteal-femoral region.

Animal Groups and Experimental Protocol

After a week of adaptation, 30 animals were randomly divided into three groups of 10 animals in each group:

- 1- Control group: 0.1 ml 0.9% sodium chloride solution was given by gavage for 30 days.
- 2- EF group: 10 kV/m (50 Hz) EF was applied 23 hours a day for 30 days and 0.1 ml 0.9% sodium chloride solution was given by gavage for 30 days (7).
- 3- EF + AST group: 10 kV/m (50 Hz) EF 23 hours/day and 100 mg/kg/day AST (dissolved in 0.1 ml solution) were given by gavage for 30 days (14).

In this experimental protocol conductive materials in the environment can change the direction and intensity of the electric field vector. Because of this situation, 1 hour was reserved for the daily routine cleaning (recovery from the wet ground) of the cage. The electric field exposure limit which is harmful to the body in the standards and regulations of the World Health Organization and Electrical and Electronics Engineering Institute was used for applied EF. EF quantity survey was measured by LF electric field meter (VX 0003 with 3 kHz internal antenna) and continuous measurement was performed by Süleyman Demirel University Engineering Faculty Electronics and Communication Department. At the end of the experiment, animals were decapitated under the anesthesia of 90 mg/kg ketamine (Alfamin, Alfasan IBV) and 10 mg/kg xylazine (Alfazin, Alfasan IBV) given intraperitoneally. Skin and sciatic nerve (bilateral) tissue samples were collected precisely and homogenized in phosphate buffer. Tissue samples obtained for oxidant / antioxidant analysis were kept at -20 °C.

Electric Fields Exposure Mechanism

In this mechanism, EF was formed between the plate using two parallel plates of the same size. The features of this mechanism consist of plates with an impact area of 0.5 m² (0.5x1.0 m) and power frequency placed between plates (to prevent the changing value of the area), fully plastic animal cages with dimensions of 40x50x20 cm³ (w x l x h) that do not interfere with the EF. The corners of the animal cages have been rounded to avoid the edge effect. In the production of the plates, 2 mm thick stainless steel was used to create a good conductivity. The two plates were spaced 50 cm wide. EF resistance was calculated according to the equation $E = V / d$. In this equation, V is the electric potential between the plates, d is the distance and E is the electric field intensity at volts/meter. The cages were placed parallel to each other and the cable

was connected to the center of the plates through the outer corner area of the cages. The set-up transformer speed of 220 Vrms/5000 Vrms and 1000 VA was used in the exposure system. According to the $E = V/d$ equation, the average intensity of the electric current between the plates was calculated as $5000\text{ V} / 0.5\text{ m} = 10.000\text{ V/m}$ (10 kV/m) in the EF group. Multimeter voltage Max 3000 TRMS Model was used for measurement (Chauvin Arnoux, Paris, France). The first voltage, second voltage, and EF intensity of the power transformer are in the range of 219-229 Vrms, 4975-5202 Vrms, and 9951-1045 V/m, respectively. Digital Gauss / Tesla meter was used in the EF purity test from the MF ground. Maximum MF strength is 0.001 mT. Undesired high-frequency fields in the experimental room were measured using the HI-3804 Electromagnetic Field Survey Meter-Industrial Compliance meter (Holaday Industries, Inc, UK) (7). The electrical field application method has been illustrated in di Figure 1.

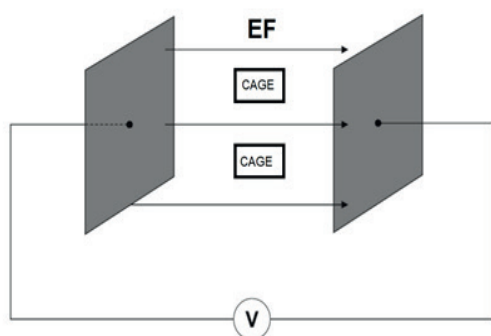


Figure 1. Electric field exposure mechanism

Biochemical Analysis

Skin and sciatic nerve tissue samples were placed in 9 times their weight in phosphate buffer (pH 7.4). It was first homogenized in a homogenizer (IKA Ultra-Turrax T25 Basic; Labortechnik, Staufen, Germany) then in a sonicator (UW - 2070 Bandelin Electronic, Germany). Tissue samples were prepared for malondialdehyde (MDA), superoxide dismutase (SOD), and catalase (CAT) enzyme analysis.

Skin and Sciatic Nerve MDA Levels

Draper and Hadley's double heating method was used to measure MDA which is a lipid peroxidation product. Trichloroacetic acid (TCA) was added to the samples first and vortexed, boiled for 15 minutes, then centrifuged for 10 minutes. Then, thiobarbituric acid (TBA) was added to these samples and boiled again. Absorbance measurements of the samples were performed at 532 nm (Shimadzu UV-1601, Germany) in the spectrophotometer. Results are given as nmol/gr.Hb and calculated with the standard absorbance values obtained from the same samples (15).

Skin and Sciatic Nerve SOD Enzyme Activities

SOD activity was measured by the spectrophotometric method using Randox brand commercial kit applied to an Olympus AU 2700 (Japan) autoanalyzer. SOD activity values of skin and sciatic tissues were expressed as U/ mg protein (16).

Skin and Sciatic Nerve CAT Enzyme Activities

CAT activity was studied according to the Aebi method. First, the first reagent with a value of 7.0 pH was prepared. The second reagent was then prepared with H₂O₂ and the amount taken from the first reagent. The tissue sample was mixed with the first reagent and the second reagent. Then, the absorbent values at 0 and 30th seconds were examined. Absorbances were measured in the spectrophotometer at 240 nm. Results were given as U/ mg protein (17).

Statistical Analysis

Data analysis was performed using the IBM SPSS software program (SPSS, version 22.0, Chicago, USA). First of all, the Kolmogorov-Smirnov test was used to examine the compatibility of the data to normal distribution. According to the Kolmogorov-Smirnov test, comparisons between control and treatment groups were made using one-way variance analysis (ANOVA) followed by the Bonferroni post hoc test. Data are presented as mean \pm standard deviation ($X \pm SD$) values. The value of $p < 0.05$ was considered significant.

RESULTS

Biochemical Results

Skin and Sciatic Nerve MDA Results

When EF is applied, MDA levels increase significantly in the skin and sciatic nerve tissues compared to the control group ($p = 0.013$, $p = 0.011$, respectively). MDA level in the skin and sciatic nerve tissue decrease significantly with AST treatment compared to the EF group ($p = 0.046$, $p = 0.039$, respectively). The results of the groups are presented in Figure 2 and Figure 3.

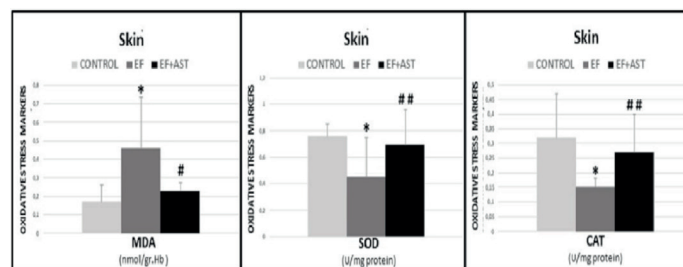


Figure 2. Oxidative stress markers of skin tissue. Data are presented as mean \pm SD. Relationships between groups and results were evaluated using one-way ANOVA (post hoc Tukey test). * $p < 0.05$ EF group compared to control, # $p < 0.05$, ## $p < 0.01$ EF + AST group compared to EF group

Skin and Sciatic Nerve SOD Enzyme Activity Results

When the electric field is applied, SOD enzyme activity decreases in the skin and sciatic nerve tissue compared to the control group, but the decrease is found statistically significant only in the skin ($p = 0.018$). With AST treatment, SOD enzyme activity increases significantly in the skin and sciatic nerve tissue compared to the EF group ($p = 0.001$, $p = 0.009$, respectively). The results of the groups are presented in Figure 2 and Figure 3.

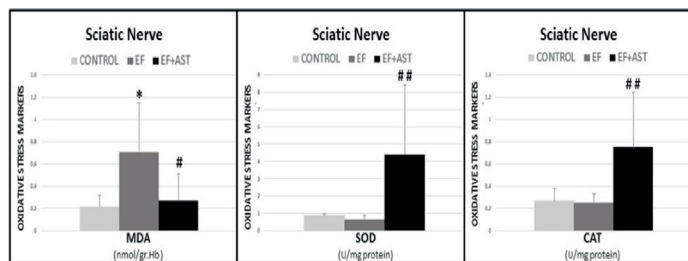


Figure 3. Sciatic nerve oxidative stress markers. Data are presented as mean \pm SD. Relationships between groups and results were evaluated using one-way ANOVA (post hoc Tukey test). * $p < 0.05$ EF group compared to control, # $p < 0.05$, ## $p < 0.01$ EF + AST group compared to EF group

Skin and Sciatic Nerve CAT Enzyme Activities Results

CAT enzyme activity decreases in the skin and sciatic nerve tissue in the EF group compared to the control group. However, this decrease is found statistically significant only in the skin ($p = 0.014$). In the skin and sciatic nerve tissue, the CAT enzyme activity increases significantly in the group treated with AST compared to the EF group ($p = 0.004$, $p = 0.008$, respectively). The results of the groups are presented in Figure 2 and Figure 3.

DISCUSSION

The information in the literature about the radioprotective effects of AST on EF-stimulated skin and sciatic nerve damage is quite lacking. In this study, it is thought that AST could reduce oxidative stress associated with EF through its possible antioxidant effects. Oxidative stress will cause cell damage to the skin and sciatic nerve. This research is conducted to test our hypothesis and fill the gap in the literature. In the study, an animal model is created that is subjected to long-term 50 Hz EF and AST. The results of the study show the antioxidant effects of AST on the skin and sciatic nerve stimulated by EF.

The free radicals are reactive molecules produced during the conversion of nutrients into energy with oxygen. Free radical formation is an oxidation reaction that occurs based on oxygen (18). The epidermis acts as the primary barrier against external attackers such as infection and radiation. This first barrier immediately responds to stress-related damage caused by external factors. Ultraviolet lights and EMA lead to the formation and overproduction of reactive oxygen species (ROS) by stressing epidermal cells (19). Ayata et al. have researched that radiation increases lipid peroxidation (LPO) through oxidative stress and changes the activities of antioxidant enzymes in the skin (20). Simon et al. have stated that exposure to the 900 MHz frequency causes a temporary change in epidermal homeostasis that can alter the skin's ability to protect against external factors (21). Ceyhan et al. have explained that exposure to 2.45 GHz EMR causes a significant increase in MDA level in skin tissue (22). Harakawa et al. investigated the effects of 50 Hz EF on plasma LPO and antioxidant activity in rats. They have reported that LPO levels increased in rats with oxidative stress (2). In our study, we showed that it increases MDA level in skin tissue at 50 Hz EF, thus

it may cause oxidative stress. Very limited studies have been reported regarding the level of MDA in skin tissue stimulated at 50 Hz EF. For this reason, we think that our results can contribute significantly to this area. However, the mechanism should be detailed in future studies.

Free radicals are essential for physiological processes, especially in brain metabolism. EMAs cause ROS increase and disruption of cellular antioxidant defense mechanisms by changing the free radical mechanism (23). In previous studies, EMAs have been reported to cause oxidative stress in the nervous system (cortex, cerebellum, hippocampus) (24-26). Kerimoğlu et al. have reported that exposure to 900 MHz EMF increased MDA levels in the spinal cord (27). Bilgici et al. exhibited that EMAs increase MDA levels in the brain (28). Also, it has been shown that long-term exposure to 900 MHz EMA causes oxidative stress by increasing MDA levels in the sciatic nerve (29). Although there is no literature investigating the oxidative effects of EF on the sciatic nerve in female rats, it is found in our study that MDA levels increased in the sciatic nerve tissue at 50 Hz EF. The body has antioxidant mechanisms such as CAT and SOD that protect against the harmful effects of ROS. In previous studies, the effects of EMAs on antioxidant capacity in the nervous system and skin have been reported differently. Meral et al. have reported that EMA causes a decrease in the level of CAT in the brain (30). Kerimoğlu et al. have reported a decrease in SOD levels in the spinal cord (27). Ragy MM (2015) showed that EMA reduced total antioxidant capacity in the brain (31). Özgüner et al. have found that 900 MHz radiation increased the CAT level in the skin tissue while decreasing the SOD level (9). In another study, it has been reported that high serum SOD values are detected in the EMA group (32). In the present study, when 50 Hz EF is applied, CAT and SOD enzyme activities decrease in the skin and sciatic nerve tissue, but the decrease is significant only in the skin tissue. The differences in the literature in terms of changes in the number of antioxidant molecules caused by exposure to EMA may have resulted from differences in tissues, application lengths, and application methods.

The development of supplements and dietary supplements to prevent skin aging and maintain its youthful appearance is a rapidly growing area of research. AST is an important antioxidant that prevents oxidative damage and anti-aging effects on the skin (33). In the present study, we investigate the effects of AST to improve oxidative damage in skin tissue stimulated by long-term EF. AST shows the oxidative damage caused by EF after stimulated by increasing the CAT and SOD activities. It is known that AST has neuroprotective effects with its strong antioxidant properties. However, the effects of AST against nerve damage are not fully known. Sharma et al. have shown that AST can have healing effects in nerve damage and can be an alternative in the treatment of neuropathic pain (34). Our study reveals that AST can prevent 50 Hz EF-associated oxidative damage in sciatic nerve tissue by increasing SOD and CAT enzyme activities. Due to the limited number of studies on this subject, future studies are needed to explain the mechanism.

CONCLUSION

The results of this study show that long-term 50 Hz EF exposure creates oxidative stress in the skin and sciatic nerve tissue. Besides, it suggests that long-term AST therapy may prevent sciatic nerve and skin damage, and may be protective against environmental stimuli such as EF. Considering the widespread use of electrical devices today, study results are also important for engineering. It will encourage the healthier use of technological equipment. It will also contribute to updating the exposure limits established for countries. Future studies are needed to explain EF and AST activity on the skin and sciatic nerve.

Competing interests: The authors declare that they have no competing interest.

Financial Disclosure: There are no financial supports.

Ethical approval: Burdur Mehmet Akif Ersoy University Animal Experiments Local Ethics Committee committee approval was received for this study under the protocol number 2020-683.

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