



Protective Effect of *Ocimum basilicum* on Brain Cells Exposed to Oxidative Damage by Electromagnetic Field in Rat: Ultrastructural Study by Transmission Electron Microscopy

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Abstract

Objective: Basil herb (*Ocimum basilicum*) has long been used in human nutrition. Nowadays antioxidant role of this herb is known more. The aim of this study was to study the anti-oxidative property of sweet basil to protect central nervous system against oxidative damages of electromagnetic field (EMF) and its affective sequences.

Materials and Methods: Forty Albino male Wistar rats were randomly allocated to four groups, 10 rats per each. Group 1 received normal diet (control group), group 2 was exposed to 50 Hz EMF for 8 weeks (EMF group). Group 3 was exposed to 50 Hz EMF and fed with basil extract (0.5 g/kg body weight) for 8 weeks (treatment group) and group 4 was fed with basil extract (0.5 g/kg body weight) for 8 weeks and named as herbal group. At the end of eighth week 5 mL blood was taken from all rats for biochemical analysis and for ultra structural study of brain neuron samples was taken.

Results: The results showed level of superoxide dismutase (SOD), glutathione (GSH) peroxidase and catalase activity (CAT) were significantly increased in herbal and treatment groups as compared to EMF group ($P < 0.05$). Level of malondialdehyde (MDA) was significantly decreased in treatment group as compare to EMF group ($P < 0.05$). Ultra structural evaluation of EMF group showed brain nucleus has a lot of heterochromatic changes and mitochondria have been ovulated and have swelling figure this changes were less in treatment group.

Conclusion: Antioxidant capacity of basil extract can cause to decrease oxidative effects of EMF on brain tissue and in rats.

Keywords: Brain, Neurons, *Ocimum basilicum*, Oxidative damage

Introduction

Oxidative damage is included not only in the etiology of many neurodegenerative disorders such as amyotrophic lateral sclerosis (ALS), Huntington disease, ataxia telangiectasia, Parkinson disease (PD) and Alzheimer disease (AD) but also seen in acute disorders resulting in neuron loss by environmental hazard factors (1). Due to recent increase in the use of electromagnetic field (EMF) producing equipments such as mobile phones, both epidemiological and experimental studies have been motivated. Today, there is a growing concern in regard to the indisputable reports on the harmful effects of the microwaves. Exposure to EMF at even low

frequencies (900-1800 Hz) causes certain established pathological consequences such as increased permeability of the blood brain barrier, disturbed neurons function, alteration in electroencephalography (EEG), disturbed regional cerebral blood flow, oxidant and antioxidant imbalance, neurotransmitter imbalance and genomic responses (2). Cellular oxidation and free oxygen radical release has been introduced as possible cellular injury mechanism that is accompanied with cognitional and affective sequences (3). Following this hypotheses, numerical antioxidative substances have been introduced to protect the central nervous system from the oxidative effect of EMF. It has been reported

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earlier that exposure to extremely low frequency EMF (ELF-EMF) can change animal behavior and cerebral blood flow in aged AD transgenic mice. Also it could modulate gene expression, cell differentiation and survival of neural cell populations (4,5).

Ocimum basilicum (from Labiatae family) has been used traditionally in Iranian and Indian medicine as folklore remedy for a wide spectrum of ailments and is also incorporated into a number of herbal medicinal preparations. It is used as treatment of cold and persistent coughs. Some investigations have shown its various protective effects including radiation protective efficacy, preventive potential against some chemicals, anti-inflammatory effect, stimulant agent in central nervous system, bactericidal activity, modulatory effect on glutathione and improvement in cognitional task, antioxidant property, ulcer protective, anti-diarrheal and blood-sugar (BS) lowering efficacy (6,7). Basil safety in animal and human models has been confirmed (8). Antioxidant effect of basil was shown by Khaki in 2011 on increased serum antioxidant (9). The objective of this research was to study the protective effect of *Ocimum basilicum* (sweet basil) on neurons of brain in rats exposed to EMF.

Materials and Methods

Animals

Albino male Wistar rats aged 8 weeks (weight = 220 ± 10 g) were housed in standard cages (14"×9"×8") inside a well-ventilated room kept at $20 \pm 2^\circ\text{C}$ with 12-hour dark-light cycle. They were obtained from Tabriz University of Medical Sciences, Central Animal Facility. All animals had free access to a standard pellet diet and water. Animals were exposed to radiation from experimental field (0.1 T) for 6 hours continuously per day for 6 weeks. Animals were free to move about in the cage during the exposure period. Sufficient ventilation and avoidance to impose heat shock to rats was controlled. Rats were brought back to the home cages following exposure. All interventions were done during a constant time period from 8:00 AM to 13:00 PM.

Forty rats were randomly allocated to four groups, 10 rats per each. Group 1 received normal diet and named as control group; group 2 was exposed to 50 Hz EMF for 8 weeks and named EMF group; group 3 was exposed to 50 Hz EMF and fed with basil extract (1.5 g/kg body weight) for 8 weeks and named as treatment group and finally group 4 was fed with basil extract (1.5 g/kg body weight) for 8 weeks and named as herbal group. All the experiments were performed in accordance with the European Animal Ethics Committee.

Extract Preparation

Fresh basil was prepared from local shopping in Tabriz. Superfluous materials were rubbed off and drained. Dried plants were steeped in methanol (80°C) and then the extracts were exploited in vacuum condition. Prepared extract was dried and used in maximum 2 days. Extracts were kept in refrigerator before being used in lab. All of the extract preparation processes were done in Pharmacognosy lab of Tabriz University of Medical Sciences, Tabriz, Iran.

Extract Administration

Prepared extract was dissolved in aqua's water. Exposed rats were fed with basil extract as 0.5 g/kg of body weight by gavage method. Feeding was done at least 1 hour before exposure to EMF.

Statistical Analysis

The data were analyzed using SPSS software (version 17). Data were expressed as means with SEM. The means of measures in two groups were compared by independent *t* test. Analyses of detected scores in nuclei and mitochondria value parameters were done by one-way analysis of variance (ANOVA) test followed by Tukey's HSD test. $P < 0.05$ was considered as significant.

Malondialdehyde Concentration Measurement in Serum

Free radical damage was determined specifically by measuring malondialdehyde (MDA). The MDA, formed as an end-product of lipid peroxidation (LPO), was treated with thiobarbituric acid to generate a colored product measured at 532 nm (MDA detection kit, Nanjing Ji-ancheng Bioengineering Institute, Nanjing, China).

Superoxide Dismutase Activity Measurement in Serum

The activity of superoxide dismutase (SOD) was measured by following the method of Beyer and Fridovich (8).

Glutathione Peroxidase Activity Measurement in Serum

The glutathione (GSH) peroxidase activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25mM H_2O_2 in the presence of reduced GSH (10mM), nicotinamide adenine dinucleotide phosphate (NADPH) (4mM) and 1 U enzymatic activity of GSH reductase (GR) (9).

Catalase Activity Measurement in Serum

Serum catalase activity (CAT) was determined according to the method of Beers and Sizer, as described by Hajhosseini et al (10) by measuring the decrease in ab-

sorbance at 240 nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer. The reaction mixture (3 mL) contained 0.1 mL of serum in phosphate buffer (50mM, pH 7.0) and 2.9 mL of 30mM H_2O_2 in phosphate buffer (pH 7.0). An extinction coefficient for H_2O_2 cm^{-1} was used for calculation. The specific activity of CAT was expressed as moles of H_2 reduced per minute per mg protein at 240 nm. An amount of 40.0 $M^{-1} cm^{-1}$ was used for calculation. The specific activity of CAT was expressed as moles of H_2O_2 reduced per minute per mg protein.

Transmission Electron Microscopy

For transmission electron microscopy (TEM) the brain samples were cut into piece (2×2 mm) and fixed in 2.5% glutaraldehyde (pH=7. 4) for 6-8 hours at 4°C. They were washed and post fixed in 2% OSO for 1 hour at 4°C. Brain tissue was dehydrated through ascending grades of ethanol and embedded in araldite CY212. Semithin sections (1 μm) were cut and stained with toluidine blue. Ultra thin sections (60-70 nm) were cut and stained with uranyl acetate and alkaline lead citrate. Morphometric study was done by evaluating the value of nucleus to cell, euchromatin to nucleus, mitochondrion to cell and rER to cell of organelles of neurons in all figures by Photoshop software 08.

Results

Results of Malondialdehyde Concentration in Serum

Administration of basil extract as 0.5 g/kg of body weight for 8 weeks significantly decreased MDA concentration in the herbal and treatment groups compared to EMF and controls ($P=0.17$; Table 1).

Results of Superoxide Dismutase Concentration in Serum

Administration of basil extract as 0.5 g/kg of body

weight for 8 weeks significantly increased SOD concentration in the herbal and treatment groups compared to EMF and controls ($P=0.17$; Table 1).

Results of Glutathione Peroxidase Activity in Serum

Administration of basil extract as 0.5 g/kg of body weight for 8 weeks significantly increased GPX concentration in herbal and treatment groups compared to EMF and control group ($P=0.17$; Table 1).

Results of Catalase Activity in Serum

Administration of basil extract as 0.5 g/kg of body weight for 8 weeks significantly increased CAT concentration in herbal and treatment groups compared to EMF and control group ($P=0.17$; Table 1).

Results of Transmission Electron Microscopy Study

Electromicrograph section of brain cortex did not show any changes in herbal and control groups whereas in the studied group, nucleus had a lot of heterochromatic changes and mitochondria were ovulated and swollen. Also the red blood cells were broken in brain artery and edema was seen in prevascular area (Figure 1A-D).

Results of Morphometric study

This analysis was done by evaluating the value of nucleus to cell, euchromatin to nucleus, mitochondries by selected 100 ultra thin section, randomly (Table 2).

Discussion

Comparable with those present in some residential areas (0.2-5.0 mT), occupational exposure to ELF-EMF at magnetic field levels has increased the risk of neurodegenerative diseases in some subjects. However, epidemiological studies have not found substantial positive associations between the occurrence of neurodegen-

Table 1. The Effect of Basil Extract as 0.5 g/kg of Body Weight for 8 Weeks on MDA, SOD, GSH Peroxidase, Catalase of the EMF, Treatment and Herbal Goups in Comparison to the Control Group (n = 10)^a

Samples Groups	Control	EMF ^b	Treatment ^c	Herbal ^d	P Value
MDA	5.05 ± 1.74	8.05 ± 1.05	4.05 ± 0.05	2.55 ± 1.05	0.022
SOD, u/g Hb	1000 ± 1.74	750 ± 2.11	900 ± 1.11	1300 ± 2.11	0.042
GSH peroxidase, u/mg Hb	125 ± 8.54	90.5 ± 5.55	100.5 ± 5.55	150.4 ± 8.54	0.072
Catalase, u/mg Hb	306.1 ± 12.81	250.4 ± 9.64	280.4 ± 0.55	350.2 ± 0.55	0.069

Abbreviations: SOD, superoxide dismutase; Hb, hemoglobin; MDA, malondialdehyde; GSH, glutathione; EMF, electromagnetic field.

^aData are presented as Mean ± SD; ^b50 Hz per day; ^c50 Hz per day + 0.5 g/kg per day; ^d0.5 g/kg per day.

Table 2. The Effect of Basil Extract as 0.5 g/kg of Body Weight for 8 Weeks on Value of Nucleus to Cell, Euchromatin to Nucleus, Mitochondrium to Cell and rER to Cell Organelles of Neurons in the EMF, Treatment and Herbal Groups in Comparison to the Control Group (n=10)^a

Groups	Herbal	Control	EMF	Treatment
V (V) Nucleus to Cell	0.367± 0.023	0.348 ± 0.017	0.289 ± 0.012	0.327 ± 0.02 ^b
V (v) Euchromatin to Nucleus	0.829 ± 0.25	0.802 ± 036	0.624 ± 0.13	0.745 ± 0.27 ^b
V (v) Mitochondrium to cell	0.321 ± 0.034	0.298 ± 0.034	0.526 ± 0.33	0.499 ± 0.28 ^b
V (v) rER to Cell	0.231 ± 0.11	0.220 ± 0.013	0.178 ± 0.030	0.219 ± 0.014 ^b

Abbreviations: SOD, superoxide dismutase; Hb, hemoglobin; MDA, malondialdehyde; GSH, glutathione; EMF, electromagnetic field.

^aData are presented as Mean ± SD; ^bP<0.05

erative disease and EMF exposure (6,7). This could be due to the selection and appropriateness of endpoints appraised, the intensity and time of EMF exposure, the target cell phenotype evaluated, the heterogeneous characteristics and small number of subjects studied and the wide variability of exposure levels between individuals. While considering the above elements it is essential to focus on epidemiological studies that evaluate mechanisms by which EMFs may impact neuronal processes and thus support the selection of defined future endpoints. It is in infancy, but proceeds to protective agents

in front of increasing environmental hazard factors, especially natural base agents have been motivated. There is more motivation to study the herbal extracts as anti-oxidative agents because herbal-based medications are accompanied with lower imposed side effects and are faced in today's society (8).

ELF-EMFs are a form of energy associated with the use of electrical power, generating magnetic field at a frequency of 50 Hz and a flux density primarily ranging between 0.2 and 5 mT. In this study, the magnetic flux density selected, 1 mT, is 2- and 10-fold the reference

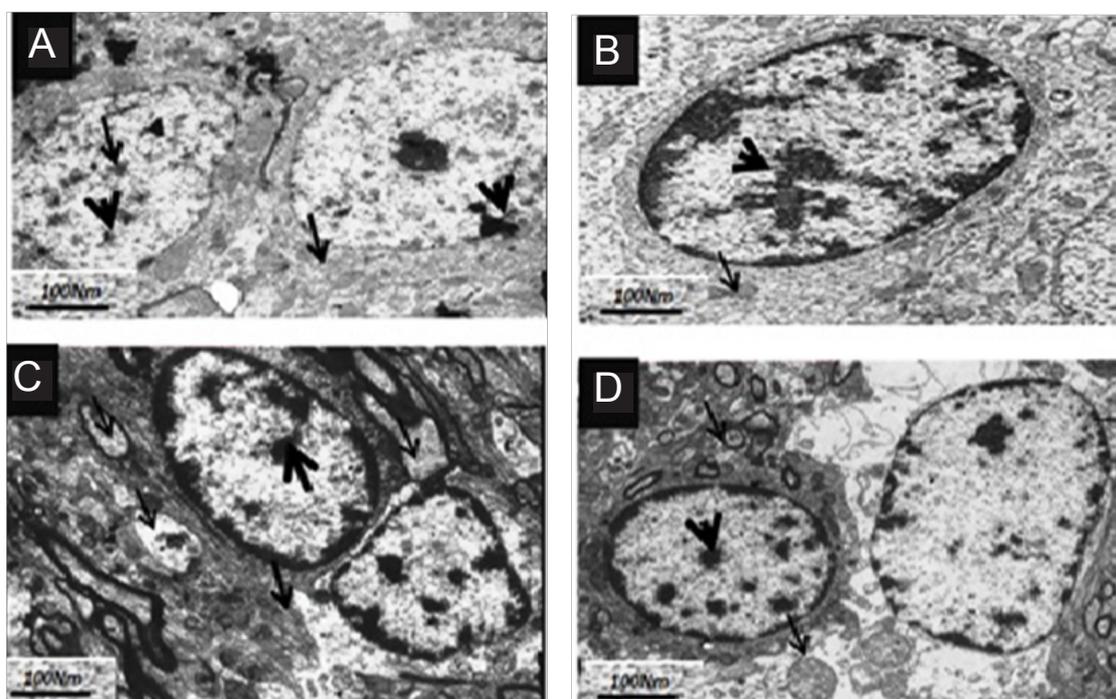


Figure 1. Electromicrograph a Section of Brain Cortex at the End of Treatment. As shown, there are no observed changes in shame (basil extract) and control groups whereas in the studies group nucleus has a lot of heterochromatic changes (arrow head), mitochondria have been ovulated and have swollen figure (arrow). Control (A), Shame (B), EMF Treated (C), Basil + EMF Treated (D), (×10 000).

levels as proposed by the European Community for Occupational and General Public Exposure. One of the most studied intensities in medical research highlighting the biological actions of ELF-EMF is the 1 mT flux density; as during the recent years there has been profound public concern of the impact of ELF-EMFs on human health and welfare, associated with both industrial and domestic use (10).

Epidemiological researches have focused on adult primary brain tumors, childhood leukemias as well as breast cancer, the potential for miscarriage and neurodegenerative disorders (11). Oxidative stress is a pathological state in which the balance of oxidant generation and detoxification is favored toward a pro-oxidant state. Also antioxidant defenses are overwhelmed, reactive species accumulate and damage to nucleic acids, proteins and membrane lipids sets in. Mitochondrial dysfunction and oxidative stress are the two mechanisms involved in the pathogenesis of ALS (12,13). Antioxidant depletion and oxidative stress are associated with blast-induced brain injuries (14-16). Under these conditions, oxidative stress can result in DNA damage, peroxidation of cellular and vascular structures, inhibition of the mitochondrial electron transport chain and oxidation of cellular proteins, leading to secondary damage in brain and lung tissues after these acute insults (17,18). Consistent with these findings, small pharmacological doses of antioxidant (vitamin E, vitamin C, or lipoic acid) loading by increasing hemoglobin oxygenation and reducing LPO have reduced blast-induced oxidative stress in the lung (19). The antioxidant N-acetylcysteine amide (NACA) by blocking inflammatory chemokine mRNA expression in the lung greatly reduced pulmonary inflammation after blast exposure (6). These findings suggest that by opposing oxidative stress conditions that lead to permanent brain damage and functional disability, antioxidants have the power to block the molecular cascades triggered by the blast exposure. Previously, we demonstrated that treatment with N-acetylcysteine (NAC) plus 2,4-disulfonyl a-phenyl tertiary butyl nitron (HPN-07) shortly after blast exposure can reduce both temporary and permanent hearing threshold shift and hair cell loss in the cochlea (20). In view of this, we point towards the possible combinatorial treatment of antioxidants to also block damage within the CNS caused by blast overpressure.

In this study our results showed basil has antioxidant potential as it can rebound the side effect of EMF on neuron cell morphology via increase in serum antioxidant levels by decrease in oxidative stress. This finding is in agreement with our previous investigation that showed EMF exposure imposed stress on animals fol-

lowed by depression like behavior in exposed animals. The sedative effect of basil without stimulatory effect on locomotor activity has been confirmed (9,10). It has been demonstrated that swimming is sensitive to serotonergic compounds and that climbing is sensitive to drug with selective effects on noradrenergic transmission (21). However, true mechanism of antidepressant effect of the *Ocimum basilicum* is unknown but behavioral parameters in forced swimming test confirmed potential anti-depressant effect as serotonergic agents. Some previous studies have investigated basil antioxidative property in vital organs (3,22). The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (23). Comparing the mentioned studies with our study shows similar results in their efficacy; nonetheless basil is a non expansive material in general population with less probable hazard side effects that people have used from several and several hundreds years ago and acceptable in folklore culture with perfect flavor in food.

Our study limitations included fixed dose of the feeding extract, dose relation effect of basil on locomotor activity, comparison with different types of known antidepressants synchronically and effect of basil on vital organs. We tried to study the cost effective and easy obtained herbal substance to evaluate its protective efficacy in EMF exposure. Although the development of EMF associated technology cannot be ceased, our young generation could be protected from the hazardous effects of radiation through extensive detailed study of herbal medicine drugs. The aim of the study was to compare the antioxidant effect of the plant in decreasing the deleterious effect of EMF.

Conclusion

Basil extract as a herbal medicine, protects brain cells from the harmful effects of EMF by regulating the antioxidant enzymes in serum. This saves the neurons from irreversible cell injury. The results show the beneficial effects of using this herb in daily diet in the population in order to protect their health when living in hazardous environmental areas exposed to EMF.

Ethical issues

This research was done by animal NIH ethic guidelines of Tabriz University of Medical Sciences, Tabriz, Iran.

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None to be declared.

Conflict of interests

The authors declare no conflict of interests.

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