



Protection effects of basil on cardiac cells apoptosis after chronic exposure to electromagnetic field (EMF) in rats by TUNEL assay

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Abstract

With developing in electronic means and increase in using these concern about the public health hazards of chronic exposure to electromagnetic field has gained more attention Medicinal use of basil belong to ancient medicine in asia such as Iran. Wistar male rat (n=40) were allocated into four groups, control (n=10) and test groups (n=30), that subdivided into groups of 3, the extract group were received of basil extract (1.5g/kg body), second extract group were received of basil extract (1.5g/kg body) and emf group that exposed to 50 Hz for 40 consequence day. In end of study the heart tissue of rats in whole groups were removed and prepared for pathology analysis by tunel method. Serum MDA, percentage of apoptotic cells and artery hyperemia significantly were increased in experimental group that has exposed to 50Hz EMF (p<0.05). the level of TAC in groups which received 1.5g/kg body basil extract significantly were increased (p<0.05) in comparison to control group. Morphology of heart in both experimental and control group were similar. Results revealed that administration of 1.5g/kg body of basil extract significantly decreased the apoptotic rate and protects cells by presents its antioxidant role.

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Introduction

Apoptosis is a highly regulated pathological and physiological process that regulates the balance between pro-death and pro-survival cell signals (khaki *et al.*, 2008). All apoptotic signaling pathways discovered thus far in extra-cardiac cell types have also been found to play a crucial role in induction of apoptosis in the cardiac cells, and therefore we will give only a brief overview of the mechanisms here as countless excellent reviews topic these basic mechanisms in detail (Regula KM *et al.*, 2005). In recent years the researches in order to understand the mechanisms of traumatic effects on vital tissues including the cardiovascular system, nervous, through oxidation and release of free radicals (Irmak *et al.*, 2002). Increasing enthusiasm in the wake of antioxidants has been taken and biologically active substances in plant extracts such as *Ocimum basilicum* plants in comparison with synthetic chemical drugs and their side effects such as NSAIDs, Prostaglandins, lipoproteins as a natural resource and more compatibility with critical systems have been considered. Basil belongs to the family Labiateae commonly known as Tulsi (khaki *et al.*, 2011). It grows in India, Pakistan, Sri Lanka, and Burma. Basil is relatively short lived compared to other plant and for maintaining medicinal properties should be kept in certain conditions of moisture and temperature. It in addition to being a seasoning and nutritional plant in the Pharmacopoeia as a medicinal plant has been introduced. Basil has also been used externally for the topical treatment of acne, insect stings, snake bites, and skin infections (Supawan *et al.*, 2007).

Some studies have suggested that Basil contains some antioxidants such as rosmarinic acid (Tada *et al.*, 1996) that has beneficial effects Alzheimer's disease and some other diseases. Furthermore, there are a number of reports on the antibacterial (Lis-Balchin *et al.*, 1998) and anti-fungal properties of this herb (Basilico *et al.*, 1999). Increasing attention to the potential effects of electromagnetic waves on cardiovascular system tissue, it was the task of pumping blood to the viewpoint of electromagnetic

waves of very high radiation levels (Jauchem *et al.*, 2001) concluded that on the basis of their study of heart rate variability from cell phones do not cause noticeable effects on heart rate regulation in healthy males and females in contrast. The present study was designed to investigate the protective effects of Basil extract on EMF effects on function and ultrastructure of heart tissue.

Materials and methods

Preparation of extract

Aerial parts of *O. basilicum* were purchased from a local store. The explant was authenticated by F.F. Fresh aerial parts of the plant were extracted by maceration with EtOH-H₂O (80:20) to produce a total extract (hydroalcoholic extract, HAE), which included total phenols and flavonoids from the plant.

Experimental animals

Two month study on 40 male wistar rats weighing approximately 220±10 g was performed for 6 consecutive weeks. Mice were kept in plastic cages under laboratory conditions at a temperature of 20+/-2 c with controlled light for 12 hours against 12 hours of darkness in the laboratory and were randomly divided in 4 groups. The environmental conditions (temperature and humidity) in all the animal holding areas were continuously monitored.

Animals were maintained under standard conditions (NIH). Rats were allocated to four groups, a control group (n = 10) and three treatment groups (n = 30). The first control group was gavage by normal saline (2 cc) daily for 6 weeks. The first treatment group had daily exposure of 8 hours in period of 6 weeks in the electromagnetic field (0.1 Tesla). The second group received Basil extract (*Ocimum Basilicum*) daily amount of 0.7 g / kg body in 6 weeks. The third treatment group had exposure to electromagnetic waves (electromagnetic field 8 hours daily in 6 weeks 0.1 Tesla) and was gavage by the extract of basil (*Ocimum Basilicum*) (0.7 g / kg body) for 6 weeks simultaneously. The pathologic samples were prepared from rat heart tissue and fixed in 10%

formalin solution and after preparing microscopic sections, samples stained with Hematoxylin – Eosin.

Measurement of Serum Total Antioxidant capacity (TAS)

TAS was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a

peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS⁺, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L), (Khaki *et al.*, 2011).

Table 1. Cardiac cells Apoptosis, TAC,MDA, Artery hyperemia percentage and Heart weights of rats with exposed to EMF and *O. basilicum* Extract.

O. basilicum + (EMF)	O. basilicum (1.5 g/kg body weight).	EMF (50Hz)	control	groups
11.05 ± 0.05*	3.45 ± 0.01	15.33 ± 0.05*	4.01 ± 0.03	Cardiac cells apoptotic cell (%)
0.75 ± 0.05*	2.25 ± 0.05*	05.01 ± 0.05*	1.05 ± 0.05	Total Antioxidant capacity (TAC) C(mmol/ml)
6.05 ± 0.05*	4.22 ± 0.05*	8.01 ± 0.05*	5.05 ± 0.05	Malondialdehyde (MDA) C(mmol/ml)
3.40 ± 0.03	4.57 ± 0.03	3.00 ± 0.01*	4.50 ± 0.05	Heart weight's (Gram)
1.5 ± 0.01*	0.04 ± 0.03	3.90 ± 0.05*	0.05 ± 0.01	Artery hyperemia (%)

Data are presented as mean ± SE.

* Significantly different at $p < 0.05$ level (compared with the control group).

Measurement of serum MDA

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL. Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard.

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) method for analysis of apoptosis

The in-situ DNA fragmentation was visualized by TUNEL method (Huang HFS *et al.*, 1995). Briefly, dewaxed heart tissue sections were predigested with 20 mg/ml proteinase K for 20 min and incubated in phosphate buffered saline solution (PBS) containing 3 % H₂O₂ for 10 min to block the endogenous peroxidase activity. The sections were incubated with the TUNEL reaction mixture, fluorescein-d UTP (in

situ Cell Death Detection, POD kit, Roche, Germany), for 60 min at 37°C. The slides were then rinsed three times with PBS and incubated with secondary anti fluorescein-POD-conjugate for 30 min. After washing three times in PBS, diaminobenzidine-H₂O₂ (DAB, Roche, Germany) chromogenic reaction was added on sections and counterstained with hematoxylin. As a control for method specificity, the step using the TUNEL reaction mixture was omitted in negative control serial sections, and nucleotide mixture in reaction buffer was used instead. Apoptotic cells were quantified by counting the number of TUNEL stained nuclei per sections, cross sections. Cross sections of 100 heart tissues per specimen were assessed and the mean number of TUNEL positive apoptotic cells per cross-section was calculated.

Results

Cardiac apoptotic cells

Number of Apoptotic cells colored brown, in EMF group was (15.33 ± 0.05) and in Ob, received group was (3.45 ± 0.01) and in Ob+EMF was (11.05 ± 0.05) and in control group was (4.01 ± 0.03) respectively. These changes was significant as p value less than 0.05 ($P < 0.05$), (Table 1).

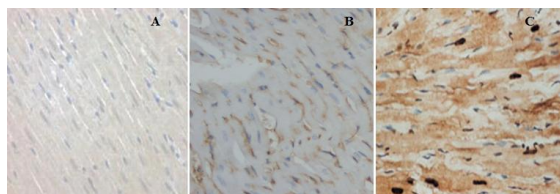


Fig. 1. A) heart ventricular section from a control rat group; shows the normal muscle tissue (arrow) and histological structure of the myocytes spaces, detecting by TUNEL assay, (40 ×).

Photomicrograph B) heart ventricular section from a EMF rat group; shows the dark brown stain muscle fiber (arrow) and histological structure of the myocytes spaces (star), detecting by TUNEL assay, (40 ×).

Photomicrograph C) heart ventricular section from a EMF+ O. basilicum rat group; shows the dark brown stain muscle fiber (arrow) and histological structure of the myocytes spaces (star), detecting by TUNEL assay, (40 ×).

Results of total blood anti-oxidant capacity

Amount of total blood anti-oxidant in EMF group was (05.01 ± 0.05) and in Ob, received group was (2.25 ± 0.05) and in Ob+EMF was (0.75 ± 0.05) and in control group was (1.05 ± 0.05) respectively. These changes was significant as p value less than 0.05 ($P < 0.05$), (Table 1).

Results of MDA (malondi aldehyde) level in blood

MDA level in in EMF group was (8.01 ± 0.05) and in Ob, received group was (4.22 ± 0.05) and in Ob+EMF was (6.05 ± 0.05) and in control group was (5.05 ± 0.05) respectively. These changes was significant as p value less than 0.05 ($P < 0.05$). Statistic analysis Dunnett (one side) shows significant differences between experimental groups in comparison to control group ($P < 0.05$), (Table 1).

Pathological results

Heart ventricular section from a control rat group; shows the normal muscle tissue and histological structure of the myocytes spaces. heart ventricular section from a EMF rat group that exposure with 50 Hz ; shows increasing in dark brown stain muscle fiber nuclei , and histological structure of the myocytes spaces was developed, pathology of heart ventricular section from a EMF+O. basilicum rat that exposure with 50 Hz and receiving 1.5 mg/kg of O. basilicum Extract for treatment ; shows the dark brown stain muscle fiber nuclei was decreased in observation when compared to EMF group and histological structure of the myocytes spaces was limited to many area (Fig. 1A, 1B, 1C).

Discussion

In addition, strong scientific evidence now also has been put forward, demonstrating that apoptotic cell death plays a pivotal role in the development of heart failure. Studies using genetically modified mice clearly indicate a direct, causal relation between levels of apoptosis and the progression towards advanced heart failure (Khaki *et al.*, 2008). Most notably, the group of Kitsis recently generated transgenic mice that express a conditionally active caspase exclusively in the myocardium, and demonstrated that very low levels of myocyte apoptosis (23 myocytes per 10^5 nuclei) caused a lethal, dilated cardiomyopathy in otherwise normal hearts (Wencker *et al.*, 2003). Advances in the technology world around us is undeniable and the increasing use of media such as mobile phones, computers , has an important part of everyday life in the world. In recent years considerable researches have been reviewed about the hazards of these advantages that electromagnetic waves emitted by mobile phones are in their heads. Any electrical device can be a source of electromagnetic field (EMF). Radiofrequency (RF) energy is a type of nonionizing radiation that is not strong enough to cause ionization of atoms and molecules (Erogul *et al.*, 2006). Low level of EMF can emit by cellular phones, so because of global using of this device;

estimation of the unforeseen risks from mobile communication has become a social and ethical problem. Although many studies have been done on the hazards of Rf (radiofrequency) waves emitted by mobile phone use, definitive result is undefined so it seems that more researches are needed to overcome drawbacks to prove relationship between EMF and health risks. (Blettner M *et al.*, 2009). In recent studies effects of EMF exposure in various fields including tumor progression, cancers, diseases of the central nervous system, cardiovascular, reproductive, immune system has been evaluated and although there was limited evidence for this association; noticeable results have been obtained (Leventhal *et al.*, 2004). Development of various heart diseases and daily exposure with emf, hypothesized an association between exposure to magnetic fields and acute cardiovascular disease (CVD). Reveals that some of ELF-EMF effects on cardiovascular system parameters occurs in a specific frequency or exposure time (window effect) (Jeong *et al.*, 2004) reveals an association between elevated magnetic field exposure and mortality of employer in electric utility industry jobs from arrhythmia-related causes and acute myocardial infarction (AMI), (Bellieni *et al.*, 2007)

Expressed that EMF generated by incubators can alter heart rate variability in newborns specially in prelatures. (Andrzejak *et al.*, 2008) study demonstrated that the mobile phone may influence heart rate variability by changing autonomic balance, (Jeong *et al.*, 2004) showed 1-day exposure to ELF-EMF suppressed the values of QT intervals in ECG by affecting ventricular repolarization and increased basal HR but (Mezei *et al.*, 2005) did not support the hypothesis that exposure to magnetic fields is a risk factor for cardiovascular mortality reported that there is not association between heart rate and in arterial blood pressure, so it means that more studies are needed to access reliable conclusions. In spite of these controversies new researches about this issue are clearer, (Roshangar *et al.*, 2012) concluded that EMF exposure can affect structure and function of

cardiovascular system and may facilitate myocardial infarction by nuclear changing of cardiomyocytes. Reactive oxygen species (ROS) are natural consequences of oxidative cell metabolism. Over production of ROS and imbalance of oxidant/antioxidant system are effective factors in the oxidative stress of cellular structures such as lipids, proteins and nucleic acids (Meral *et al.*, 2007). Superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which cause oxidative damages in lipids and DNA (Gülçin *et al.*, 2006b). Various environmental factors may intervene in this Phenomenon including longterm exposure to ELF-MF (Frahm *et al.*, 2006). Cellular damage caused by oxidative stress of exposure to electromagnetic radiation can induce apoptosis in various tissues of the body (khaki *et al.*, 2011). Free radical scavenging enzymes such as catalase, superoxide dismutase (SOD), glutathione peroxidase (GPX) are the first line cellular defense against oxidative injury (Sharma *et al.*, 2001). Animal studies conducted significant decrease in TAC (total antioxidant activity) such as SOD, GPX, vitamins E and A concentrations and increase of MDA (a product of polyunsaturated fatty acid peroxidation and used as an indicator of oxidative stress in cells and tissues) and plasma selenium concentration in erythrocytes and plasma after EMF exposure (Sharifian *et al.*, 2009). Antioxidant potency has been received much attention as one of protective mechanisms in foodstuffs (Niwano *et al.*, 2011), so Increase the intake of foods rich in antioxidant compounds (e.g. polyphenols, carotenoids, Vitamin A, b-carotene, curcumin, Allium cepa, quercetin, caffeine, chlorogenic acid, ellagic acid and bixin) due to their well-known healthy effects is recommended (khaki *et al.*, 2011).

The herbal extracts as natural resources and isolation of active antioxidant like flavonoids molecules are headed (Katalinic *et al.*, 2006). Many plants have the benefit of potential antioxidant activities and Several plants found to render radioprotection e.g. Ginkgo biloba and

Podophyllum hexandrum (Arora *et al.*, 2005). Many traditional plants are used for treatment of various diseases throughout the world as uncomplicated and consequent therapy like Basil (Hasani-Ranjbar *et al.*, 2009). Among various properties of Basil including ability of treatment of diabetes, cardiovascular diseases, neurodegenerative disorders, antifungal, antimicrobial, antiviral, antiapoptotic Benefits (Sharma *et al.*, 2001; Kaya *et al.*, 2008); according to the electromagnetic environment around us and role of EMF in occurring oxidative stress, antioxidant activity and phenolic compounds of Basil is of most interest (Javanmardi *et al.*, 2003; Kruma *et al.*, 2008). Our results showed heart ventricular section from a control rat group; shows the normal muscle tissue and histological structure of the myocytes spaces. Heart ventricular section from an EMF rat group that exposure with 50 Hz; shows increasing in dark brown stain muscle fiber nuclei, and histological structure of the myocytes spaces was developed, pathology of heart ventricular section from an EMF+ *O. basilicum* rat that exposure with 50 Hz and receiving 1.5 mg/kg of *O. basilicum* Extract for treatment; shows the dark brown stain muscle fiber nuclei was decreased in observation when compared to EMF group and histological structure of the myocytes spaces was limited to many areas and according to our results in table-1, this treatment effect belonged to anti-oxidant effect of *O. basilicum* that cause to increasing total antioxidant capacity in serum and decreasing MDA levels in serum, so this herb as strong antioxidant has protective cells from EMF damages side effect and this result is agree with other researchers results (Javanmardi *et al.*, 2003; Khaki *et al.*, 2012). Other study showed antioxidant and radical scavenging activity of basil containing phenols like rosmarinic acid, makes it to a possible food supplement and natural pharmaceutical applications (Gülçin *et al.*, 2007;). Vitamin E that is known one of the most important antioxidants, is contributed to antioxidant activity of the lipophilic extracts of basil that can be effective in radiation related disorders (Sgherri *et al.*, 2011). In this study malondialdehyde

(MDA) level in the Basil extract used groups significantly decreased and total antioxidant capacity (TAC) was increased, so we reach the conclusion that Basil extract beneficial effects on cardiovascular disorders caused by electromagnetic field exposure, is significant.

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