International Journal of Biosciences (IJB)
ISSN: 2220-6655 (Print) 2222-5234 (Online)
Vol. 2, No. 10(2), p. 1-10, 2012
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Amelioration of myocardial apoptosis by using *Ocimum basilicum* in rats after exposure to electromagnetic field (EMF): light and transmission microscopic study

Amir Afshin Khaki[1], Arash Khaki[2*]

[1]*Department of Anatomical Sciences, Islamic Azad University, Bonab- Branch, Iran*

[2]*Department of Veterinary Pathology, Islamic Azad University, Tabriz- Branch, Iran*

Received: 09 September 2012
Revised: 28 September 2012
Accepted: 29 September 2012

Key words: Apoptosis,Emf, GPX, myocardial cells, Ocimum basilicum, TAC.

Abstract

Apoptotic cell death plays a pivotal role in the development of heart failure. Exposure to EMF that arises from electronic means has harmful effect on public health, and cause to cell injury like apoptosis Medicinal use of ocimum basilicum dates back to ancient Iran, China and India. It has been used since ancient time as medicinal and food origin as an antioxidant's ocimum basilicum has a useful effect on many tissues as a protective on emf harmful Sid effects.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 ocimum basilicum extract (1.5g/kg body), second extract group were received of ocimum basilicum extract (1.5g/kg body) and emf group that exposed to50 Hz for 40 consequence day. for 40 consequence day. Animals were kept in standard conditions. In end of study the heart tissue of Rats in whole groups were removed and prepared for pathology and biochemical analysis. 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, GPX in groups which received 1.5g/kg body ocimum basilicum 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 ocimum basilicum extract significantly decreased the apoptotic rate and protects myocardial cells by presents its antioxidant role.

*Corresponding Author: Arash Khaki ✉ arashkhaki@yahoo.com

Introduction

Heart diseases, infarction and heart failure is on of common cause of morbidity and mortality in worldwide (Braunwald *et al.,* 2000). Research's showed abnormalities in cell membrane and cytoskeletal organization (Chien *et al.*, 1999), adrenergic signaling (Marks *et al.*, 2002), intracellular calcium handling (Marks *et al.*,2002), and myocardial energetics have been observed, the molecular and cellular mechanisms that mediate the pathogenesis of heart failure are poorly understood (Luo *et al.*, 1994).Nowadays, myocyte apoptosis has been noted in failing human hearts. Apoptosis is a highly regulated pathological and physiological process that regulates the balance between pro-death and pro-survival cell signals. 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 (khaki *et al* .,2008;Regula K.M *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).Ocimum Basilicum belongs to the family Labitateae commonly known as Tulsi and using as people traditional medicine.It grows in parts of Asia India , Pakistan ,Sri Lanka , iran . These plants are useful to treat certain disease like heart disorders and splenomegaly. O. basilicum 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. Reported higher risks for hypertension and coronary artery disease in workers that run radio technic and communication equipments.It is known that antioxidant effect of Basil is beneficial and it was demonstrated in previous researches that done by khaki and colleges, so the present study was designed to investigate the protective effects of Basil extract on apoptosis induces by EMF exposer on 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 (*O. 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 (*O. 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 light 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).

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.

Glutathione peroxidase (GPX) activity measurement in serum

GPx activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H2O2 in the presence of reduced glutathione (10 mM), NADPH, (4 mM), and 1 U enzymatic activity of GR (Yoshikawa etal.,1993).

TUNEL 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 % H_2O_2 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 antifluorescein-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 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.

Transmission electron microscopy

For transmission electron microscopy (TEM) the heart ventricle samples were cut into piece (2×2 mm) and fixed in 2.5%glutaraldehyde (PH=7. 4) for 6-8 h at 4°C. They were washed and post fixed in 2% OSO for 1 h, at 4°C. The tissue was dehydrated through ascending grades of ethanol and embedded in araldite CY212. Semi thin 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.

Results

Cardiac apoptotic cells

Number of Apoptotic cells colored brown, in EMF group was (16.03 ±0.05) and in *O. basilicum*, received group was (6.05 ±0.05) and in *O. basilicum* +EMF was(9.05 ±0.05) and in control group was(4.01 ±0.05) respectively. These changes was significant as p value less than 0.05 ($P<0.05$), (Table 1).

Table 1. Myocardial cells Apoptosis, TAC,MDA ,GPX, Mitochondria blebs , Muscle fiber degeneration and Heart weights of rats witch exposed to EMF and O. basilicum Extract.

O. basilicum + (EMF)	*O. basilicum* (1.5 g/kg body weight).	EMF (50Hz)	Control	groups
9.05 ±0.05*	6.05 ±0.05	16.03 ±0.05*	4.01 ±0.05	Myocardial 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)
11.5 ±0.01*	138.4±0.7	93.90±0.05*	125±0.7	GPX (u/mg Hb)
0.55 ±0.05*	0.01 ±0.05	0.75 ±0.05*	0.01 ±0.05	Mitochondria blebs (%)
5.05 ±0.05*	0.00 ±0.01*	8.55 ±0.05*	0.01 ±0.01	Muscle fiber degeneration (%)

Data are presented as mean ± SE.

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

Results of total blood anti-oxidant capacity

Amount of total blood anti-oxidant in EMF group was (05.01 ±0.05) and in *O. basilicum*, received group was (2.25 ±0.05) and in *O. basilicum* +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 *O. basilicum*, received group was (4.22 ±0.05) and in *O. basilicum* +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 Dennett (one side) shows significant differences between experimental groups in comparison to control group (P<0.05), (Table 1).

Results of slutathione peroxidase (GPX) activity in serum

Glutathione peroxidase (GPX) level in in EMF group was (93.90±0.05) and in *O. basilicum*, received group was (138.4±0.7) and in *O. basilicum* +EMF was (11.5 ±0.01) and in control group was (125±0.7) respectively. These changes was significant as p value less than 0.05 (P<0.05).Statistic analysis Dennett (one side) shows significant differences between experimental groups in comparison to control group (P<0.05), (Table 1).

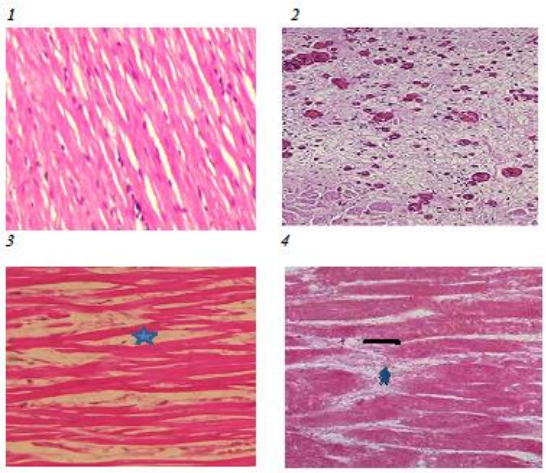

Fig. 1. Heart ventricular section from a control rat group; shows the normal muscle tissue (arrow) and histological structure of the myocytes spaces, detecting by H&E assay, (40 ×).

Fig. 2. Heart ventricular section from a EMF rat group; shows hyperemia muscle fiber degeneration (arrow) detecting by H&E assay, (40 ×).

Fig. 3. Heart ventricular section from a EMF rat group; shows the and histological structure of the myocytes spaces(star), detecting by H&E assay, (40 ×).

Fig. 4. Heart ventricular section from a EMF+ O. basilicum rat group; shows the regeneration of

muscle fiber(arrow) and histological structure of the myocytes spaces(star), detecting by H&E assay, (40 ×).

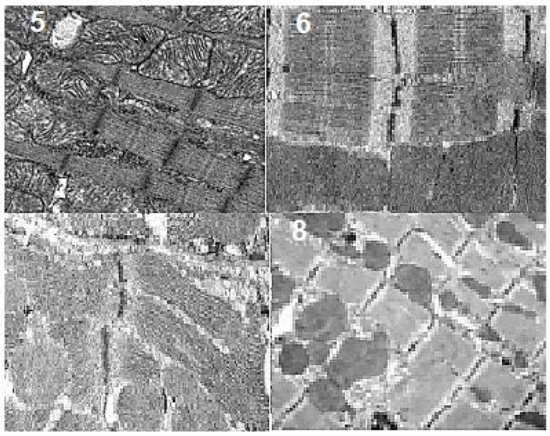

Fig. 5. Transmission Electron micrographs of the myocardial tissue & sarcomere of the control group, are shown by a normal and regular structural,(arrow) (X5000).

Fig. 6. Transmission Electron micrographs of the myocardial tissue & sarcomere of the EMF group, are shown by lose of area in sarcomeres and regular structural (arrow), (X5000).

Fig. 7. Transmission Electron micrographs of the myocardial tissue & sarcomere of the EMF group, are shown by irregular structural of myocardial cells, sarcomeres were ruptures (arrow),(X5000).

Fig. 8. Transmission Electron micrographs of the myocardial tissue & sarcomere of the EMF + O. basilicum group, are shown by structural of myocardial cells and sarcomeres were backed to normal(arrow), fibrosis are seen (star),(X5000).

Results of mitochondria blebs in myocardial cells

Mitochondria blebs level in EMF group was (0.75 ±0.05) and in *O. basilicum*, received group was (0.01 ±0.05) and in *O. basilicum* +EMF was (0.55 ±0.05) and in control group was (0.01 ±0.05) respectively. These changes was significant as p value less than 0.05 (P<0.05).Statistic analysis Dennett (one side) shows significant differences between experimental groups in comparison to control group (P<0.05), (Table 1).

Results of ventricle Muscle fiber degeneration

Ventricle Muscle fiber degeneration in EMF group was (8.55 ±0.05) and in *O. basilicum*, received group was (0.00 ±0.01) and in *O. basilicum* +EMF was (5.05 ±0.05) and in control group was 0.01 ±0.01) respectively. These changes was significant as p value less than 0.05 (P<0.05).Statistic analysis Dennett (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 (arrow) and histological structure of the myocytes spaces, in EMF group hyperemia muscle fiber degeneration , enhanced in myocytes spaces were seen, ultra structural study of the myocardial tissue & sarcomere of this group, are shown by lose of area in sarcomeres and irregular structural of myocardial cells, sarcomeres were ruptures ,in EMF+ *O. basilicum* group)heart ventricular section from a EMF+ *O. basilicum* rat group; shows the regeneration of muscle fiber(arrow) and histological structure of the myocytes spaces coming to normal form,also ultra-structural study of myocardial cells and sarcomeres showed these parts were backed to normal and fibrosis are seen in parts of myocardial cells(micrograph 1,2,3,4,5,6,7&8).

Discussion

Researcher's studies conformed that, in addition to necrosis, apoptosis also plays a role in the process of cells damage after myocardial infarction, which has pathological and therapeutic implications. Necrosis is present by the quick loss of cellular homeostasis, fast swelling as a result of the accumulation of water, electrolytes and starts with plasma membrane rupture and cause to the disruption of cellular organelles. Programmed cell death (Apoptosis) is, unlike necrosis, a highly regulated and energy requiring process. it is characterized by shrinkage of the cell and the nucleus. The nuclear chromatin is condensed into sharply delineated masses, and eventually breaks up. Oxidative stress through H_2O_2 and the NO donor, N-acetyl-Snitroso-

DL-penicillinaminamide (SNAP), induced apoptosis in ventricular cardiomyocytes isolated from a rat heart.In isolated rat cardiomyocytes, a correlation between the apoptotic effect of SNAP or YC-1 (a direct activator of soluble guanylyl cyclase) and the increased activity of soluble guanylyl cyclase (that is, the intracellular cGMP content) was seen (Taimor *et al* ., 2000). 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 *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 (Khaki *et al* .,2011). 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). 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 (Sharma *et al* .,2001). 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 various diseases throughout the world as uncomplicated and consequentive therapy like Basil (Hasani-Ranjbar *et al* .,2009). Amoung virous properties of Basil including ability of treatment diabetes, cardiovascular diseases, neurodegenerative disorders, antifungal, antimicrobial, antiviral,antiappoptotic Benefits (Kaya *et al* .,2008); according to the electromagnetic environment around us and role of EMF in Occuring oxidative stress , atioxidative activity and fenolic 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 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, lose of mitochondria cristae, blebs of mitochondria happen 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 and according our results in table-1,this treatment effects belonged to anti-oxidant effect of *O. basilicum* that cause to increasing total antioxidant capacity and GPX in serum and decreasing MDA levels in serum, and with this antioxidant effect confirmation cause to decrease rate of mitochondrial blebs in EMF group which received 1.5 mg/kg of *O. basilicum, studies* in past time researchers demonstrated mitochondria is very important for cells as energy provider , this results is agree with other researchers results(Javanmardi *et al*.,2003;Khaki *et al* 2012; Gülçin *et al*., 2006b).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) with Glutathione peroxidase (GPX) levels in serum was increased, and programed cell death percentage was significantly decreased in osmium basil groups.so we reach the conclusion that basil extract beneficial effects on cardiovascular disorders such as apoptosis caused by electromagnetic field exposure, is significant.

Acknowledgment

We would like to thank, Islamic azad university, Bonab Branch-Iran for give grant and financial support of this research.

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