Effect of Ocimum basilicum on Ovary tissue Apoptosis after exposed with extremely low frequency electromagnetic fields (ELF-EMF) in Rats

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

1- Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2- Department of Pathology, Rasht Branch, Islamic Azad University, Rasht-Iran

Abstract: Medicinal use of basil, Ocimum basilicum, dates back to ancient times in Iran, China, and India. This herb has been used since ancient times as a medicine and food and it is known that the antioxidant effect of O. basilicum is beneficial to protect tissue and decreasing carcinogenic effect of EMF, so it was hypothesized that this herb might also provide protection ovarian tissue from reactive oxygen spaces (ROS). Female wistar rats (n = 40) were allocated to four groups, a control group (n = 10) and three treatment groups (n = 30). The first treatment group received O. basilicum extract (1.5 g/kg body weight), the second extract group received O. basilicum extract (1.5 g/kg body weight) and EMF exposure at 50 Hz for 40 consecutive days, whilst the third group received only EMF exposure for 40 consecutive days. At the conclusion of the test period rat ovary tissues were removed from all group members. Ovary tissue preparation was performed and analyzed for apoptosis. There was a significant increase in apoptosis in EMF group when compared with other groups ($P<0.05$). EMF has negative effect on ovary histology in rats by increasing ROS. However, these side effects are less seen in the EMF group that received O. basilicum extract. Therefore, it is recommended that usage of O. basilicum extract in modern country has fewer side effects of industrial as one of female cancer (ovary cancer) problems.
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1. Introduction

The antioxidant capacity of phenolic compounds, flavonoids, and foods rich in these compounds, has been repeatedly demonstrated in various in vitro and in vivo systems [1]. Ocimum basilicum (Basil) is an annual herb of the Lamiaceae family, which is widely cultivated in Asia as a nourishing food and herbal medicine. O. basilicum is widely used in folk medicine to treat a wide range of diseases. For example, the aerial part of O. basilicum is traditionally used as an antispasmodic, aromatic, digestive, carminative, stomachic, and tonic agent. O. basilicum has also been used externally for the topical treatment of acne, insect stings, snake bites, and skin infections [2,3].An electromagnetic field (EMF or EM field) is a physical field produced by electrically charged objects. EMFs affect the behavior of charged objects in the vicinity of the field. The EMF extends indefinitely through space and determines electromagnetic interaction [4,5]. EMF is one of the four fundamental forces of nature; the others are gravitation, the weak interaction, and the strong interaction. The EMF can be viewed as a combination of an electric field and a magnetic field. The increased use of power lines and modern electrical devices is of concern as a public health hazard, and chronic exposure to EMF has attracted considerable attention. Exposure to EMF adversely affects spermatogenesis by the Sertoli and Leydig cells [6]. Magnetic fields of 50 Hz also induce cytotoxic and cytostatic changes in the differentiating spermatogonia of mice [7]. Furthermore, the study about effects of EMF in female rats showed: During the development of ovarian follicle in human, recruitment of a cohort of follicles occur, with only one destined to ovulate. In 2007 solimany rad and roshangar conformed previous researchs that done by Gougeon and present the remainder of the cohort undergoes atresia by uncertain stimuli and mechanisms. It appears that growth factors and related peptides may are involved in this process [8-10]. It is suggested that follicular atresia in the ovary results from apoptosis [11], The present study was designed to investigate about protective effects O. basilicum as anti-oxidanton ELF-EMF effects on ovary cells apoptosis.

2. Material and Methods

A total of 40 female Wistar rats were maintained for use in this study. Rats were housed together (10 per cage) and fed on a compact diet in the form of granules and water. The diet contained all the essential ingredients, including, vitamins and minerals. The environmental conditions (temperature and humidity) in all the animal holding areas were continuously monitored. Temperature was maintained in the range of 23 °C and humidity was maintained at 35–60%. Light was provided on a 12 h light/dark cycle from 0700 h to 1900 h. All animals were treated in accordance to the Principles of Laboratory Animal

Care [NIH]. The experimental protocol was approved by the Animal Ethics Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz University of Medical Sciences. Rats were allocated to four groups, a control group (n = 10) and three treatment groups (n = 30). The first treatment group received O. basilicum extract (1.5 g/kg body weight), the second extract group received O. basilicum extract (1.5 g/kg body weight) and ELF-EMF exposure at 50 Hz for 40 consecutive days, while the third group received only ELF-EMF exposure for 40 consecutive days. Animals were maintained under standard conditions.

2.1. ELF-EMF-producing system

The equipment was based on the Helmholtz coil, which operated following Fleming's right hand rule. The equipment produced an alternating current of 50 Hz, which created an EMF of 80 G. The intensity of the EMF was controlled using a transformer. The equipment had two main parts. In the first part, there were two copper coils placed one above the other and separated by a distance of 50 cm. A cylindrical wooden vessel was placed between the coils (the exposure area), the interior of which contained a chamber for holding the caged experimental animals. The second part was the transformer, which controlled the input and output voltage using a voltmeter and the current with an ampere meter. A fan was used as required, to prevent increases in temperature inside the chamber. Four cages at a time were placed within the chamber, with or ten rats per cage.

2.2. Surgical procedure

On day 40, a sodium pentobarbital solution (40 mg/kg) was administered intra-peritoneally as an anesthetic, and the peritoneal cavity was opened with a lower transverse abdominal incision. The ovary tissues immediately were removed from the control and experimental groups. The weight of the testes for each group member was recorded. Animals were then decapitated between 10:00 h and 12:00 h.At the end of 4 weeks of treatment, ovary was dissected from each rat ,24 h after the last administration Then tissue preparation was performed to investigate ,artery hyperemia and ovarian cells apoptosis by TUNEL method.

2.3. TUNEL analysis of apoptosis

The in-situ DNA fragmentation was visualized by TUNEL method (Khaki et al.,2008). Briefly, dewaxed 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 ovarian sections were incubated with the TUNEL reaction mixture, fluorescein-dUTP (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- H_2O_2 (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 ovarian cross section. 100 cross sections of per specimen were assessed and the mean number of TUNEL positive dark brown cells per each cross-section was calculated.

2.4. Extract preparation

Fresh basil was prepared from local shopping in Tabriz. Superfluous materials were rub off and were drained. Dried plants were steeped in methanol (90°) Merck company, then extract were exploited in vacuum condition. Prepared extract were dried and used in maximum two days.

2.5. Measurement of Serum Total Antioxidant capacity (TAC)

TAC 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).

3. Results

Our finding showed, ovarian apoptotic granulosa cells percentage significantly was decreased following administration of O. basilicum extract (1.5 g/kg body weight) in compared to the control group($P<0.05$). Exposed to 50 Hz of ELF-EMF caused a significant increase in the apoptotic granulosa cells percentagees. When 50 Hz of ELF-EMF was administrated together with O. basilicum extract (1.5 g/kg body weight).apoptotic granulosa cells percent was significantly decreased ($p < 0.05$) in granulosa cells ,respectively Artery hyper- emia significantly decreased ($P<0.05$) from,results indicated

antioxidants ability of basil couse to increased TAC level significantly (P<0.05). These results Indicating the protective effect of O. basilicum against ELF-EMF- induced granulosa cells apoptosis, other results showed percentage of large antral follicle and Ovary weight's were significantly decreased in groups with exposed to 50 Hz of ELF-EMF($p < 0.05$) but following administration of O. basilicum extract (1.5 g/kg body weight) can re uptake this decreasing, in other hand large antral follicle in group that receiving O. basilicum was significantly increased when compared to other study groups . artery hyperemia was significantly increased in groups with exposed to 50 Hz of ELF-EMF($p < 0.05$) and O. basilicum (1.5 g/kg body weight) can modify this harmful effect of ELF-EMF (Table:1).

Table 1- Granulosa cells Apoptosis, Large antral Follicle & Artery hyperemia percentage, TAC and ovary 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
11.05 ±0.05✶	3.45 ±0.01	15.33 ±0.05✶	4.01 ±0.03	Granulosa apoptotic cell (%)
9.05 ±0.05✶	13.25 ±0.05✶	05.01 ±0.05✶	10.05 ±0.05	Large antral Follicle (%)
1.40±0.371	1.57±0.73	1.00±0.01✶	1.50±0.05	Ovary weight's(Gram)
1.5 ±0.01✶	0.04 ±0.03	3.90±0.05✶	0.05 ±0.01	Artery hyperemia (%)
1.1 ±0.05	2.01 ±0.05✶	0.75 ±0.05✶	1.8 ±0.05	TAC(mmol/ml)

Data are presented as mean ± SE.

✶ Significantly different at $P < 0.05$ level (compared with the control group).

4. Discussion

In fertility is one of the major problems in matchs life, about 35 percent of infertility is regard to woman [12]. The importance of many of these factors is not yet clearly understood. A better understanding of underlying mechanisms in fertility and better study results clarifying the effectiveness of nutritional and biochemical factors are important to improve diagnosis and treatment. Smart choices for better foods might prevent body from many diseases [13,14]. It has been suggested that lifetime of free radicals depended to electric and magnetic fields at environmental levels and it may extend the and result in DNA damage [6,15]. EMF by affecting biochemical reactions cause to product unpaired electron such as a superoxide ion, nitrogen oxide and hydroxyl radical, ion channels, synthesis of macromolecules could have a harmful effect on cellular metaboli sm and cause to cells damages [15-18].Plants and natural products are extensively used in several traditional systems of medicine, so screening these products for radio-protective compounds has several advantages, because they are usually considered non-toxic and are widely accepted by humans. Many natural antioxidants, whether consumed before or after radiation exposure, can confer some level of radioprotection. In addition to beneficial effects accrued from established antioxidants, such as, vitamin C and E, and their derivatives, vitamin A, beta carotene, curcumin, Allium cepa, quercetin, caffeine, chlorogenic acid, ellagic acid, and bixin, protection is also conferred by several novel molecules, including, flavonoids, eppigallocatechin, and other polyphenols [19-22]. the findings of the present study indicate that ELF-EMF cause to apoptosis of granulosa cells and this is responsible for initiation of follicular atresia and degeneration. In this study EMF could significantly increasing arteriole hyperemia and decreasing in ovary weights and number of large antral follicles, so it seems EMF as an environmental factor, could disturb folliculogenesis by inducing apoptosis in granulosa cells. Regarding the universal increase of infertility rate [23], further studies are needed to clarify the relation between EMF exposure and infertility in mammals.EMF-induces as one apoptosis agent in alterations in the oocyte and could be considered as a pre apoptotic status of oocyte. Irregular morphology of nucleus could be an indication of changes in nuclear skeleton. A change in cytoskeletal proteins and degradation of nuclear lamin is considered as a trigger of apoptosis cascade [24].Although the mechanisms underlying follicular reversible or irreversible cell damage are not well known at this time, DNA damage, which can be initiated by oxidative stress such as free radicals role, it has been proposed as a possible mechanism that leads to the activation of the apoptotic cascade in atretic follicles [25]. In support of this idea; it is shown that EMf has a pro-oxidant effect [21] and it is proposed that the effect of EMF is mediated by production of free radicals [22]. According to Peluso et al in 1977, graulosa cells in atretic follicles undergo nuclear condensation and cytoplasmic blebbing. Based on roshangar & solimany rad research in 2007 type of changes occurred in EMF-exposed granulosa cells and followed by appearance of apoptotic bodies, a characteristic of late apoptosis. our findings well agrees with pro-oxidant effect of EMF[24] and that, oxidants are well known apoptosis-inducing factors

[25]. A disturbance in the pro-oxidant/antioxidant system has been defined oxidative stress. Reactive oxygen species (ROS) are very reactive molecules ranked as free radicals owing to the presence of one unpaired electron such as a superoxide ion, nitrogen oxide and hydroxyl radical, administration of this extract able to counterbalance the negative effect of ELF-EMF on ovary tissue. previous study showed that 2 hours of 60 Hz EMF exposure immediately altered the metabolism of free radicals, decreased SOD activity in plasma, decreased GSH content in the heart and kidney, but did not induce immediate lipid peroxidation[21], EMF is able to generate destructive reactive oxygen species including superoxide, hydrogen peroxide and hydroxyl radical and frequently used to produce oxidative and necrotic damages[20]. The role of EMF in the induction of apoptosis and oxidative damage has also been reported. This could be indicative of free radical scavengering properties of Ocimum basilicum [22]. The results of other study showed the anti-oxidant ability of Ocimum basilicum in the enhancement of protective effects of EMF exposures rats resulting from decrease of apoptosis in testis and vein congestions decreasing. This study demonstrated that the administration of Ocimum basilicum can overcome reproductive toxicity of EMF effects. In conclusion This natural extract (Ocimum basilicum) as an anti-oxidant can protect ovary tissue and follicles and it also able to reduce apoptosis in ovary tissue.

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Correspond Author:
Arash khaki,
Postal address: Women's Reproductive Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
Tel:0098-9143138399

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