

Full Length Research Paper

Effect of *Ocimum basilicum* on ovary tissue histopathology after exposure to electromagnetic fields (EMF) in rats

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Plants continue to be a major source of medicines, as they have been throughout human history. *Ocimum basilicum* 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 electromagnetic field (EMF), so it was hypothesized that this herb might also provide protection to ovarian tissue against reactive oxygen species (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 treatment 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 fibrosis and vein congestion. However, these side effects are less seen in the EMF group that received *O. basilicum* extract. Usage of *O. basilicum* extract could be one of the useful methods as antioxidant therapy against EMF exposure in industrial area.

Key words: Apoptosis, electromagnetic field (EMF), fibrosis, *Ocimum basilicum*, ovary.

INTRODUCTION

Herbs help to protect cells from the damaging effects of oxidation. Factors like stress, aging, electromagnetic field (EMF) and pollution causes high level of free radicals in body which damage DNA and causes many diseases such as infertility. Substances like vitamin E, vitamin C, or beta carotene act as anti-oxidant nutrients in the body. Vitamin E and beta-carotene protect cell membranes and vitamin C removes free radicals from inside the cell (Alexandropoulou et al., 2006). *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 (Supawan et al., 2007; Khaki et al., 2011). An 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

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electromagnetic interaction (Schüz et al., 2009; Emre et al., 2011). 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 (Martínez-Sámano et al., 2010). Magnetic fields of 50 Hz also induce cytotoxic and cytostatic changes in the differentiating spermatogonia of mice (Khaki et al., 2008). Furthermore, the study on effects of EMF in female rats showed that during the development of ovarian follicle in human, recruitment of a cohort of follicles occur, with only one destined to ovulate. Many researchers confirmed the observation of the previous studies that the remainder of the cohort undergoes atresia by uncertain stimuli and mechanisms. It appears that growth factors and related peptides may be involved in this process (Wei and Giudice, 1997). It is suggested that follicular atresia in the ovary results from apoptosis (Williams and Smith, 1993). The present study was designed to investigate the protective effects *O. basilicum* as anti-oxidant on EMF effects on ovary cells apoptosis.

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 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 EMF exposure at 50 Hz for 40 consecutive days, while the third group received only EMF exposure for 40 consecutive days. Animals were maintained under standard conditions.

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 ten rats per cage.

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 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.

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-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 ovarian cross section. 100 cross sections per specimen were assessed and the mean number of TUNEL positive dark brown cells per each cross-section was calculated.

Extract preparation

Fresh basil was prepared from local shop in Tabriz. Superfluous materials were rubbed off and drained. Dried plants were steeped in methanol (90°) (Merck company) then extract were obtained in vacuum condition. Prepared extract were dried and used in a maximum of two days.

RESULTS AND DISCUSSION

Our finding showed, ovarian apoptotic granulosa cells percentage significantly decreased following administration of *O. basilicum* extract (1.5 g/kg body weight) in comparison to the control group ($P < 0.05$). Exposure to 50 Hz of EMF caused a significant increase in the apoptotic granulosa cell percentages. When 50 Hz of EMF was administered together with *O. basilicum* extract (1.5 g/kg body weight) apoptotic granulosa cells percent was significantly decreased ($p < 0.05$) in granulosa cells. Artery hyperemia significantly decreased ($p < 0.05$). The

Table 1. Granulosa cells apoptosis, fibrosis & vein congestion percentage of rats exposed to EMF and *O. basilicum* extract.

<i>O. basilicum</i> + (EMF)	<i>O. basilicum</i> (1.5 g/kg body weight)	EMF (50Hz)	Control	Group
11.05 ±0.05*	3.45 ±0.01	15.33 ±0.05*	4.01 ±0.03	Granulosa apoptotic cell (%)
0.08 ±0.05	0.00 ±0.03	1.01 ±0.05*	0.00 ±0.00	Fibrosis (%)
2.5 ±0.01*	0.04 ±0.03	5.01±0.05*	0.03 ±0.00	Vein congestion (%)

Data are presented as mean ± SE. *Significantly different at $p < 0.05$ level (compared with the control group).

obtained result indicates the protective effect of *O. basilicum* against EMF- induced granulosa cells apoptosis. The other parameters like percentage fibrosis and vein congestion were significantly increased in groups exposed to 50 Hz of EMF ($p < 0.05$) but following administration of *O. basilicum* extract (1.5 g/kg body weight) can modify harmful effect of EMF (Table 1).

Fibrosis is the formation of excess fibrous connective tissue in an organ or tissue in a reparative or reactive process. This is as opposed to formation of fibrous tissue as a normal constituent of an organ or tissue. Infertility is one of the major problems in life style, about 35 percent of infertility is reported in woman (Mosher et al., 1991). One of the infertility type that was observed in women with cystic fibroid has not been well studied. It has long been associated with female infertility, the underlying causes have not been fully examined and remain unclear. Previous study of mouse models of cystic fibroid can provide important insights into the human condition and allow for experiments not feasible in humans. Smart choices for better foods might prevent body from many diseases (Reddy et al, 2006; Suryavathi et al, 2005). It has been suggested that lifetime of free radicals depended on electric and magnetic fields at environmental levels and it may result in DNA damage (Michael et al., 1999; Lai and Singh, 2004). EMF by affecting biochemical reactions increases the generation of unpaired electron such as a superoxide ion, nitrogen oxide and hydroxyl radical, ion channels, synthesis of macromolecules could have a harmful effect on cellular metabolism and be the cause of cells damages (Levin, 2003; Lai and Singh, 2004; Khaki et al; 2008, 2012). Herbal 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 radio-protection. 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 also exhibit various levels of protection; protection is also conferred by several novel molecules, including, flavonoids, eppigallocatechin, and other polyphenols (Kirtikar et al., 1991; Khaki et al., 2009a,b,c).

The findings of the present study indicate that EMF can cause programmed cell death of granulosa cells and this is responsible for initiation of follicular atresia and degeneration. In this study EMF could significantly increase vein congestion and fibrosis, so it seems EMF as an environmental factor, could disturb follicular maturation by inducing apoptosis in granulosa cells. Regarding the universal increase of infertility rate (Boivin et al., 2007) further studies are needed to clarify the relation between EMF exposure and infertility in mammals. EMF-induces as an one of theapoptosis promoting agent 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 (Khaki et al., 2008). 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, has been proposed as a possible mechanism that leads to the activation of the apoptotic cascade in atretic follicles. In support of this idea; it is shown that EMf has a pro-oxidant effect (Regoli et al., 2005) and it is proposed that the effect of EMF is mediated by production of free radicals (Khaki et al., 2011). According to Peluso et al. (1977), granulosa cells in atretic follicles undergo nuclear condensation and cytoplasmic blebbing. Our findings well agrees with pro-oxidant effect of EMF (Regoli et al., 2005) and that, oxidants are well known apoptosis-inducing factors (Chandra et al., 2000; Jiang et al., 2002, 2005). A disturbance in the pro-oxidant/antioxidant system has been defined as 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 test extract was able to counterbalance the negative effect of EMF on ovary tissue. Earlier study showed that 2 h of 60 Hz EMF exposure immediately altered the metabolism of free radicals, decreased superoxide dismutase (SOD) activity in plasma, decreased reduced glutathione (GSH) content in the heart and kidney, but did not induce immediate lipid peroxidation (Khaki et al., 2009). EMF is able to generate destructive ROS including SOD and hydroxyl radical and frequently used to produce oxidative and necrotic damages (Khaki et al., 2008). The role of EMF in the induction of apoptosis and oxidative damage has also

been reported. This could be indicative of free radical scavenging properties of *O. basilicum* (Polat et al., 2006). The results of other study showed the anti-oxidant ability of *O. basilicum* in the enhancement of protective effects of EMF exposed rats resulting from decrease of apoptosis in testis and vein congestions decrease. This study demonstrated that the administration of *O. basilicum* can overcome reproductive toxicity of EMF effects. In conclusion this natural extract (*O. 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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ABBREVIATIONS

EMF, Electromagnetic field; **PBS**, phosphate buffered saline solution; **ROS**, reactive oxygen species; **SOD**, suoeroxide dismutase.

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