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*Full Length Research Paper*

# Effect of *Ocimum basilicum* on apoptosis in testis of rats after exposure to electromagnetic field

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**Medicinal use of basil, *Ocimum basilicum*, dates 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 spermatogenesis, so it was hypothesized that this herb might also provide protection to sperm parameters. Male 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 electromagnetic field (EMF) exposure at 50 Hz for 40 consecutive days, whilst the third group received only EMF exposure for 40 consecutive days. Animals were maintained under standard conditions. At the conclusion of the test period, rat testes tissues were removed from all group members. Tissue preparation was performed and analyzed for apoptosis by TUNEL method. There was a significant increase in apoptosis in EMF group when compared with other groups (P<0.05). EMF has negative effect on testis histology in rats. However, these side effects are less seen in the EMF group that received *O. basilicum* extract. Therefore, it is recommended that the usage of *O. basilicum* extract in modern country has reducing side effects on industrial induced infertility.**

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

## 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 (Alexandopoulou 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). 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 electro-magnetic 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

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differentiating spermatogonia of mice (Khaki et al., 2008b) Little is known about the effect of EMF on the cytoarchitecture of the boundary tissue of the seminiferous tubules, which perform a number of crucial functions, including, mechanical support and transport of nutrients to the spermatozoa (Roychoudhury et al., 2009) and sperm discharge by maintaining pressure on the tubules (Iorio et al., 2007). Other studies have investigated transitory effects of EMF on the testes, but no previous study has reported the possibility of recovery from the potentially harmful effects of EMF exposure after an exposure-free period. Furthermore, the effects of EMF at the gonadal ultra-structural level have only infrequently been studied. The present study was designed to investigate about protective effects *O. basilicum* on EMF effects on testis apoptosis.

## MATERIALS AND METHODS

A total of 40 male 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 to 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 or 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 testes were then immediately 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, testis was dissected from each rat, 24 h after the last administration. Then tissue preparation was performed to investigate vein congestion and apoptosis by TUNEL methods.

### TUNEL analysis of apoptosis

The *in-situ* DNA fragmentation was visualized by TUNEL method (Khaki et al., 2008a). 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% $H_2O_2$ for 10 min to block the endogenous peroxidase activity. The 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 germ cells were quantified by counting the number of TUNEL stained nuclei per seminiferous tubular cross section. Cross sections of 100 tubules per specimen were assessed and the mean number of TUNEL positive germ cells per tubule cross- section was calculated.

### Statistical analysis

All values were expressed as mean ± SD. Differences in mean values were compared using SPSS 15.0 by one-way ANOVA test. $p<0.05$ was considered as statistically significant.

## RESULTS

Compared to the control group, apoptotic cells percentage decreased following administration of O. basilicum extract (1.5 g/kg body weight). Exposure to 50 Hz of EMF caused a significant increase in the apoptotic cells percentage. When 50 Hz of EMF was administrated together with *O. basilicum* extract (1.5 g/kg body weight), apoptotic cells percentage was significantly decreased ($p < 0.05$) from 18.12±1.05 to 10.05±0.01 in spermatogonia, and decreasing significantly($p < 0.05$) from 20.11±0.05 to 9.05±0.05 in primary spermatocytes, respectively; vein congestion significantly decreased ($p < 0.05$) from 8±0.03 to 0.5 ±0.01. These results indicate the protective effect of *O. basilicum* against EMF- induced apoptosis (Table 1).

## DISCUSSION

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

**Table 1.** Percentage of apoptotic germinal cells in rats testis exposed to EMF and *O. basilicum* extract.

| Group | Control | EMF (50 Hz) | *O. basilicum* (1.5 g/kg body weight) | *O. basilicum* + EMF |
|---|---|---|---|---|
| Spermatogonia apoptotic cell (%) | 6 ±2.03 | 18.12±1.05* | 2.25±0.01 | 10.05±0.01* |
| Primary spermatocytes apoptotic cell (%) | 7±0.05 | 20.11±0.05* | 4.25±0.01 | 9.05±0.05* |
| Testis weight's (g) | 1.40±0.821 | 1.30±0.001 | 1.47±0.373 | 1.40±0.371 |
| Testis vein congestion (%) | 1 ±0.01 | 8±0.03* | 6 ±0.03 | 0.5 ±0.01* |

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

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, Vitamins C and E, and their derivatives, Vitamin A, β-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 (Kirtikar Basu, 1991; Khaki et al., 2009a, b, c). Infertility is one of the major problems in match's life; about 25 and 35% of infertility is regard to man and woman respectively (Mosher and Pratt, 1991). 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 as important in improving diagnosis and treatment. Smart choices for better foods might protect the body from many diseases (Reddy et al., 2006; Suryavathi et al., 2005). As all spermatogenesis stages occur in seminiferous tubule of testis, it is possible to evaluate the extent of spermatogenesis by determination of number of spermatozoa produced per one gram of testicular parenchyma (Acharya et al., 2008; Hew et al., 1993). The sperm count is considered as important parameter which asses the effects of chemical on spermatogenesis (Yousef, 2005). It has also been reported that there is a direct correlation between the epididymal sperm count and motility with fertility in animals (Dawson et al., 1992; Timmermans, 1989; Yu et al, 2005). The oxidative damage, elevated lipid peroxidation and the alteration of memberane properties can lead to germ cell death at different stages of development and decrease sperm count (Bestas et al., 2006). Accordingly, it is expected that antioxidant therapy acts as a protective defense against oxidative stress and improve fertility parameters. The ability of antioxidants such as ascorbic acid in semen to protect spermatozoa from oxidative damage has been shown by some authors (Timmermans, 1989). The main pharmacological actions of ginger and compounds isolated there include immuno-modulatory, anti-tumorigenic, anti-inflammatory, anti-apoptotic, anti-hyperglycemic, anti-lipidemic and anti-emetic action. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals. It is considered a safe herbal medicine with only few and insignificant adverse/side effects (Dawson et al., 1992). Oxidants and antioxidants have attracted widespread interest in nutrition research, biology and medicine. It has become clear that constant generation of pro-oxidants, including oxygen free radical, is an essential attribute of aerobic life (Acharya et al., 2008). 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 this extract with gentamicin was also able to counterbalance the negative effect of gentamicin on sperm count (khaki et al., 2009c). A previous study showed that 2 h 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 (Khaki et al., 2009c); 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 (Khaki et al., 2008b). The role of EMF in the induction of apoptosis and oxidative damage has also been reported. Previous study was indicative of free radical scavenging properties of *O. basilicum* (Polat et al., 2006). The results of other study showed the ability of *O. basilicum* in the enhancement of protective effects in rats resulting from decrease of apoptosis in testis, testis weights increasing; vein congestions decreasing. This study demonstrated that the administration of *O. basilicum* can overcome reproductive toxicity of EMF. This natural extract was also able to reduce apoptosis in testis.

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