

Full Length Research Paper

# Effects of basil, *Ocimum basilicum* on spermatogenesis in rats

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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 spermatogenesis, so it was hypothesized that this herb might also provide protection to sperm parameters. Male Wistar rats (n = 30) were allocated to three groups, a control group (n = 10) and two treatment groups (n = 20). The first treatment group received *O. basilicum* extract (1.5 g/kg body weight), the second extract group received *O. basilicum* extract (3g/kg body weight) 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, before sperm was collected from the epididymis and prepared for analysis. Total testosterone serum, sperm concentration, percentage of sperm viability and sperm motility were significantly increased in the experimental group, which received 1.5 g/kg body weight *O. basilicum* extract ( $p < 0.05$ ), compared to control group. LH, FSH hormones, morphology and testes weights for both experimental and control groups were similar. Results indicate that administration of 1.5 g/kg body weight of *O. basilicum* extract significantly increased sperm percentage, viability, motility and total serum testosterone. This suggested that *O. basilicum* extract may be a promising treatment for enhancing healthy sperm parameters.

**Key words:** *Ocimum basilicum*, sperm, rat, testosterone.

## INTRODUCTION

The use of herbal medicines (medicinal plants or phytotherapy) has recently gained popularity in Europe and the United State. 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 (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).

In the last few years, a marked decrease in the quality of semen has been reported (Carlsen et al., 1992). These changes in semen quality are more likely to be due to environmental factors. Chemicals and drugs which are particularly misused are among these environmental factors (khaki et al., 2008)

The present study was aimed at investigating the possible beneficial effects of *O. basilicum* as a source of natural antioxidants to aid the sperm parameters in rats.

## MATERIALS AND METHODS

### Preparation of extract

Aerial parts of *O. basilicum* were purchased from a local store.

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The explant was authenticated by F.F. Fresh aerial parts of the plant were extracted by maceration with EtOH-H<sub>2</sub>O (80:20) to produce a total extract (hydroalcoholic extract, HAE), which included total phenols and flavonoids from the plant.

### Experimental animals

A total of 30 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 h to 1900 h. All animals were treated in accordance to the principles of laboratory animal care. Rats were allocated to three groups, a control group (n = 10) and two treatment groups (n = 20). The first treatment group received *O. basilicum* extract (1.5 g/kg body weight), the second extract group received *O. basilicum* extract (3 g/kg body weight) for 40 consecutive days and animals were maintained under standard conditions (NIH).

### 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 1000 and 1200 h and blood samples were obtained. Blood samples were centrifuged at 4°C for 10 min at 250 × g and the serum obtained was stored at -20 °C prior to analysis.

### Epididymis sperm count, viability and motility

Sperm were released from the cauda epididymis by dissecting in 2 ml of Hams F10 medium containing 0.5% bovine serum albumin. After 5 min incubation at 37°C (with 5% CO<sub>2</sub>), the cauda epididymis sperm reserves were determined using the standard hemocytometric method and sperm motility was analyzed using a microscope (Olympus IX70) with a 10 × field and levels were reported as the mean of motile sperm, according to the WHO method.

### Total serum FSH, LH and testosterone hormone measurement

Serum concentrations of FSH and LH were determined in duplicated samples, by radioimmunoassay (RIA). Rat FSH and LH kits were obtained from Biocode Co. (Belgium) and used according to the manufacturer's protocol. The hormone detection sensitivities per assay tube were 0.2 ng/ml and 0.14 ng/ml for FSH and LH, respectively. Total serum concentration of testosterone was measured using a double-antibody RIA kit (Immunotech Beckman Coulter Co., USA). The testosterone detection sensitivity per assay tube was 0.025 ng/ml.

### Total antioxidant capacity (TAC) and malondialdehyde (MDA) concentration measurement in serum

A TAC detection kit was obtained from Nanjing Jiancheng Bioengineering Institute, China. According to this method, the

antioxidant defense system, which consists of enzymatic and non-enzymatic antioxidants, is able to reduce Fe<sup>3+</sup> to Fe<sup>2+</sup>. TAC was measured by the reaction of phenanthroline and Fe<sup>2+</sup> using a spectrophotometer at 520 nm. At 37°C a TAC unit was defined as the amount of antioxidants required to increase the absorbance by 0.01 units in 1 mL of serum. Free radical damage was determined by specifically measuring malondialdehyde (MDA). MDA was formed as an end-product of lipid peroxidation, which was treated with thiobarbituric acid to generate a colored product measured at 532 nm (MDA detection kit from Nanjing Jiancheng Bioengineering Institute, China).

### Statistical analysis

The ANOVA test was used to compare data for the control group and the experimental groups. The results were expressed as mean ± S.E.M (standard error of means).

## RESULTS

### Weight of individual male testes

The obtained results in this study are shown in Table 1. There was a significant difference in testes weights between the groups ( $p < 0.05$ ).

### Results of sperm motility, viability and count

Administration of 1.5 and 3 g/kg body weight *O. basilicum* extract for 40 consecutive days significantly increased sperm motility, viability and count only in the extract-treated group ( $p < 0.001$ ), when compared with the control group ( $p < 0.05$ ) (Table 1).

### Results of serum total testosterone, LH and FSH hormones measurement

Administration of 1.5 and 3 g/kg body weight *O. basilicum* extract for 40 consecutive days had no significant effect ( $p > 0.05$ ) on LH and FSH concentration in the serum, when compared with the control group (Table 1). However, there was a significant increase in the total serum testosterone level in the extract group, when compared with the control group ( $p < 0.001$ ) (Table 1).

### Results of total antioxidant capacity (TAC) and malondialdehyde (MDA) concentration in serum

Administration of 1.5 and 3 g/kg body weight *O. basilicum* extract for 40 consecutive days significantly decreased the concentration of the malondialdehyde (MDA) level in the extract groups when compared with the control group ( $p < 0.05$ ) (Table 1). Total antioxidant capacity (TAC) was significantly increased in the extract groups, when compared with group ( $p < 0.05$ ) (Table 1).

**Table 1.** The effect of 1.5 and 3 g/kg *Ocimum basilicum* extract on sperm parameters, serum FSH, LH, TAC, MDA, total testosterone and testes weight, for control group in the rats.

Groups (n = 10)	Control	(1.5 g/kg body weight <i>Ocimum basilicum</i> extract)	(3 g/kg body weight <i>Ocimum basilicum</i> extract)
Testis (g)	1.40±0.821	1.47±0.373	1.40±0.371
Sperm concentration (total count) (No of sperm/rat ×10 <sup>6</sup> )	51.90±5.36	68.60±2.34**	60.55±0.22**
Motility (%)	33.75±6.88	73±4.35**	77±1.38**
Viability (%)	60.25±1.23	94.10±1.68**	92.10±80**
Serum testosterone levels (ng/ml)	1.70±0.01	2.99±0.210**	3.01±0.01**
LH levels (ng/ml)	1.51±0.138	1.73±0.164	1.31±0.128
FSH levels (ng/ml)	22.17±1.544	22.29±1.545	20.11±1.178
Total antioxidant capacity (TAC)	0.53±0.666	0.91±0.012*	0.90±0.222*
Malondialdehyde (MDA)	4.30±0.212	2.55±0.171*	2.21±0.122*

Data are presented as mean ± SE. \*significantly different at p< 0.05level (compared with the control group). \*\*significantly different at p< 0.001 level (compared with the control group).

## DISCUSSION

The role of nutritional and biochemical factors in reproduction and sub fertility treatment is very important. 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 radicals, is an essential attribute of aerobic life (Feng et al., 2001). Several conditions can interfere with spermatogenesis and reduce sperm quality and production. More factors such as drug treatment, chemotherapy, toxins, air pollutions and insufficient vitamins intake have harmful effects on spermatogenesis and sperm normal production exposure of biological systems to electromagnetic radiation, such as ionizing radiation, results in the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). These reactive species inflict damage on various bio-macromolecules, including, DNA, lipids and proteins present in the cell (Rajesh, 2008). This damage leads to early

signs, e.g., cataract induction, haemologic deficiencies, damage to skin and fertility impairment or late sickness, e.g., cancer several years after exposure, following radiation exposure (Schüz et al., 2009). The harmful effects of EMF ionizing radiation, such as X-rays and gamma rays, have been demonstrated with gonadal tissues (Khaki et al., 2006). 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 (Rajesh,

2008; Khaki et al., 2009a,b; Khaki et al., 2010). Basil (*O. basilicum* L., family Lamiaceae) is used as a kitchen herb and an ornamental plant in the house garden (Gülçin et al., 2007). Our results confirmed that EMF increased free radical and reactive oxygen species (ROS) resulting in cell injury and decreased sperm ability, which agrees with the findings in other reports (Garip and Akan, 2010; Sharma et al., 2009). Malondialdehyde (MDA) levels in the EMF group were significantly increased and again this agreed with other researchers to confirm that EMF leads to enhanced MDA content (indicating lipid peroxidation) and increased H<sub>2</sub>O<sub>2</sub> accumulation, thereby inducing oxidative stress and cellular damage (Sharma et al., 2009; Grigor'ev et al., 2010). However, the hydroalcoholic extract of *O. basilicum* increased the antioxidant capacity and had potential beneficial effects by neutralizing free radicals in the EMF group that received it, and led to a decreased level of MDA. These results confirmed previous chemical studies of herbal antioxidant effects (Niwano et al., 2011) from

*O. basilicum* ingestion, due to the presence of flavonoids, phenylpropanoids, and rosmarinic acid in the aerial parts of the plant (Bors et al., 1977; Peluso, 2006). These reports also documented the antioxidant and radical scavenging activity of *O. basilicum* (Jayasinghe et al., 2003; Dorman and Hiltunen, 2010).

In conclusion, many herbs like *O. basilicum* are well-known to contain flavonoids and have a strong antioxidant effect that is beneficial for serum antioxidant levels, leading to improved sperm health parameters via the reduction of oxidative stress (Khaki et al., 2009b, 2010), so it seems likely that long-term use of herbs can increase testosterone levels, improve sperm parameters and increase the chance of fertility.

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