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Protective effect of date palm pollen (*Phoenix dactylifera*) on sperm parameters and sexual hormones in male NMRI mice exposed to low frequency electromagnetic field (50 Hz)

Javad Baharara[1*], Elaheh Amini[2], Farzaneh Salek-Abdollahi[3], Najme Nikdel[3], Majid Asadi-Samani[4]

[1]Research Center for Animal Development Applied Biology & Department of Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[2]Department of Animal Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
[3]Faculty of Biological Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran
[4]Medical Plants Research Center, Shahrekord University of Medical Sciences, Shahrekord, Iran

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ABSTRACT

Introduction: Exposure to electromagnetic fields (EMFs) induces harmful effects on testis and reproductive activities. In traditional medicine, date palm pollen (DPP) which has remarkable nutritional values is used for curing male infertility and impotency. The aim of this study was to investigate the protective effect of DPP in preventing the detrimental effects of low frequency electromagnetic field (50 Hz) on sperm parameters and sexual hormones.

Methods: Adult male mice were randomly divided into 7 groups of 8 and exposed to EMF 4 h/day for 10 days. In this study experimental groups received DPP with doses of 25, 50, 100 and 200 mg/kg, respectively before exposure. At the end of the experiment each group were tested for sperm parameters including: motility, count, morphology, viability and the level of luteinizing hormone (LH) and testosterone.

Results: Our results revealed that exposure to EMF induced significant reduction ($P<.001$) in sperm count, viability and progressive motility in comparison with control group. EMF caused abnormalities in sperm and significant decrease in testosterone level while there was no significant difference in level. Administration of DPP before exposure improved the sperm count, viability, motility and testosterone level in experimental groups. In addition, pretreatment with DPP prevented the sperm abnormality induced by EMF.

Conclusion: The results indicate the protective effect of DPP against EMF adverse effects on sperm parameters and sexual hormones in male mice.

Implication for health policy/practice/research/medical education:
Date palm pollen (DPP) has protective effects against EMF adverse effects on sperm parameters and sexual hormones in male mice and might be beneficial in human sexual dysfunction.

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Introduction

Nowadays, the widespread use of various electrical and telecommunication appliances that produce electromagnetic fields is increasing rapidly and results in daily exposure of humans to electromagnetic fields (EMFs), more than past few decades (1). Modern societies are pervaded by Non-ionizing 50-60 Hz low frequency electromagnetic field exposure. The probable health effects of different electromagnetic spectrum produced by electrical instruments have resulted in performance of researchers in this field (2).

Testes have 2 critical functions: spermatogenesis and male sex hormone production. However, the Leydig cells secrete testosterone which regulates the spermatogenesis also the pituitary gland secretes luteinizing hormone (LH) and follicle stimulating hormone (FSH) that regulate male reproduction. So LH stimulates Leydig cells to produce testosterone and maintains their functions (3). Male fer-

***Corresponding author**: Javad Baharara, Professor, Dept. of Biology & Research Center for Animal Development Applied Biology, Mashhad Branch, Islamic Azad University, Mashhad, Iran, Tel: + 98 511 8437092, Fax: + 98 511 8437092, Email: baharara@yahoo.com

tility depends on the semen quality that contains normal spermatozoa (quality) in the adequate number (quantity). Nevertheless different risk factors may be associated with male fertility problems such as genetic disorders, genital duct obstruction, varicocele, decreased sperm production, decreased semen quality parameters, erectile dysfunction and male impotence (4). It has been also proved that EMF has adverse effects on gonads, liver and brain. Moreover, many studies have shown that testis are very sensitive to electromagnetic fields and can be damaged by extremely low frequency irradiation exposure in which decreased number of sperms and reproductive activities may result in infertility (5). Electromagnetic fields can intensify the generation of oxidative stress and cause an imbalance between the production of reactive oxygen species (ROS) and their antioxidant defense system, inducing cellular damage (6). In testis the rates of metabolism and cell replication is high and oxidative stress can be harmful in seminiferous tubules because electromagnetic exposure and heat can affect the blood-testis barrier and cause spermatogonial degeneration. On the other hand, several studies have shown that antioxidants provide protective barrier by breaking the oxidative chain reaction in the sperm cellular content (7,8).

Herbal medicines have been popular for prevention and treatment of diseases from ancient times and in recent years herbal remedies are used as primary health care with minimum side effects around the world (9). The use of natural antioxidants from plant is increasing day by day. In addition, numerous natural antioxidants such as, vitamins C, E and their derivatives, Vitamin A, β-carotene, curcumin, *Allium cepa*, quercetin, caffeine, chlorogenic acid, ellagic acid, bixin, flavonoids, epigallocatechin and other polyphenols consumption before or after radiation exposure can protect cells against radiation induced damage (10,11). *Phoenix dactylifera* commonly known as the date palm belongs to Arecaceae or palm family. Its various parts such as leaves, barks, pits, fruits and pollens have anticancer, antioxidant, hepatoprotective, antidiabetic, antihypertensive, antiulcerative, anti-inflammatory, antiproliferative, antimutagenic, antidiarrheal, antibacterial, antifungal and antiviral potential (12,13). Date palm pollen (DPP) has been used for curing male and female infertility and impotency for thousands of years as a traditional herbal medicine. DPP contains various types of phytochemicals such as estrone, α-amirin, triterpenoidal saponins, flavonoids estrone, estradiol and estriol and a crude gonadotrophic substance (14). DPP is a good source of natural antioxidants and has remarkable health benefits and nutritional values. Many studies have shown that DPP can enhance spermatogenesis, increase sperm count and concentration of testosterone, FSH and LH (15).

Therefore, the aim of this study was to evaluate the possible protective effect of DPP in preventing the harmful effects of 50 Hz low frequency electromagnetic field on sperm parameters such as sperm count, viability, motility, morphology and sexual hormones in male NMRI mice.

Materials and Methods

Collection and administration of date palm pollen

DPP grains samples were collected from the botanical garden in Bushehr city (South of Iran). Pollen grains were washed with distilled water and then dried. The extract was prepared by mixing palm pollen grain powder with normal saline as solvent by shaking and vortexin. It was administered to mice via oral gavage before each exposure to EMF.

Animals

Adult male NMRI mice, 6-8 weeks old and weighing 25±2 g obtained from Razi Vaccine and Serum Research Institute, Mashhad, Iran. These animals were housed in the animal house under standard conditions of temperature (25°C) and 12 hours light: 12 hours dark, as well as fed pellets and water ad libitum.

Experimental design

Mice were randomly divided into 7 groups, each group consisting of 8 animals as follows: Control group (kept in normal conditions in animal house), sham exposed group (exposed to a power off EMF), EMF group (exposed to 50 Hz electromagnetic field 4 h/day for 10 days without any treatment), experimental groups (1 to 4) (received DPP with doses of 25, 50, 100 and 200 mg/kg respectively before exposure to EMF).

At the end of the experiment, animals were weighed and anesthetized with ether. Blood samples were collected from the heart. The serum was obtained by centrifugation at 3000 rpm for 10 minutes. The sera were separated and preserved in -20°C until analysis.

Then, the epididymis was carefully separated and fragmented in Dulbecco's modified Eagle's medium (DMEM) containing 5% FBS. The sperm suspension was used for the analysis of sperm parameters including motility, count, morphology, and viability. To count the sperms, after putting the sperm suspension on Neubauer's chamber, the sperms on the 4 corners of the central square were counted by light microscope (16). The sperm morphology was examined using the Papanicolaou staining method and sperm viability was evaluated by application of eosin staining (17). Sperm motility was evaluated microscopically and categories as progressive motility (rapid and slow), nonprogressive motility and immotility (18).

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), using the statistical package of SPSS version 16. In all tests $P < .05$ was considered as statistically significant.

Results

Our experimental results revealed that exposure of mice

to electromagnetic field significantly decreased ($P < .001$) the sperm count and administration of DPP before exposure increased sperm count against deleterious effects of EMF (Figure 1).

The eosin staining evaluates the viability of spermatozoa which is based on the ability of the cell membrane to exclude dyes. In this method, dye enters a nonvital (dead) cell due to the damaged plasma membrane. Our results indicated that the percentage of stained cells increased under exposure to 50 Hz electromagnetic field while pretreatment with DPP elevated the percentage of unstained spermatozoa live cells (Figure 2). Therefore the viability of sperms with intact membrane was significantly decreased ($P < .001$) in EMF group while the rates of viability enhanced in all experimental groups, although only in experimental group 4 the percentages of viability returned to the normal control value.

Figure 3 shows that sperm progressive motility had a significant reduction ($P < .001$) in EMF and increasing the concentration of DPP improved sperm progressive motility. Furthermore, electromagnetic field significantly increased ($P < .001$) immobility and nonprogressive motility in mice. However, in experimental group 4 the percentages of progressive motile sperm as well as nonprogressive and immotile spermatozoa were enhanced similar to control group. Meanwhile, there were no significant differences in the percentage of motile sperm between the sham and control groups.

In current study we used Papanicolau staining method to examine the morphology of spermatozoa. As shown in Figure 4 our results demonstrated a significant increase in the percentage of sperm with abnormal morphology in EMF and sham groups as compared to control. In addition the rates of normal sperm morphology were improved in treated groups while the best improvement for sperm morphology was observed in experimental group 4.

The experimental data show that exposure to EMF induced significant reduction of testosterone level in EMF group (0.47 ± 0.92 nmol/L) ($P < .05$) while there was no significant difference in sham exposed group (1.21 ± 1.09 nmol/L) compared to control (1.5 ± 0.46 nmol/L). In addition the amounts of testosterone hormone in experimental groups (1-4) were 0.69 ± 1.41, 0.98 ± 1.33, 1.07 ± 1.24, and 1.39 ± 0.18 nmol/L, respectively. However, the levels of this hormone in experimental groups 3 and 4 increased nearly similar to the value of control group. The amounts of LH in sham exposed and EMF groups were 1.24 ± 1.47 and 1.1 ± 0.74 IU/L. In addition, in experimental groups (1-4) the levels of LH were 1.35 ± 1.23, 1.21 ± 2.5, 1.33 ± 1.47, and 1.27 ± 0.39 IU/L, respectively. Therefore there were no significant changes of LH level ($P > .05$) in all groups compared to control group (1.3 ± 1.22 IU/L).

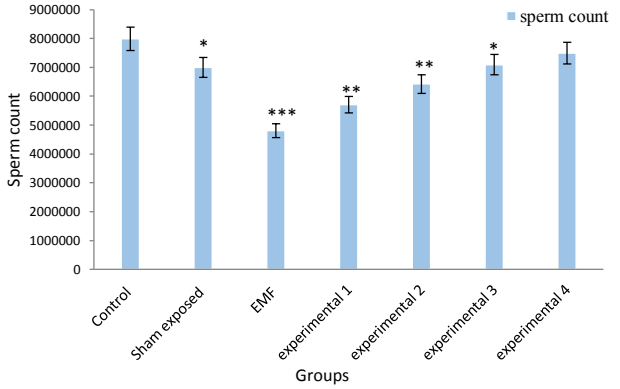

Figure 1. Effect of electromagnetic field (50 Hz) and date palm pollen treatment on total sperm count in mice. * $P < .05$, ** $P < .01$, *** $P < .001$.

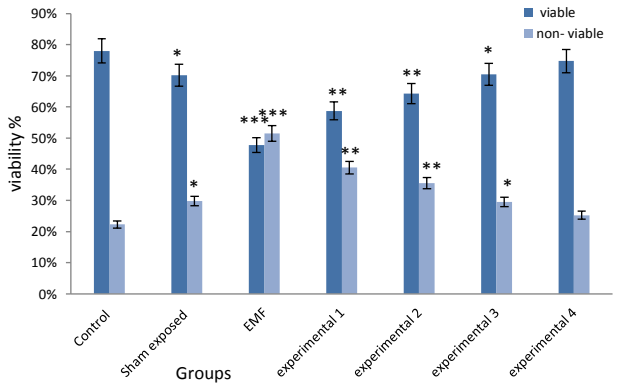

Figure 2. Effect of electromagnetic field (50 Hz) and date palm pollen treatment on sperm viability in mice. * $P < .05$, ** $P < .01$, *** $P < .001$.

Discussion

Our study showed that low frequency electromagnetic field has negative effects on sperm parameter such as viability, morphology and motility characteristics. Exposure to EMF leads to significant decrease in LH and testosterone levels which can change the hormonal balance of sex hormones and affects the reproductive activity. Daily exposure of humans to electromagnetic fields has grown concerns over the public health. Low frequency EMFs induced tissue damage and had adverse effects in organs

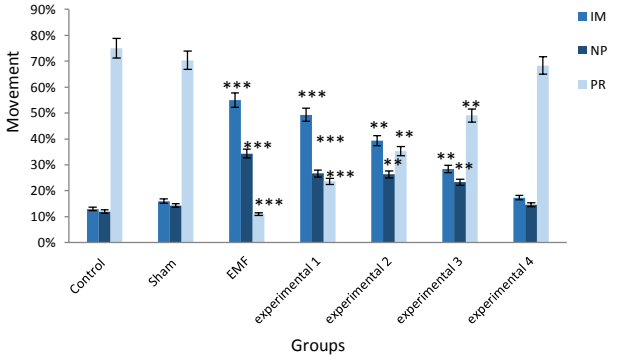

Figure 3. Effect of electromagnetic field and date palm pollen treatment on sperm motility in mice. Abbreviations: PR, Progressive motility; NP, nonprogressive motility; IM, immotility. * $P < .05$, ** $P < .01$, *** $P < .001$.

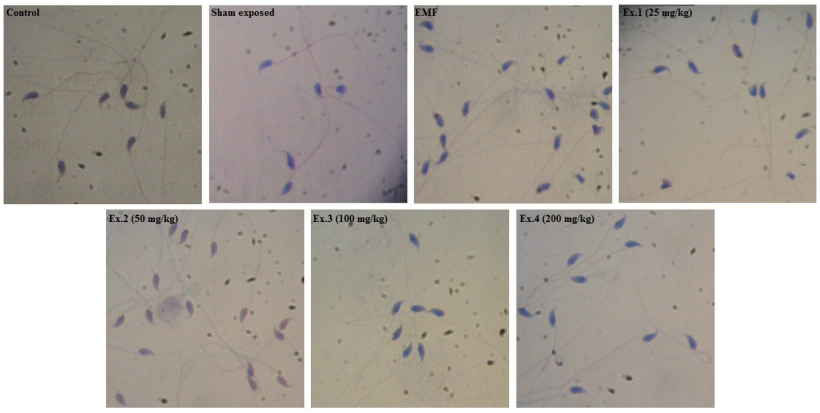

Figure 4. The protective effect of date palm pollen pretreatment in reducing sperm abnormality induced by electromagnetic field (40X) (Papanicolau staining).

particularly on testis and spermatogenesis (11). The results achieved in this study revealed that administration of DPP before exposure could prevent the detrimental effects of EMF on reproductive parameters and sex hormones in mice. The protective role of DPP may be attributed to its antioxidant contents and free radical scavenging activities. In recent years natural medicines particularly herbal medicines are extensively used to improve health conditions due to their minimum side effects, being readily accessible and inexpensive price. Herbs are an important source of phytochemicals, too (19).

There are several reports related to the protective effect of natural based substances in prevention and treatment of deleterious effects induced by electromagnetic radiation which are similar to our results. *Alstonia scholaris* is a tree belonging to family Apocyanaceae. Gupta et al (20) investigated the radioprotective potential of the *Alstonia scholaris* extract and noted that this extract could be efficient against radiation-induced biochemical alterations. So the protective effect of this extract might prove the beneficial use of plants as radio-protector. Green tea (*Camellia sinensis*) has various benefits in human health in prevention and treatment of different diseases like cancer. Accordingly in 2015 a study about the inhibitory effects of green tea on electromagnetic waves damages showed that the antioxidant property of green tea might decrease the genotoxic effects of electromagnetic radiation (21). *Ocimum basilicum* (Basil) belongs to Lamiaceae family which has been used since ancient times as an herbal remedy. Khaki et al (11) designed a study to evaluate the protective effects of *O. basilicum* on electromagnetic fields effects on testis apoptosis which revealed that the administration of *O. basilicum* may reduce the reproductive toxicity of EMF and apoptosis in testis. L-carnitine and coenzyme Q10 (CoQ10) are 2 effective antioxidants which scavenge free radicals. Ramadan et al (22) reported the protective effects of these compounds on testicular toxicity in mice induced by exposure to magnetic field.

In 2014 Arfat et al (23) revealed that DPP could enhance the reproductive activity and the levels of serum testosterone, FSH and LH in rats which might be due to the presence of gonadotropin like substances in the DPP. Cadmium is an extremely toxic heavy metal which has adverse health effects in various organs particularly testes. Prolonged exposure to cadmium caused reproductive disorder including histological alterations, reduced sperm count and motility, increased rates of sperm abnormalities, high generation of ROS and decreased serum testosterone level. El-Neweshy et al (24) reported that administration of DPP efficiently improved the spermatogenesis and sperm characteristics. Another study in 2012 examined the protective effect of DPP against testicular damages caused by cadmium toxicity in male rats and indicated the significant protective effect of DPP against testicular dysfunction through enhancing estradiol level, normalization of testosterone level and sperm function parameters (25). *P. dactylifera* pollen has various types of phytochemicals and nutrients such as carotenoids, flavonoids and phytosterols. A study revealed the preventive role of DPP against adverse side effects of chemotherapy induced infertility in male subjects. So DPP treatment decreased sperm abnormalities and improved the sperm parameters (26). DPP contains a wide range of bioactive compounds and may be efficient in prevention and treatment of oral microsites caused by radiation and chemotherapy. So, DPP may have the potential protective effect to block oxidative free radicals, prevent DNA damage, and neutralize inflammatory reactions (27). Cisplatin is a potent chemotherapy drug which induces testicular dysfunction. The impact of cisplatin on reproductive parameters of male rats was ameliorated by administration of DPP and *P. ginseng* through increasing the level of serum testosterone, sperm count, motility and viability as well as genital organs weight (28).

Conclusion

The results of the present study showed that exposure to low frequency electromagnetic field (50 Hz) can induce deleterious effects on reproductive parameters such as sperm count, viability, motility, morphology, and testosterone level which lead to decreased male fertility. In ad-

dition, pretreatment with DPP improved these parameters to normal control value. Accordingly, it is concluded that medicinal plants such as DPP can be used as efficient electromagnetic protective agent to reduce the adverse effects of EMFs on sperm parameters and sexual hormones in male mice.

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Authors' contributions

All authors had role in design, work, statistical analysis and manuscript writing.

Conflict of interests

The authors declared no competing interests.

Ethical considerations

Ethical issues (including plagiarism, misconduct, data fabrication, falsification, double publication or submission, redundancy) have been completely observed by the authors.

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