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Effects of ganoderma lucidum and melatonin on sperm in rats exposed to electromagnetic field

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ABSTRACT

Background: The aim of this study was to investigate the effect of the electromagnetic field (EMF) generated by 10 kV (50 Hz) high voltage line on the epididymal sperm characteristics and the protective effects of ganoderma lucidum (GL) and melatonin (M) in EMF exposed male rats. ***Materials and Methods:*** In this study, 64 Wistar rats were divided into 8 groups (n= 8). Rats in 26-day and 52-day experiment groups were exposed to EMF for 8 hours daily during 26 and 52 days respectively. Rats in EMF+GL-26 and EMF+GL-52 groups were treated with GL by oral gavage 20 mg/kg daily and rats in EMF+M-26 and EMF+M-52 groups received M 10 mg/kg daily during experiments. At the end of experiments, all rats were sacrificed and epididymal sperm concentration, motility and morphology were evaluated. ***Results:*** In the 26-day experiment, sperm motility of the control group were significantly lower than all groups ($P<0.05$). In the 52-day experiment, the EMF+GL52 group had the lowest tail and total morphologic defect rates and the differences between this group and EMF52 and EMF+M52 groups were significant. ***Conclusion:*** Our data demonstrated that; (1) EMF exposure can have different effects on sperm quality according to duration of exposure, (2) treatment of GL in rats exposed to EMF for 26 or 52 days increased sperm concentration and reversed negative effect of EMF on sperm morphology, respectively and (3) treatment of M in rats exposed to EMF for 26 or 52 days had negative effects on sperm concentration and sperm morphology, respectively.

Keywords: *ELF-EMF, rat, spermatozoa, melatonin, ganoderma lucidum.*

INTRODUCTION

It has been known that some of the reasons for the increasing prevalence of male type infertility are changing environmental factors, lifestyle, and exposure to pesticides and radiation from certain electronic devices and household devices that are increasingly used throughout the day [1, 2]. Power lines, computers, televisions and many small household devices are known as sources of electromagnetic fields [3-5]. When considering usage frequency of these devices, it has been estimated that extremely-low frequency electromagnetic fields (ELF-EMF) may have significant adverse effects. Many studies have been performed on the side effects of ELF-EMF. However, conflicting results have been reported regarding the alteration of spermatological and reproductive functions. While it has been suggested that EMF exposure may increase activated ejaculated sperm percentage and life

time of spermatozoa in fishes [6] and may increase human spermatozoa motility and kinematic parameters [7], some studies showed clear damage to spermatogenesis [8-13]. Besides negative effects on spermatozoa, it has been showed that EMF exposure may cause some morphological damages on testicular tissue as well [14]. Because of these opposing views, the effects of ELF-EMF exposure on sperm parameters and male type fertility is not clearly known [1].

Some previous researches reported protective effects of antioxidants against electromagnetic field exposure on living cells. Melatonin (M), n-acetylcysteine, green tea and some herbal extracts believed to have protective potency against oxidative stress condition. Melatonin has been reported to have antioxidant capability [15, 16]. Antioxidant and protective potency of M were shown in radiation induced animals with increase of living spermatozoa rate and decrease in DNA damages [15]. Melatonin application increased sperm motility and decreased morphological damages caused by mobile phone radiation exposure also [17].

Ganoderma lucidum (GL) has been reported to have antioxidant, immune modulator activator, antitumoral, anti-HIV and anti-viral potencies [18]. However, as far as we know, there is no study about the probable protective effects of GL on epididymal sperm characteristics in rats exposed to ELF-EMF.

It has been reported that the whole spermatogenic process requires 40.0 to 53.2 days (about 53 days) in rats (19, 20). Therefore, we designed two experiments with short (26 days) and long term (52 days; to cover spermatogenesis duration) in the study. The aim of this study was to investigate the effect of 26 and 52 days exposure to high-voltage induced EMF on the epididymal sperm characteristics and to examine the protective effects of GL and M in EMF exposed male rats.

MATERIALS AND METHODS

All experimental procedures approved by the Dicle University Ethics Committee on Animal Research (approval no: 2013/13) and animals obtained from Dicle University, Medical Sciences Application and Research Center (DUSAM).

Animals

In the present study, 64 male Wistar Albino rats, aged 4-6 months and weighed 250-300 g, were used. Rats were maintained under the stable condition at room temperature (23±1 °C, 12-h light/dark, photo schedule); standard laboratory animal feed and water were provided to animal *ad libitum*. Animals were adapted to laboratory condition since 7-days before beginning of the study.

ELF-EMF exposure and measurement

Two transformers that produced 10 kV (10,000 V) of high voltage were used to create ELF-MF. For transformer 1, the input was 220 V, and the output was 10 kV. For transformer 2, the input was 10 kV, and the output was 220 V and 5,000 VA. The electric and magnetic field intensity were measured with a Spectran device NF5035 (AARONIA AG, Strickscheid, Germany), with reference to the method of 6-minute measurement (ICNIRP). The ELF-EMFs values were measured in the experimental setup which had a 30 cm vertical distance to 10 kV (50 Hz) high frequency line and which was 50 cm away from the transformers. The electric and magnetic field intensity measurements in experimental setup were determined as 80.3 V/m and 2.48 mT, respectively.

Experimental Design

At first, the rats were randomly divided into two time groups (26 and 52-day experiments). Then, the rats both in 26 and 52-day experiments were divided into 4 equal study groups (n: 8) including Control-26 (C-26), EMF26, EMF+GL26, EMF+M26, Control-52 (C-52), EMF52, EMF+GL52 and EMF+M52. Except in the control (C-26) group, all the rats in 26-day experiment exposed to EMF for 8 hours per day for 26 days. The EMF+M26 group of rats were treated with melatonin (M; 10 mg/kg/day, Merck, Germany) intraperitoneally (ip) and the rats in EMF+GL26 group received ganoderma lucidum (GL; 20 mg/kg/day, Gano excel,

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Malaysia) by oral gavage for 26 days. In the 52-day experiment, the study groups were created similar to the 26-day experiment, differently, studies were continued for 52 days. Control-26 (C-26) and Control-52 (C-52) were not exposed to EMF and drug administration.

At the end of 26-day and 52-day experiments, all rats were sacrificed under general anaesthesia and epididymal sperm concentration, motility and morphology were evaluated.

Epididymal Sperm Count, Motility and Morphological Evaluation

Left epididymis of sacrificed animals was used for evaluation of sperm cell count, motility and morphology. Spermatozoa in the epididymis were counted by a modified method of Alp *et al.* [21]. Briefly, the epididymis was finely minced with anatomical scissors in 10 ml of physiologic saline, placed in a rocker for 10 min, and allowed to sit at room temperature for 2 min. After incubation, supernatant fluid was diluted 1:10 with a solution containing 5 g sodium bicarbonate, 1 ml formalin (35%), and 25 mg eosin per 100 ml of water. Total sperm number was determined using counting chambers. The cells were counted with the help of a light microscope at 200 × magnifications.

The fluid obtained from the cauda epididymis with a pipette was diluted to 2 ml with Tris buffer solution. A slide was placed on a phase-contrast microscope, and an aliquot of this solution was placed on the slide and percent motility was evaluated visually at a magnification of 400 times. Motility estimations were performed from three different fields in each sample. The mean of the three estimations was used as the final motility score. Samples for motility evaluation were kept at 37 °C.

To determine the percentage of morphologically abnormal spermatozoa in the cauda epididymis, the slides stained with eosin–nigrosin (1.67% eosin, 10% nigrosin and 0.1 M sodium citrate) were prepared. The slides were then viewed under a light microscope at 400 x magnification. Two-hundred spermatozoa were examined on each slide, and the head and tail and total abnormality rates of spermatozoa were expressed as percent.

Statistical analyses

All values were presented as mean±SEM (Standard Error of Means). Differences were considered statistically significant when calculated p values were less than 0.05. Data were analysed by One-way analyses of variance (ANOVA) and Post Hoc Tukey's test being used the SPSS/PC computer program (SPSS, Inc., IBM, Armonk, NY, version 10.0) to determine the differences among groups.

RESULTS

Results of 26 and 52-day experiments were summarized in table 1 and 2 respectively. In the 26-day experiment, sperm motility of the control group rats were significantly lower than those of all groups (P<0.05). Moreover, rats in control group had lower sperm concentration than those of the EMF+GL26 group (P<0.05). Sperm concentration was found to be lower in the EMF+M26 group than the EMF26 and EMF+GL26 group (P<0.05). All groups had similar morphological defect rates (P>0.05).

Table 1. Comparison of spermatological parameters in 26-day experiment.

Groups	Motilitiy (%)	Concentration ($x10^6$/ml)	Head defects (%)	Neck defects (%)	Tail defects (%)	Total morphologic defects (%)
C-26	53.3±7.69^a	74.6±12.17^{ab}	2.1±0.46^a	2.0±0.82^a	18.0±2.74^a	22.1±2.32^a
EMF26	76.9±3.43^b	104.3±10.17^{bc}	6.6±1.81^a	3.3±0.61^a	13.4±3.21^a	23.3±5.05^a
EMF+GL26	75.4±3.12^b	111.1±12.12^c	4.0±0.65^a	2.3±0.57^a	11.4±1.07^a	18.0±1.27^a
EMF+M26	68.6±3.73^b	71.4±6.63^a	3.1±0.77^a	1.6±0.37^a	14.6±3.29^a	19.3±3.66^a
P value	*<0.05*	*<0.05*	*>0.05*	*>0.05*	*>0.05*	*>0.05*

Within columns, means with no common letters are statistically different.

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Table 2. Comparison of spermatological parameters in 52-day experiment.

Groups	Motilitiy (%)	Concentration ($x10^6$/ml)	Head defects (%)	Neck defects (%)	Tail defects (%)	Total morphologic defects (%)
C-52	65.0±4.08[a]	99.6±6.9[a]	2.3±0.61[a]	1.6±0.48[a]	7.6±1.63[a]	10.7±1.95[ab]
EMF52	70.0±2.67[a]	86.8±5.6[a]	1.0±0.31[a]	1.1±0.55[a]	12.1±1.0[b]	14.4±1.36[b]
EMF+GL52	71.4±4.04[a]	86.4±5.4[a]	2.0±0.44[a]	0.4±0.20[a]	6.6±1.02[a]	9.0±0.79[a]
EMF+M52	68.3±3.66[a]	80.4±7.8[a]	6.7±0.94[b]	1.7±0.42[a]	12.3±1.4[b]	20.7±1.66[c]
P value	*>0.05*	*>0.05*	*=0.002*	*>0.05*	*=0.01*	*=0.001*

Within columns, means with no common letters are statistically different.

In the 52-day experiment, sperm motility and concentration values did not differ among groups (P>0.05). Compared to the control group, the rats in EMF+M52 group had significantly higher sperm head, tail and total morphological defect rates and the rats in EMF52 group had higher sperm tail defect rate (P<0.05). The EMF+GL52group had the lowest tail and total morphologic defect rates and the differences between this group and EMF52 and EMF+M52 groups were significant.

DISCUSSION

Infertility is one of the most common diseases and affects between 17 and 25% of couples [22]. Of these, male factor infertility is responsible for approximately 50% of the infertility cases [22, 23]. The most common cause of males' infertility is their inability to produce enough healthy and active sperm [22]. Extremely low frequency (50 and 60 Hz) ELF-EMFs are associated with the production, transmission, and use of electricity; thus the potential for human exposure is very high [24]. Therefore, the possible adverse effects of EMF on reproduction have been studied in both experiments involving animals and humans. However, conflicting results have been reported regarding the alteration of spermatological and reproductive functions. While some studies showed clear damage to spermatogenesis [8-13, 23, 25-27], a number of studies indicated that exposure to EMF did not induce any adverse effects on spermatogenesis and reproductive capacity in experimental animals and human [28, 29]. Moreover, it has been suggested that ELF-EMF exposure of spermatozoa can improve motility and that this effect depends on the field characteristics [7]. Significant increases in the values of the motility and of the other kinematic parameters have been observed when spermatozoa were exposed to an ELF-EMF with a square waveform of 5mT amplitude and frequency of 50 Hz in human [7]. Similarly, an increase in the percentage of activated ejaculated sperm and a prolongation of their viability were shown in fish after in vitro sperm exposure to magnetic fields up to 100 mT [6].

We have evaluated the effect of 26 and 52 days exposure to high-voltage induced EMF on the epididymal sperm characteristics and examined the protective effects of GL and M in EMF exposed male rats. We found that, interestingly, EMF exposure for 26 days increased the epididymal sperm motility significantly (P<0.05) and raised the concentration numerically (P>0.05). However, sperm motility and concentration values did not differ among groups (P>0.05) in the 52-day experiment. Moreover, unlike the 26-day exposure, EMF exposure for 52 days increased tail defects in spermatozoa. Contrary to our findings, Aydın *et al.* [24] reported that ELF exposure time of 1 to 3 months did not affected the epididymal sperm characteristics in rats. According to the results of previous researches and our current study, EMF appears to have positive or negative effects on epididymal spermatozoa. However, it seems that there may be a relationship between exposure time to EMF, waveform, source type of electromagnetic field and positive or negative effects of EMF on epididymal spermatozoa.

Protective effects of M on spermatozoa in EMF exposed animals have been shown in previous studies [15-17]. Melatonin treatment reversed the effects of EMF for sperm count, testosterone level and DNA fragmentation in rats [15]. In radiofrequency radiation-exposed mice, M inhibits pre-meiotic spermatogenesis arrest in male germ cells through its anti-oxidative potential and ability to improve DNA repair pathways, leading to normal sperm count and morphology [17]. However, in our study, M treatment showed a harmful effect rather than a protective effect in rats exposed to EMF. When compared to rats exposed EMF only, rats treated with M together with EMF exposure had lower sperm concentrations in 26-day experiment (P<0.05) and had higher head and

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total morphological defect rates in 52-day experiment (P<0.01). Moreover, M treatment increased significantly head and total morphological defect rates compared to the control group in 52-day experiment while control and EMF exposed rats had similar values in terms of these parameters.

Antioxidant, immune modulator activator, antitumoral, anti-HIV and anti-viral potencies of GL has been reported previously [18]. This is the first study indicating the protective effects of GL treatment on epididymal sperm characteristics in rats exposed to EMF. Treatment of GL in rats exposed to EMF increased sperm concentration significantly compared to the control group in 26-day experiment while control and EMF exposed rats had similar concentration values. The EMF+GL52 group had the lowest tail and total morphologic defect rates and the differences between this group and EMF52 and EMF+M52 groups were significant in 52-day experiment. Although EMF exposure for 52 days increased the tail defect ratio significantly (P<0.05), treatment of GL in rats exposed to EMF reversed this negative effect of EMF on tail morphology.

It has been concluded from the study that; (1) while EMF exposure for 26 days affected positively the sperm production and motility in male rats, this positive effect disappeared when EMF exposure time was extended to 52 days, moreover, it started to affect sperm morphology negatively, so it can have different effects on sperm quality according to duration of exposure, (2) treatment of GL in rats exposed to EMF for 26 or 52 days increased sperm concentration and reversed negative effect of EMF on sperm morphology, respectively and (3) treatment of M in rats exposed to EMF for 26 or 52 days had negative effects on sperm concentration and sperm morphology, respectively.

***Conflicts of interest*:** *Declared none.*

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