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Simultaneous Effects of Exposure to Microwaves and Noise on Male Rats' Sperm Parameters and Total Antioxidant Capacity

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A B S T R A C T

Background: There is currently great concern about the possible adverse effect of microwave radiation from cell phones. In addition, noise is one of the physical pollutants of modern societies.
Objectives: The present study aimed to examine the separate and simultaneous effects of cell phone microwaves, noise, and their effects on sperm parameters and total antioxidant capacity in adult male rats.
Material and Methods: An experimental study was conducted on 28 Wistar adult male rats (200 - 250 g). Randomly selected animals were divided into four groups; control (C), microwave (M), noise (N), and noise plus microwave (NM) groups. In all groups, a sperm analysis was performed based on World Health Organization (WHO) standards and the mean of the sperms' total antioxidant capacity was determined by a Ferric Reducing Ability of Plasma (FRAP) assay. The data were analyzed by a one way ANOVA statistical technique, followed by a Tukey's test using SPSS (version 16) software and $P < 0.05$ was considered significant.
Results: The findings of the study demonstrated that sperm viability and motility, in the exposure to cell phone waves group (group 2) and the simultaneous exposure to cell phone waves and noise group (group 4), decreased significantly compared to the control group ($P < 0.05$). Moreover, the total antioxidant capacity of sperm in all exposure groups decreased significantly compared to the control group ($P < 0.05$).
Conclusion: Exposure to cell phone waves can decrease sperm viability and motility in adult male rats. These waves can also lower rat sperms' total antioxidant capacity which results in oxidative stress. Exposure to severe noise levels can cause a significant decrease in the total antioxidant capacity of sperm in adult male rats, resulting in oxidative stress.

Keywords: Cellular Phone; Noise; Oxidative Stress

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▸Implication for health policy/practice/research/medical education:
The study discusses effect of microwaves and noise on reproductive male. Reading this article is recommended to the specialists in the field of occupational and environmental health, health policy makers as well as general population.

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1. Background

Microwaves are part of a wide range of electromagnetic waves with a frequency range of 300 MHz - 300 GHz (1). The evidence indicates that these waves are harmful to humans and based on their; intensity, frequency, type, and exposure duration, create biological effects (2). Furthermore, there is great concern about the possible adverse effects of cell phone microwaves. Researchers have warned people of the harmful effects of this type of radiation on the; brain (3), heart (4), thyroid (5), skin (6), kidneys (7), eyes (8), liver (9), and reproduction (10) tissues, while some researchers have reported contradictory results (11, 12). There have been few studies on the effects of cell phone waves on sperm parameters. The Wdowiak, et al. (10) study, revealed that cell phone waves decreased the motility and percentage of sperm with normal morphology in people who used cell phones. In addition, a study by Yan, et al. (13, 14) showed that these waves decreased; motility, viability, and count of sperms with normal morphology.

However, the issue in question is; cell phone waves may cause oxidative stress by enhancing lipid peroxidation and changing antioxidant activities in the body (15). Oxidative stress is a process in which the normal balance between oxidants and antioxidants is changed in such a way that it leads to increasing levels of oxidants and biological damage (16). Furthermore, few studies have examined the effects of cell phone waves on antioxidants. The results of one study indicated that cell phone waves can; increase lipid peroxidation, decrease total thiol concentrations and the total antioxidant capacity of blood plasma, resulting in oxidative stress (15). Other studies, however, have shown that these waves have no effect on the antioxidant system (17). A study by De Luliis, et al. revealed that cell phone waves can decrease sperm motility and viability through reactive oxygen species (18).

On the other hand, unwanted sound (noise) is one of the main physical pollutants of today's societies and one of the harmful factors in the workplace. It is estimated that more than 600 million people in the world are exposed to extreme noise in their workplace (19). Various investigations on the effects of noise on the health of working people have indicated that noise, in addition to hearing loss (20), also has other adverse effects such as increasing blood pressure and changes in heart rate (21). Like the possible effects of cell phone waves, noise can distort antioxidant balance through the mechanism of producing free radicals and as a source of oxidative stress, this creates the possibility of the development of many diseases, including cancer (22, 23). It has been reported that exposure to noise levels of more than 90 dB are considered to be a source of oxidative stress and sharp noise can increase the levels of free radicals in the body and consequently destroy normal cell function and its integrity (23). Yildirim, et al. (24) investigated the effects of noise on lipid peroxidation and blood antioxidant enzymes of textile workers who were exposed to severe noise levels, 105 dB (A), for eight hours a day. Their findings indicated that severe noise creates oxidative stress. Furthermore, the effect of noise on male reproductive activity was also studied. The results of another study indicated that noise of 90 dB intensity can decrease sperm parameters and showed that severe noise may affect fertility levels (25). However, there is a need to conduct further studies in this regard.

2. Objectives

This study aimed to assess the effects of sound waves and severe noise on sperm parameters and total antioxidant capacity in adult male rats. In addition, the simultaneous effect of cell phone waves and severe noise on sperm parameters and total antioxidant capacity was investigated as a new study.

3. Materials and Methods

This experimental research was carried out on 28 Wistar male rats (200-250 g) in the Fertility and Infertility Research Center at Kermanshah University of Medical Sciences. The animals were purchased from the Pasture Institute of Iran and kept in the animal house according to recommended conditions in terms of (26); temperature (21-23° C), light (12 hours light and 12 hours darkness), ventilation and food. Medical ethics laws of Tarbiat Modares University concerning laboratory animal work and handling were followed.

The rats were randomly assigned into four groups (n = 7) based on the study design:

Group1: Control group which were in experimental conditions, but the rats were not exposed to cell phone simulated waves or noise.

Group 2: This group was exposed to cell phone simulated waves (915 MHz frequency) for fourteen days.

Group 3: This group was exposed to noise [100dB (A)] for fourteen days.

Group 4: This group was exposed to cell phone simulated waves and noise, simultaneously for fourteen days.

3.1. Design and Construction of Exposure Cylinder and Radiation Chamber

A - Design and construction of the exposure cylinder: To expose the animals to cell phone simulated waves and noise, a plexiglas cylinder consisting of an external cylinder (radius = 15 cm, height = 30 cm) and an internal cylinder (radius = 5 cm, height = 30 cm) was prepared. The animals were put between the internal and external space during the experiments and they had free access to all of the spaces. The internal cylinder was intended to prevent the animals from entering close to the field of the monopole antennae (this was installed vertically in the

center of the internal cylinder) of the simulation generator from which cell phone waves (*Figure 1*) were emitted vertically into the center of the internal cylinder, because measuring the density of the waves is not accurate in the near field.

Figure1.

Cell Phone Simulated Wave Generator

B- Design and construction of the radiation chamber: This chamber was designed to prevent the reflection of microwave radiation from the GSM antennae. This cubical chamber (120 × 120 × 120 cm) was made of neopan. Pyramids with cubical bases (6 × 10 × 10 cm) and a height of 30 cm made of sponge, were put on the inner walls of the chamber. Graphite was put on the pyramids to absorb the microwaves. The outer walls of the chamber were covered with aluminum foil to prevent external microwaves from entering the radiation chamber (27). It should be mentioned, that the exposure cylinder was placed in the center of the radiation chamber.

3.2. Exposing rats to Cell Phone Simulated Waves

The vertical antenna (monopole) of the cell phone stimulation generator was placed in the center of the internal cylinder and the density was measured; 5, 10, and 15 cm from the antennae and at a height of 5 cm from the floor of the exposure cylinder by the portable system (Holaday, Texas, USA). The average density in the aforementioned distances was 1.60 mw/cm^2.

The rats in group 2 were exposed to microwaves (915 MHZ) for eight hours a day for fourteen days.

C - Exposing rats to noise: The rats in this group (group 3) were exposed to noise (700-5700 Hz), at a combination of three octave band sounds (1000, 2000, and 4000 Hz), and a sound level pressure of 100 ± 0.9 dB (A) for eight hours a day for 14 consequent days. The noise was created at the intended frequency using signal software and was performed on the computer using Cool Edit software, Arizona, USA. The noise produced was amplified through two loudspeakers. The intensity and frequency of the noise in the exposure cylinder was measured by a sound level meter (CEL-450, CASELLA, and Bedford, England).

D - Exposing rats to cell phone simulated waves and noise simultaneously: The animals in this group (group 4) were exposed to cell phone simulated waves and noise simultaneously. The exposure conditions in this group were similar to those in groups 2 and 3.

3.3. Measuring Sperm Parameters

The animals in all of the four groups were anesthetized with chloroform at the end of the experiment. After opening the anterior wall of the thorax, blood was taken from the heart. The cauda of the epididymis was separated and segmented in HAM's/F10 (Gibco, Manchester, UK) containing 10% bovine serum which had been balanced in the incubator previously at a temperature of 37° C and 5% CO2. After 45 minutes, a sperm analysis was performed according to World Health Organization (WHO) instructions (28) in the following manner:

Sperm motility: This was examined and classified in accordance with the WHO recommended method (a, fast progressive; b, slow progressive; c, non-progressive; d, non-motile) in ten microscopic fields. Total number of sperms of both a and b were determined as the motility percentage of each sample.

Sperm viability: Supra Vital staining was performed to identify live sperms. One drop of medium containing sperm was put on the slide and then this was mixed with a small drop of Eosin B (0.5% in saline). The coverslip was immediately put over the drop and analyzed at an enlargement of 400x. In each slide, 100 sperms were counted and the percentage of sperms was determined. Sperm count: A Neubauer Haemocytometer (Horsham, Germany) was used to count the sperms. One drop of the diluted sample was put on a slide, and then all of the sperms in the central square were counted, and consequently the sperm count in 1 ml was calculated.

Sperm morphology: After staining, sperms in 10 microscopic fields were analyzed and classified into two groups according to WHO classification: 1- normal sperms, 2- abnormal sperms (a, sperms deficient in heads; b, sperms deficient in necks; c, sperms deficient in cauda). The percentage of sperms with a normal morphology was then determined. After the sperms were analyzed they were centrifuged at a temperature of 4° C for 15 minutes at 2 500 RPM. After separating the supernatant, a 1 cc buffer phosphate solution containing EDTA (29) was added to the remaining sperm cast. The samples were preserved at a temperature of -70° C until the time of measurement.

3.4. Measuring the Total Antioxidant Capacity of Sperm in Rats

The total antioxidant capacity of sperm in the rats was measured by a FRAP (Ferric reducing ability of plasma) Test (30). The basis of this method is the ability of plasma in decreasing the capacity of fFerric ion (Fe3+) to fFerro (Fe2+). The complex made from the fFerro ion by TPTZ (2, 4, 6-Tripyridyl Triazine) is blue in an acid solution and its maximum absorption is 593 nanometers. The samples were put in the incubator (37 ° C) for 20 minutes and then centrifuged for 10 minutes at a temperature 4° C and 3 000 RPM. Using a sampler, 850 μliter of supernatant was separated from the cast. Then, the sperms of the remaining sperm cast (which contained a 150 μliter buffer solution) were broken by a sonikator machine (Labsonic, Goettingen, Germany). While breaking the sperms, the samples were put in a salt and ice powder container so that the heat produced from the sample was transferred to the ice powder and did not affect the antioxidant capacity of the sample. After breaking the sperms, the sample was centrifuged (4° C) again at 8 000 RPM. Then, the supernatant was separated from the cast and at the time of the FRAP solution preparation it was kept in the freezer (4° C). It was measured immediately after the solution was prepared. FRAP test method: Firstly, standard solutions with concentrations of; 125, 250, 500 and 1000 μM were prepared from FeSO4.7 H2O. Then, TPTZ powder (0.0247 g) was dissolved in 7.5 ml HCl (40 mM) to prepare a TPTZ solution. Next, 7.5 ml of FeCl3. 6 H2O solution (20 mM) and 75 ml buffer acetate solution (concentration = 300 mM and pH = 3.6) were added to the TPTZ solution to make a FRAP solution. The chemicals used for the FRAP test were purchased from the Merck Company (Darmstadt, Germany). After preparing the FRAP solution, 1.5 ml of the solution was added to 150 μliter distilled water and was put in the water bath (37° C) equipped with a shaker for five minutes. Then, 50 μliter of the experimental sample or standard groups was added to the tubes and put in a water bath 37° C equipped with a shaker again for ten minutes and the complex absorption rate in the wave length of 593 nm was immediately recorded by a spectrophotometer (Jenway 3620D, Cambridge, England).

In this study, all of the samples were duplicated and measured to enhance the accuracy of the analysis.

3.5. Statistical Analysis

The results were indicated by mean ± SD and analyzed by a one-way ANOVA and Tukey's post-hoc using SPSS software (version 16)(IBM, New York). $P < 0.05$ was considered significant.

3. Results

The results of the present study indicated that the mean of sperm viability in the control group was 87.64 ± 1.82% and in experimental groups 2, 3, and 4, the means were 81.14 ± 2.87, 83.93 ± 3.32, and 79.00 ± 3.99%, respectively. The results indicated a significant decrease in sperm viability in both groups, including the exposure to cell phone simulated waves group (group 2, P value = 0.039), and the simultaneous exposure to cell phone simulated waves and noise group (group 4, P value = 0.002) compared to the control group (group 1). Comparing the two groups showed that there was a further reduction in sperm viability in group 4 than in group 2, which was not statistically significant (*Table 1*). Moreover, the findings of the study showed that the mean of sperm motility in the control group was 49.96 ± 4.59% and in experimental groups 2, 3, and 4, the means were 40.91 ± 4.11, 42.76 ± 5.16, and 39.89 ± 2.32%, respectively. The results indicated a significant decrease in sperm motility in both groups including the exposure to cell phone simulated waves group (group 2, P value = 0.013) and simultaneous exposure to cell phone simulated waves and noise group (group 4, P value = 0.004) compared to the control group (group 1). Comparing the two groups showed that there was a further reduction in sperm motility in group 4 than in group 2, which was not statistically significant (*Table 1*).

Table 1. Comparison of Sperm Parameter Means in Control and Exposure Groups

Groups[a]	Sperm Count (×106), Mean ± SD	Sperm Viability (%), Mean ± SD	Sperm Motility (%), Mean ± SD	Sperm Normal Morphology (%), Mean ± SD
1	58.56 ± 6.01	87.64 ± 1.82	49.96 ± 4.59	82.06 ± 4.60
2	62.14 ± 8.92	81.14 ± 2.87[b]	40.91 ± 4.11[b]	81.78 ± 3.96
3	59.65 ± 8.43	83.93 ± 3.32	42.76 ± 5.16	82.25 ± 7.48
4	56.87 ± 6.74	79.00 ± 3.99[c]	39.89 ± 2.32[c]	80.31 ± 5.86

[a] Group 1 = control; group 2 = exposed to cell phone simulated waves (915 MHz frequency) for fourteen days; group 3 = exposed to noise [100dB(A)] for fourteen days; group 4 = exposed to cell phone simulated waves and noise simultaneously for fourteen days.
[b] Comparison of control group ($P < 0.05$)
[c] Comparison of control group ($P < 0.01$).

Regarding sperm count and normal morphology, no statistical decrease was observed in any of the exposure groups compared to the control group ($P > 0.05$). The comparison of the exposure groups with each other

showed no statistical difference (P > 0.05) in terms of these two parameters (*Table 1*). The mean of the sperms total antioxidant capacity in the control group was 406.35 ± 64.12 μ mole per 60 million sperm and the means in groups 2, 3, and 4 were 297.92 ± 92.76, 299.20 ± 28.32 and 255.78 ± 60.75 μ mole per 60 million sperm, respectively. The comparison of total antioxidant capacity in the exposure and control groups indicated that there was a statistically significant decrease (group 2, P value = 0.044; group 3, P value = 0.048; group 4, P value = 0.001) in all three exposure groups in terms of their sperm total antioxidant capacity (*Table 2*).

Table 2. Comparison of Total Antioxidant Capacity Means of Sperm in Control and Exposure Groups

Groups[a]	Total Antioxidant Capacity (μmole per 60 Million of Sperm), Mean ± SD	*P*Value, Versus Control Group
1	406.35 ± 64.12	
2	297.92 ± 92.76	0.044
3	299.20 ± 28.32	0.048
4	255.78 ± 60.75	0.001

[a] Group 1 = control; group 2 = exposed to cell phone simulated waves (915 MHz frequency) for fourteen days; group 3 = exposed to noise [100 dB(A)] for fourteen days; group 4 = exposed to cell phone simulated waves.a Group 1 = control; group 2 = exposed to cell phone simulated waves (915 MHz frequency) for fourteen days; group 3 = exposed to noise [100 dB(A)] for fourteen days; group 4 = exposed to cell phone simulated waves.

4. Discussion

The sperm analysis revealed that sperm viability and motility in the exposure to cell phone simulated waves group and the simultaneous exposure to cell phone simulated waves and noise group, decreased significantly compared to the control group (P < 0.05). However, there was no significant difference regarding sperm morphology and count in these two groups. Furthermore, the total antioxidant capacity of the sperm in all of the exposure groups decreased significantly compared to the control group. A study by Agarwal, et al. (14) concerning the effect of cell phone waves on cell phone users indicated that cell phone waves have a negative impact on; sperm count, viability, and normal morphology, and this effect becomes more severe as daily duration increases. The results of the present study are, to a certain extent, in line with the findings of the Agarwal, et al. research, because in the present study, cell phone simulated waves decreased sperm motility and viability. The findings obtained in this study are compatible with the results of the Erogual, et al. study (31) in which cell phone waves decreased sperm motility, but had no effect on sperm count. In the present study too, sperm motility decreased, but it had no impact on the sperm count which was observed in group 2 (exposed to cell phone simulated waves).

The findings of this study, however, are contrary to the results of the Kesari, et al. (32) research, in which cell phone use decreased sperm counts in cell phone users, whereas in the present research, simulated cell phone waves had no effect on sperm counts in rats. It should be noted that in the Kesari et al. study, human samples were used, while in this research laboratory animals were used, which is a notable factor. The differences between exposure duration in the cited studies should also be taken into account. Regarding oxidative stress and protective antioxidants, and their relationship with the reproduction system, new aspects of human infertility are introduced. One of the cells that are very sensitive in this regard is sperm. Mammals' sperm membranes are full of unsaturated fatty acids and they are sensitive to oxidation. On the other hand, abnormal sperms are responsible for the overproduction of reactive oxygen species (ROS) which result in oxidative stress and these are introduced as one of the causes of male infertility (33). With regard to the impact of cell phone waves on total antioxidant capacity, the results of this study revealed that cell phone waves can decrease sperms' total antioxidant capacity. This finding is in line with the results of the Mailankot et al. study (34), in which cell phone waves increased lipid peroxidation and decreased glutathione antioxidant in the testis and epididymis of rats, which is in line with the findings of the present study. The results of this research are compatible with the outcomes of the Meral et al. (35) study, in which the frequency and radiation pulse are compatible with those of the present study. It can also be concluded, from the findings of this study, that cell phone waves may, in addition to affecting sperm parameters, cause oxidative stress in the body and consequently create various diseases. Thus, with the widespread use of cell phones globally, more studies are required in this regard.

Concerning the effect of noise severity, the results of this study indicated that noise levels at 100 dB (A) can decrease the total antioxidant capacity of sperm and consequently result in oxidative stress. These findings are in line with the Yamashita et al. (36) study. That study found that noise cannot affect sperm parameters in the short term (two weeks exposure), but it could cause oxidative stress through influencing total antioxidant capacity, which was intensified by exposure to noise and mobile phone waves simultaneously. It seems that there

is a pressing need to recognize the effects of noise and mobile phone waves as a source of oxidative stress, and these should be controlled by limiting personal contact with these two harmful factors. Accordingly, these results suggest that in order to reduce the risks of exposure to these factors in people who have high levels of contact with these irritants, such as airport staff, they should receive a high proportion of antioxidants in their diet. It also needs to be remembered that this study cannot be generalized to humans, because of the harm that microwave and noise pose for people.

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Authors' Contribution

Masud Ghanbari: Participated in all parts of experiment, Seyed Bagher Mortazavi: Desinged and conducted the project, Ali Khavanin: Consult and co desineger of the project, Mozafar Khazaei: Participated in experimental methods.

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