

Effects of mobile phone radiation and noise pollution on serum levels of LDH, thyroid hormones, liver enzymes, cortisol, and reproductive system in male Wistar rats

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Abstract

The expansion of mobile phones and noise pollution in the human environment has given rise to dangerous outcomes. In this study, the effects of exposure to traffic noise and cell phone radiation on the serum levels of LDH, thyroid hormones, liver enzymes, cortisol, and reproductive system function and testicular histology of male rats have been analyzed. Male Wistar rats were exposed to mobile radiation (930 MHz) and noise pollution (100 dB, 700 to 5700 Hz). After eight weeks of exposure, serum levels of LDH, thyroid hormones, liver enzymes, cortisol, and male reproductive hormones were measured using the enzyme-linked fluorescence assay method. Testicular tissue was also examined by H&E staining. Data were analyzed using ANOVA. The results showed that prolonged exposure to cell phone radiation and noise pollution increased the LDH levels, reduced the T3, T4, glutamic-pyruvic transaminase, cortisol, testosterone, LH, FSH levels, and decreased the number of spermatogonia and spermatocytes, serum glutamic-pyruvic transaminase and alkaline

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phosphatase. Conclusively, long term exposure to mobile phone radiation and noise pollution leads to increased LDH, decreased T3, T4 and SGPT level, and had destructive effects on testicular tissue leading to decreased serum testosterone level as well as decreased spermatogonia, spermatocyte and Sertoli cells count.

Keywords: Mobile phone, Noise pollution, LDH, Endocrine hormones, Reproductive system, Wistar rats

1 Introduction

The increasing use of microwaves-generating devices, such as mobile phones in everyday life, as well as the increase in noise pollution in our environment, has raised many concerns about the effects of mobile phone radiation and noise on human health. In recent years, noise pollution has been identified as one of the main causes of damage due to the increase in industry and population growth, as well as the expansion of communications and transportations (Habib et al., 2007). Over the last century, the amount of stress produced by humans has increased significantly and has doubled in the past ten years (Hunashal & Patil, 2012). Adverse effects of noise pollution are divided into four categories: 1- physical effects, such as hearing impairments; 2- physiological effects, such as high blood pressure and cardiac arrhythmias; 3- psychological effects, such as sleep disorders; 4- human performance effects, such as reduced efficiency and misunderstanding (Rahma et al., 2013). In vitro study has reported that radiofrequency radiation induces various cancers and DNA or chromosomal damage (Singh et al., 2018). Several studies have shown that electromagnetic radiation can affect the physiology of many internal organs, including the liver (Rajaei and Mohammadian, 2012) and endocrine glands, such as the pituitary gland (Manikandan et al., 2006) and the hypothalamus (Babisch, 2003). Exposure to simulated cell phone radiation has been reported to alter the endocrine system of mice receiving these radiations (Baharara et al., 2004) and many studies suggested that noise (noise stress or noise pollution), as one of the environmental stresses, can have similar effects to other parts of the central nervous system, cardiovascular, auditory, and endocrine systems (Seidman and Standring, 2010). Today, the widespread use of mobile phones and the increase in noise pollution, as a source of environmental stress in various societies, have caused human health hazards in the world. Many offices and workplaces are located in noisy parts of the city and workers have to use their mobile phones for long hours in these places. So far as we know, the reports and conclusive evidences about the destructive effects of noise pollution and cell phone radiation on various organs of the body are limited. Therefore, to investigate the effects mobile phone radiation and noise pollution on endocrine system as well as muscular and liver function, we have evaluated the destructive effects of mobile phone radiation and noise pollution separately and in combination on serum levels of lactate dehydrogenase (LDH), thyroid hormones (T3 and T4), liver enzymes (serum glutamic pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT)), alkaline phosphatase (ALP), cortisol, and reproductive system in male Wistar rats.

2 Materials and Methods

2.1. Animal studies

This study was performed on 40 adult male Wistar rats (6-8 weeks old with a mean body weight of 150 g), bred at the Vivarium of the Pasteur Institute of Iran, under conventional laboratory conditions. All animals were suited in the same room without near sources of electric and magnetic surface from the cages and were housed collectively (7 animals in each cage with dimensions of 30 × 40 × 40 cm). The rats were kept in a pure (lacking any metallic fittings) polycarbonate cage and given ad libitum access to standard laboratory food and water. The housing room was maintained at 24°C with 42 ± 5% relative humidity and a 12–12 h light-dark cycle (light on 06:00–18:00 h). All animal studies had been approved by the Medical Ethics Committee (MEC) and were performed according to the guidelines of the MEC on the use and care of laboratory animals.

To produce noise pollution, the sound of a noisy street in a big city was recorded using a digital recorder (RX-DT 707, Panasonic, Taiwan). The recorded sound was amplified (Pyramid Studio Pro Pa-6009, Pioneer Corporation, Tokyo, Japan) and delivered to speakers. The sound intensity was controlled and regulated by a sonometer (TES-1358, TES Electrical Electronic Corp., Taiwan). Noise frequencies were between 8 and 10 kHz. The audio set including minute patterns, with 1-minute silences between them was used, which prevented any animal adaptation to stressful conditions (Sarkaki et al., 2000). During the experiment, the separate noise receiving groups were kept in a room and the other groups were not exposed to the noise.

The mobile phone radiation was produced by a mobile test phone (NOKIA 3110, Nokia Mobile Phones Ltd.), connected to a Communication Test Set Personal Computer (PC) Developer's Kit with PC and appropriate software module. Mobile radiation exposure was performed in the same room where all animals were located. Mobile phone, as a source of micro-waves radiation, was put in each cage. Each mobile phone was put in a small, perforated polycarbonate cell to prevent damage that could be caused by the rats as well as chewing by them. The animals were allocated into nine experimental groups and one control group. Each group consisted of 4 animals, was kept in 10 experimental cages). The first Group (I) was kept in a quiet environment as the control Group, and II, III and IV were exposed to mobile phone radiation for 1, 3, and 6 hours per day (MP1, MP3 and MP6 groups) during the light period, respectively, for 60 days. Group V, VI, and VII were exposed to noise pollution for 1, 3, and 6 hours per day (N1, N3, and N6 groups) during the light period, respectively, for 60 days. Group VIII, IX, X were exposed to combined mobile phone radiation and noise pollution for 1, 3, and 6 hours per day (MP1+N1, MP3+N3, and MP6+N6 groups) during the light period, respectively, for 60 days. At the end of the exposure, the rats were anesthetized using ketamine (50 mg/kg, IM), and blood sampling was taken. Then, testis biopsies were performed after sacrificing.

2.2. Chemiluminescence spectroscopy

Levels of LDH, thyroid hormones (T3 and T4), liver enzymes, cortisol, and reproductive hormones (LH, FSH, and testosterone) were assessed, using the chemiluminescence method (Electro Chemiluminescence, Alexis device model Elecsys E411, Roche, Geislingen, Germany). Serum levels of Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic

Transaminase (SGPT), and Alkaline Phosphatase (ALP) were measured by spectrophotometry, which was determined by Pars Azmay Company's quantitative plasma enzyme diagnostic kit, using Kinetic Photometry method.

2. 3. Histological analysis

For histological evaluation, the testis was excised from the scrotal sac and was cut into small pieces, and specimens were placed in a 10% formalin fixative solution immediately. After fixing for light microscopic observation, the specimens were dehydrated with ethanol, infiltrated, and embedded in paraffin. Sections with three-micron thickness were prepared using a rotary microtome (RM2125, Leica Biosystems Nussloch GmbH, Heidelberg, Germany) and stained with hematoxylin and eosin (H&E). The micrographs were taken by a light microscope equipped with a digital camera (Moticam pro 280; Motic Instruments Inc., Xiamen, China) and evaluated using the Image Tool (version 6 UTHSCSA, San Antonio, Texas, USA) software. The morphological changes in tissues such as inflammation and regeneration of the seminiferous tubules were studied. Morphometric analysis of tubules and diameters or thicknesses of seminiferous tubules were also evaluated accordingly.

2. 4. Statistical analysis

The statistical analysis was performed using Sigma Stat Software Package (SPSS Science, Chicago, IL, USA). The data obtained from the evaluation of serum hormone levels and histological parameters were statistically evaluated using one-way analysis of variance and subsequently Dunnett's test. The results were represented as mean \pm standard deviations and $p < 0.05$ was regarded as statistically significant.

3 Results and Discussions

3. 1. Effect of mobile phone radiation and noise pollution on serum levels of LDH

Compared to control group, serum levels of LDH had no significant alteration in MP1, MP3, MP6, N1, MP1+N1, and MP3+N3 groups. LDH serum level increased significantly in N3 and N6 groups ($p < 0.01$), however, significantly decreased in MP6+N6 groups ($p < 0.05$) compared with control animals (Figure1).

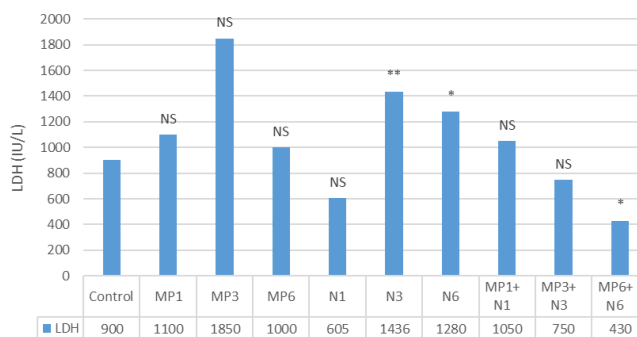


Figure 1. Serum LDH levels in control, MP1, MP3, MP6, N1, N3, N6, MP1+N1, MP3+N3, and MP6+N6 groups. NS indicates no significant difference compared to control group. * indicates a significant difference compared to control group (*: $p < 0.05$), (**: $p < 0.01$).

3. 2. Effect of mobile phone radiation and noise pollution on serum levels of T3 and T4

Although serum level of T3 had no significant alteration in MP1 group, it significantly decreased in other experimental groups compared with control group. Serum levels of T4 had no significant alteration in MP1 and MP3+N3 groups, however, significantly decreased in other experimental groups ($p < 0.01$, $p < 0.001$) (Figure 2).

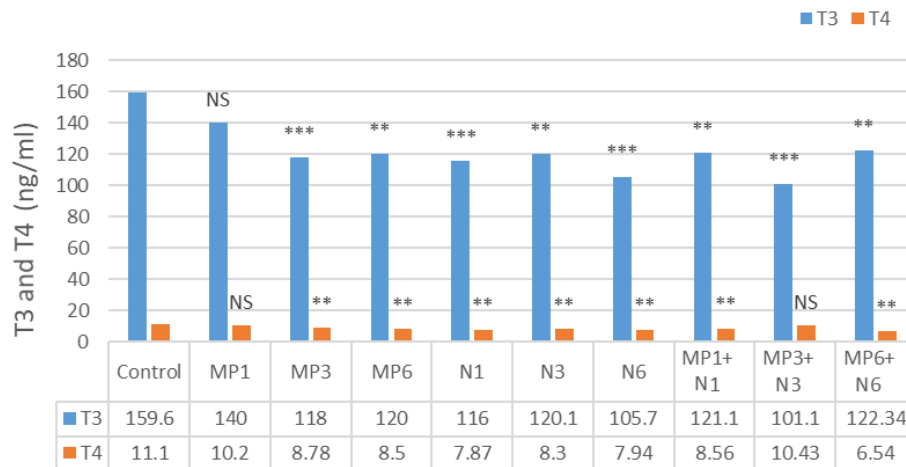


Figure 2. Serum levels of T3 and T4 in control, MP1, MP3, MP 6, N1, N3, N6, MP1+N1, MP3+N3 and MP6+N6 groups. NS indicates no significant difference compared to control group. * indicates a significant difference compared with control group (*: $p < 0.05$), (**: $p < 0.01$), (***: $p < 0.001$).

3. 3. Effect of mobile phone radiation and noise pollution on serum levels of SGPT, SGOT, and ALP

Serum SGOT and ALP levels had no significant alteration in experimental groups compared with control group. Serum SGPT level showed no significant alteration in MP1 group, however, significantly decreased in other experimental groups compared with control group ($p < 0.05$, $p < 0.001$, respectively) (Figure 4).

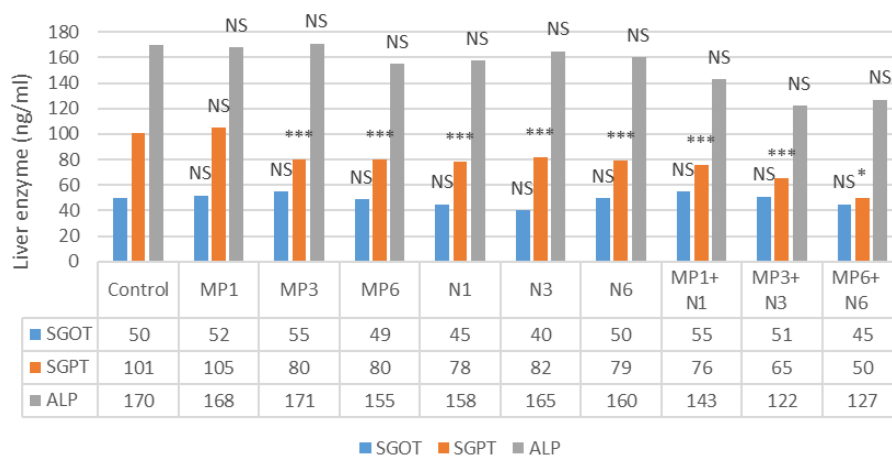


Figure 4. Serum levels of SGOT, SGPT and ALP in MP1, MP3, MP6, N1, N3, N6, MP1+N1, MP3+N3 and MP6+N6 groups. NS indicates no significant difference compared to control group. * indicates a significant difference compared to control group (*: $p < 0.05$), (**: $p < 0.01$). (***: $P < 0.001$).

3. 4. Effect of mobile phone radiation and noise pollution on serum level of cortisol

Serum levels of cortisol had no significant alteration in MP1, MP3 and N6 groups compared to control group, however, significantly increased in N1, N3 and MP3+N3 ($p<0.05$), MP6+N6 ($p<0.01$), and MP3 and MP6, MP1+N1 groups ($p<0.001$) compared to control group (Figure 5).

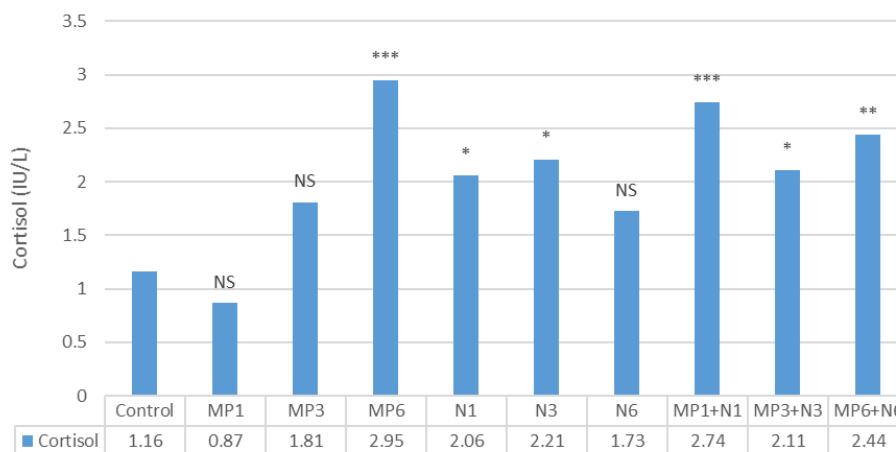


Figure 5. Serum cortisol levels in control, MP1, MP3, MP6, N1, N3, N6, MP1+N1, MP3+N3 and MP 6+N6 groups. NS indicates no significant difference compared to control group. * indicates a significant difference compared with control group (*: $p<0.05$), (**: $p<0.01$), (**: $p<0.001$).

3. 5. Effect of mobile phone radiation and noise pollution on serum levels of testosterone

Serum levels of testosterone in MP1 and MP3 groups had no significant alteration compared to control group, however, significantly decreased in MP6, N6, MP3+N3 and MP6+N6 ($p<0.001$), N3 and MP1+N1 ($p<0.01$) and N1 ($p<0.01$) groups compared to control animals (Figure 6).

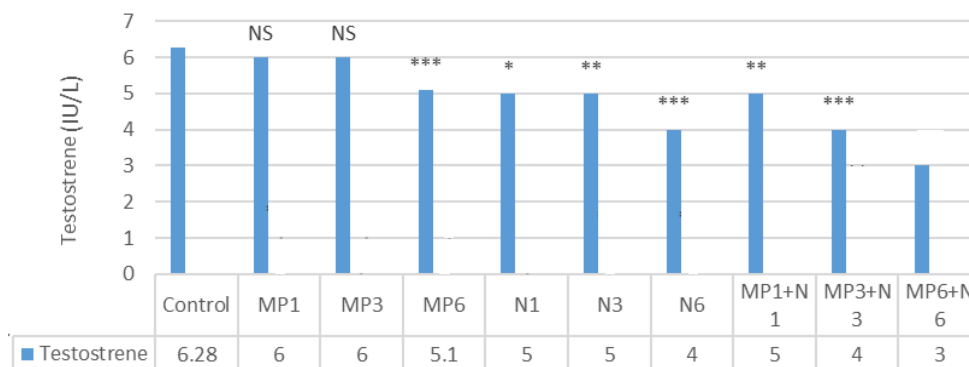


Figure 6. Serum testosterone levels in control, MP1, MP3, MP 6, N1, N3, N6, MP1+N1, MP3+N3 and MP6+N6 groups. N.S indicates no significant difference compared to control group. * indicates a significant difference compared to control group (*: $p<0.05$), (**: $p<0.01$), (**: $p<0.001$).

3. 6. Effect of mobile phone radiation and noise pollution on testicular tissue cells

Results of the histological analysis showed that the spermatogonia cells count in MP1, N1

and MP1+N1 did not significantly change, however decreased significantly in MP3, MP6, N3, N6, MP3+N3 and MP6+N6 groups compared with control group ($p<0.001$).

The spermatocytes count did not significantly change in MP1 group, however, significantly decreased in MP3, MP6, N3, N6, MP1+N1, MP3+N6, MP6+N6 groups ($p<0.001$) and N1 ($p<0.05$) compared to control group. Sertoli cell count significantly decreased in MP6+N6 group compared with control animals ($p<0.05$). (Figure 7)

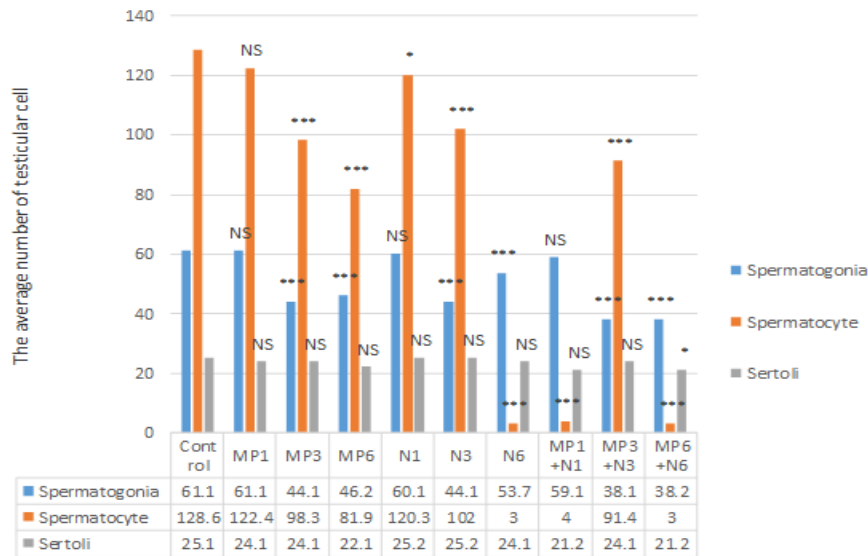


Figure 7. Spermatogonia, spermatocytes and Sertoli cells count in control, MP1, MP3, MP 6, N1, N3, N6, MP1+N1, MP3+N3 and MP6+N6 groups. NS indicates no significant difference compared to control group. * indicates a significant difference compared with control animals (*: $p<0.05$), (**: $p<0.001$).

3. 7. Histological observations

Figures 8 and 9 represent histological changes in seminiferous tubules in control, MP1+N1, MP3+N3 and MP6+N6 groups indicating increased deformity and decreased spermatogonia, soermatocyte cells count in MP1+N1, MP3+N3 and MP6+N6 groups and decreased Sertoli cells count in MP6+N6 group.

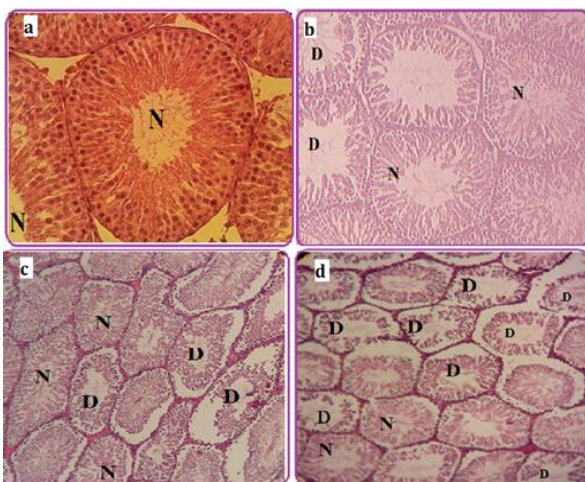


Figure 8. Morphology of seminiferous tubules (400x) in control (a), MP1+N1 (b) and MP3+N3 (c) and MP6+N6 (d) groups. Increased deformed tubules (D)/ normal tubules (N) are observed in b, c and d groups (scale bar: 100 μ m).

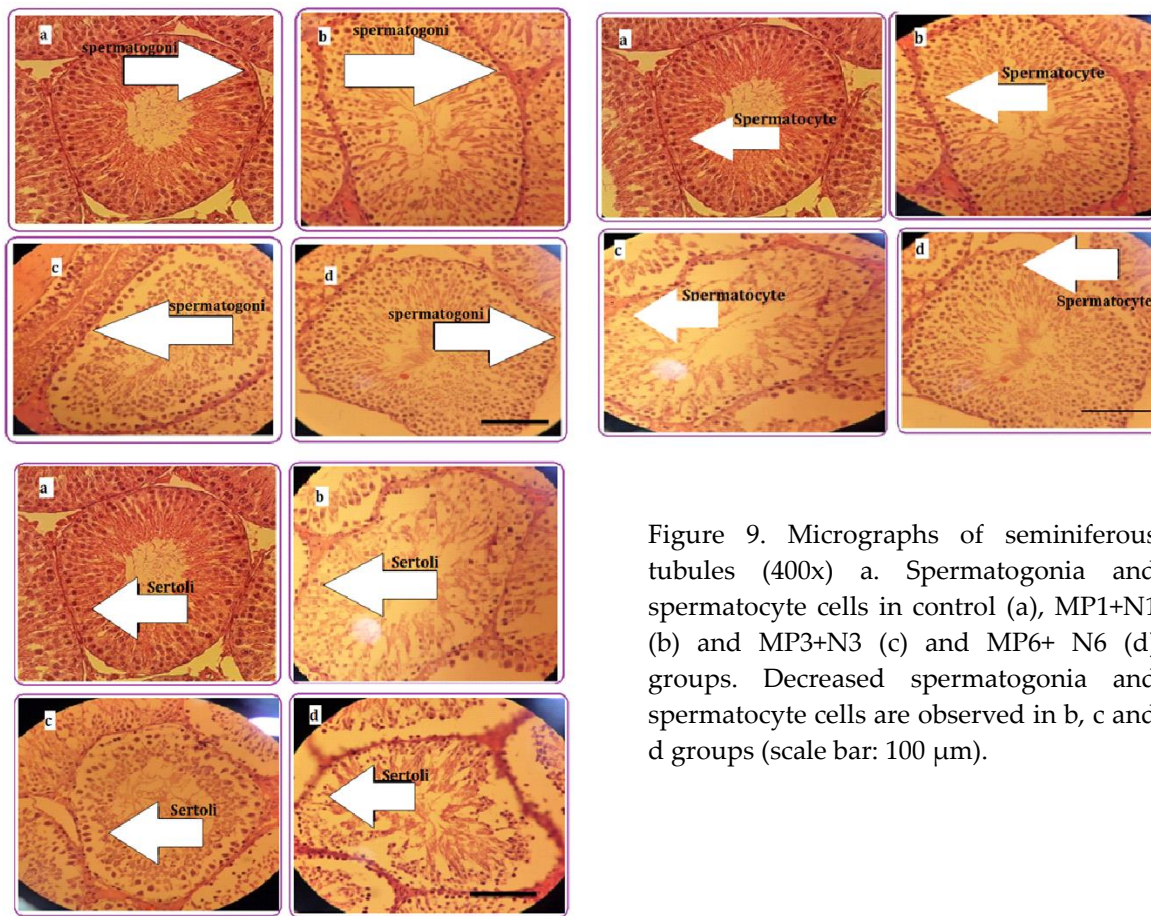


Figure 9. Micrographs of seminiferous tubules (400x) a. Spermatogonia and spermatocyte cells in control (a), MP1+N1 (b) and MP3+N3 (c) and MP6+ N6 (d) groups. Decreased spermatogonia and spermatocyte cells are observed in b, c and d groups (scale bar: 100 μ m).

3. 8. LDH

Our findings indicated that long term exposure to combined mobile phone radiation and noise pollution leads to increased serum levels of LDH. It has been shown that noise pollution affects the activity of neuropeptides in hypothalamic and extra hypothalamic sites (Michaud et al., 2003). Increased noise pollution can increase the activity of the sympathetic system and thus may impair the function of the endocrine system (Bhanap, 2013). In line with our results, there are reports revealing a definite association between high noise pollution and increased myocardial infarction. Noise pollution also increases the serum levels of endocrine hormones (Gohari and Ahmadi, 2014), which in turn, may influence metabolic pathways leading to a change in LDH production. Noise pollution also has been reported to affect gastric secretions suppressing the immune system's response, resulting in serum LDH levels change (Fyhri & Aasvang, 2010). Consistent with our findings, previous studies have shown that 4 minutes of daily exposure to 1800 MHz electromagnetic radiation reduces the serum level of the LDH (Pawlak et al., 2014).

3. 9. Thyroid hormones

We have shown that the long term exposure to combined mobile phone radiation and noise pollution results in decreased thyroid gland function in rats. In line with our findings, it has been shown that daily exposure to 1800 MHz electromagnetic radiation reduces the secretion of thyroid hormones (Pawlak et al., 2014). It has also been reported that 900 MHz electromagnetic

radiation affects the function of the thyroid gland and decreases the serum levels of T3 and T4 compared to the control group (Koyu et al., 2005). In a similar study, exposure to 250 MHz electromagnetic radiation significantly decreased the mean concentrations of T3 and T4 in rats (Lu et al., 1980). Decreased secretion of T4 after exposure to different kinds of stress may be due to a defect in the hypothalamic-pituitary-thyroid axis caused by inappropriate secretion of TSH. It can also occur because of the decreased activity of the type 2 deiodinase enzyme. This enzyme converts T4 to T3 in the tissue, which is activated by TSH (Kioukia-Fougia et al., 2002). In contrast, the findings of a research show that the exposure to leakage radiation from a micro radiation oven after two months could increase the concentration of T3 and T4 hormones (Jelodar and Beyzaee, 2010). Blood concentration of T3 and T4 in the group exposed to electromagnetic radiation with a frequency of 265 MHz was found to be increased in dogs (Magin et al., 1977).

3. 10. Liver enzyme

Despite SGOT and ALP, serum levels of SGPT enzyme decreased significantly in rats exposed to noise pollution and mobile phone radiation. Noise is known as one of the most important physical and environmental damaging factors, and its harmful effects on living organisms and biological systems have been systematically reported in addition to hearing loss. Noise stress increases free radicals and ultimately causes oxidative stress leading to significant effects on heart, kidneys, and liver (Pinar et al., 2011), in turn, may effect on SGOT and SGPT biosynthesis. There are strong reports about the destructive effects of noise pollution on SGPT levels in the liver, heart, and kidneys (Demirel et al., 2009; Holovská et al., 2015; Hassoy et al., 2013; Zare et al., 2007). In contrast to our findings, it has been found that exposure to cell phones was associated with liver cell damage and increased apoptosis, resulting in decreased liver enzymes production in including SGPT (Rajaei and Mohammadian, 2012; Saadat et al., 2010). A significant decrease in SGPT levels was also reported in the exposure of electromagnetic radiation in guinea pigs (Zare et al., 2007). A research carried out by workers in the field of electromagnetic radiation showed a decrease in SGOT serum level in rats exposed to electromagnetic radiation (Saadat et al., 2010).

3. 11. Cortisol

Serum cortisol level significantly decreased in animals exposed to long term combined mobile phone radiation and noise pollution. Mobile phone radiation and noise pollution may act as strong stressors (Babisch, 2002; Thomée et al., 2011) affecting on body function leading to increased serum cortisol level.

3. 12. Reproductive system hormones

The results of our study showed that long term exposure to mobile phone radiation and noise pollution has destructive effects on testicular tissue leading to decreased serum testosterone level as well as decreased spermatogonia, spermatocyte and Sertoli cells count. In line with our findings decreased male sex hormones level has been reported in animals exposed to long-term noise exposure (Qassemian et al., 2019; Chamkori et al., 2016). It was also reported that loud noises reduce serum levels of testosterone, FSH, and LH leading to reduced

spermatogonia cells count (Swami et al., 2007). Decreased levels of the FSH and LH are probably due to a decrease in GnRH, which is caused by the effect of stress on hypothalamus (Diab et al., 2012) leading to decreased testosterone levels (Knol, 1991; Goodman et al., 2012; Zirkin and Papadopoulos, 2018; Lamb, 2019), which in turn may result in destructive effects on testicular tissue and decreased spermatogonia, spermatocyte and Sertoli cells count (Waheeb and Hofmann, 2011). A study study in 2007 has shown that noise pollution with an intensity of 100 decibels causes inevitable changes in the testicular tissue (Swami et al., 2007).

4 Conclusion

Our findings indicated that long term exposure to combined mobile phone radiation and noise pollution leads to increased serum levels of LDH, decreased thyroid gland hormones and SGPT level, and had destructive effects on testicular tissue leading to decreased serum testosterone level as well as decreased spermatogonia, spermatocyte and Sertoli cells count.

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Conflict of interests

The authors declare that there are no competing interests.

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