

## EFFECT OF 3G/4G MOBILE PHONE RADIATIONS ON MICE TESTIS

HARVINDER SINGH<sup>1,2</sup>, MEENAKSHI SHARMA<sup>3</sup>, KAILASH CHANDRA YADAV<sup>4</sup> AND  
SUNIL KUMAR DHATWALIA<sup>1\*</sup>

<sup>2</sup>Government College Nalagarh, Solan, HP, India

<sup>1</sup>Department of Zoology, SBAS, Maharaja Agrasen University, Solan, HP, India

<sup>3</sup>Department of Zoology, Sri Sai University, Palampur, HP, India

<sup>4</sup>College of Fisheries Science and Research Centre, (CSAUAT Kanpur), Campus-Etawah, UP, India

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### ABSTRACT

The current study investigated the long-term effects of radiofrequency radiations (RFRs) emitted from third generation (3G) and fourth generation (4G) mobile phone on testis of Swiss albino mice. Total thirty six healthy mice were divided in to three groups of twelve mice each. Group I was served as control. The mice of Group II were exposed to 3G mobile phone, while Group III was exposed to 4G mobile phone for a period of 120 days for 4 hours daily at a distance of 6-8 cm during video call. Six mice from each group were sacrificed after 120 days, while the remaining six mice from each group were kept unexposed for 30 days to note the recovery. Results indicated that Long-term exposure to mobile phone radiation causes hypo-spermatogenesis, leydig cell hypoplasia and decrease in the sperm count, sperm motility and level of serum testosterone. The severity was more in the 4G exposed groups than 3G exposed groups. Signs of recovery were reported.

**KEY WORDS :** 3G/4G mobile phone, Radiofrequency radiations, Testis, Histopathology, Serum testosterone

### INTRODUCTION

The biological effects of radiofrequency electromagnetic radiations (RF-EMF) are a rising field of interest, in context to environmental impacts on human health (Ragy, 2014; Adebayo *et al.*, 2019). Several electronic devices create damaging electromagnetic fields (EMFs) which affect human health (Ongel *et al.*, 2009). Among these devices, the mobile phones have upraised the alarm in concern to public health all over the globe. The prolonged exposure of the reproductive organs against RF-EMF generated by mobile has been the subject of major scientific research (Özorak *et al.*, 2013). It was reported earlier that RF-EMF caused impairment of tissues in various organs including brain (Kang *et al.*, 1997), testis (Zare *et al.*, 2007; Al-Mayyahi *et al.*, 2020; reviewed by Singh and Sharma, 2020), kidney (Al-Mayyahi *et al.*, 2020; Singh *et al.*, 2020), liver (Moradpour *et al.*, 2020) and blood (Singh *et al.*, 2012; Singh and Bagai, 2013; Christopher *et al.*,

2020). The harmful effect of RF-EMF exposure was also confirmed on spermatogenesis, sertoli cells, leydig cells (Aydin *et al.*, 2007; Khayyat, 2011), and serum testosterone level (Wang *et al.*, 2003; Ozguner *et al.*, 2005). The present study was designed and performed as an experimental approach to investigate the effect of 3G/4G mobile phones on serum testosterone and morphological changes in the testis of male Swiss albino mice. The experiment was further extended to notice recovery in the tissues after one month post exposure.

### MATERIALS AND METHODS

**Animals:** Adult Swiss albino mice of male sex, 6-8 weeks old were used for the experimentation. Experiments were carried out as per institutional guidelines for animal care and use in the Maharaja Agrasen University. All the animals (control and experimental) were subjected to the similar environmental conditions (temperature  $25 \pm 3^\circ\text{C}$ ,

relative humidity of  $60 \pm 10\%$  and light/dark cycle of 12/12 hours), except the exposure field. Animals were fed with a standard pellet diet and water with ad-libitum.

**Ethics Statement:** Experiments were conducted after taking the permission from institutional ethical committee (IEC) of Maharaja Agrasen University, Baddi, Solan, HP in its meeting held on dated 10.10.2019 (Approval No. MAU/SBAS/2019/206).

**Experimental Design and Exposure Conditions:** A total of thirty six mice were randomly divided in to three groups having twelve mice in each group. The Group I was served as control. The Group II was exposed to 3G mobile phone (SAR = 0.406 W/Kg and 0.562W/Kg for body and head respectively), while Group III was exposed to 4G mobile phone (SAR = 0.458 W/Kg & 1.520W/Kg for body and head respectively) during video call from a distance of 6-8 cm for 4 hours daily, 02 hours each in the morning and evening for 120 days. The average power density (PD) at a distance of 6-8 cm was measured as 0.998 mW/cm<sup>2</sup> for 3G and 1.032 mW/cm<sup>2</sup> for 4G mobile phone during video call with Electrosmog Meter (MECO- 2790; Mecon Pvt. Ltd.). After exposure of 120 days the six mice from each group were sacrificed, while remaining six mice in each group were kept unexposed for 30 days, to observe the recovery if any due to removal of radiation exposure and labeled as 3GR (3G recovery group) and 4GR (4G recovery group). Mobile phones were programmed in auto answer mode. Both the groups have been exposed with similar set of mobile phone as well as from same service provider

**Evaluation of Biochemical Parameters:** After the completion of experiment, six mice from each exposed and recovery group were sacrificed along with normal controls and blood samples were immediately processed for serum testosterone by Competitive Immunoassay method (enhanced chemiluminescence method) using VITROS ECi. Immunodiagnostic Systems.

**Histological Studies:** The testis were removed carefully and fixed in Bouin's fixative (Pearson, 1968). The sections were stretched in hot water on albumin coated slides and stained with Delafield's Hematoxylin and Eosin Technique (H&E) to study histology (Baker, 1945). The DPX mounted sections were observed under light microscope (Leica DC 100, PC I Interface Digital Camera).

**Statistical Analysis:** Serum testosterone level were

tested for significance by using one-way ANOVA followed by post hoc Tukey's test; p values of <0.05, <0.01 and <0.001 were considered significant. Results were expressed as mean  $\pm$  S.D.

## RESULTS

### Histological Studies

**Control:** The Testicular sections of control mice showed normal architecture and proper arrangement of cells in the seminiferous tubule and the interstitium. Developing spermatogonial cells and germinal epithelium did not indicate any degeneration (Fig. 2A).

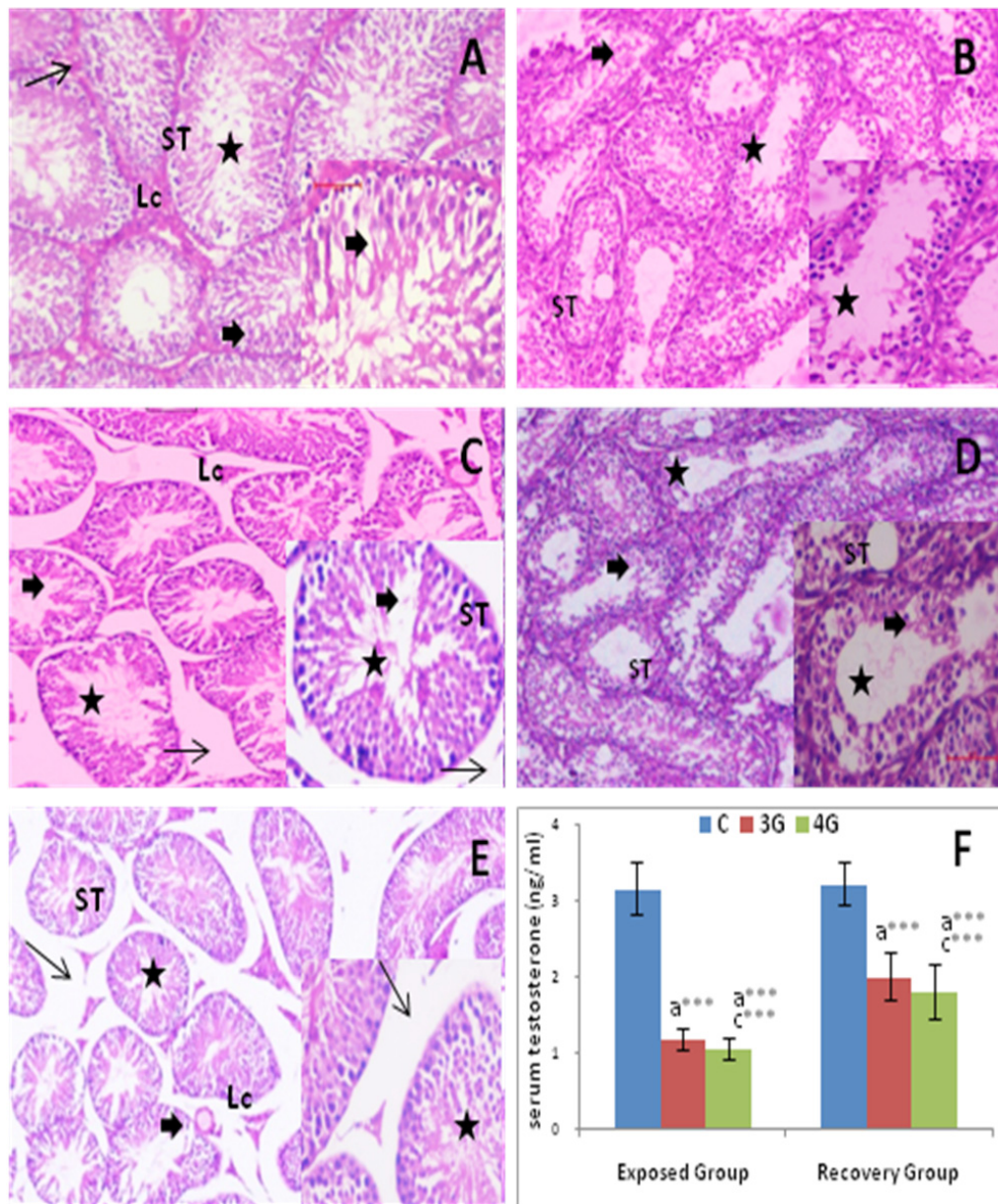
**3G and 4G:** The testis of the exposed mice showed minor degenerative changes, which includes disorganized arrangement of germ cells, spermatogenic arrest with no sperms in the lumen of seminiferous tubules and Leydig cell hypoplasia. Also, thickness of germinal epithelium was decreased due to dislodged cells (Fig. 2B, D). Sperm count and sperm motility were also reduced significantly ( $p < 0.05$ ) as compared to the control.

**3GR and 4GR:** The testis of both the recovery group appeared normal. However the Leydig cells hypoplasia and increased intertubular space were also evident (Fig. 2C, E). The sperm count and sperm motility did not showed any significant change as compared to the control.

**Serum Testosterone Level:** In the present study, serum testosterone level was significantly reduced by 38.23% in 3G ( $p < 0.001$ ) and 44.34% in 4G ( $p < 0.001$ ) exposed groups as compared to the control group (C). However, no significant change was observed among the 3G and 4G exposed groups in respect to serum testosterone level (Fig. 1F). The serum testosterone showed a significantly recovery ( $p < 0.001$ ) in both the recovery groups, compared to the control and exposed groups. The decrease in serum testosterone level was more pronounced in 4G exposed groups (Fig. 1F).

## DISCUSSION

The present study was designed and performed as an experimental approach to investigate the effect of 3G/4G mobile phones on serum testosterone and morphological changes in the testis of male Swiss albino mice. We have reported laydigs cell hypoplasia consequently decrease in the level of



**Fig. 1.** Photomicrographs (H/E- 40X and 100X) showing the effect of cell phone RF-EMR on the testis histology of mice. Control (A) showing normal seminiferous tubules (St) with intertubular connective tissue and Leydig cells (Lc), 3G Exposed (B), 4G Exposed (D) showing degenerated and disorganization of some seminiferous tubules, Leydig cell hypoplasia ( $\rightarrow$ ) detachment of the spermatogonia from the basal membrane (thick black arrow), spermatogenic arrest, no sperms in the lumen of seminiferous tubules ( $\star$ ), 3G Recovery (C) and 4G Recovery (E) with wider intertubular space (narrow black arrow) with normal spermatogenesis, and bar diagram (F) displaying the comparative effect of exposure and withdrawal of 3G and 4G mobile phone electromagnetic radiation on serum testosterone in mice.

serum testosterone and decrease in the sperm, which can be seen as an empty lumen of seminiferous tubules in both the exposed groups (Fig. 1B, D). The above changes may be due to the heating effect of RFR which may adversely affect the process of spermatogenesis (Saunders and Kowalczyk, 1981a) or oxidative stress through

reactive oxygen species (ROS) production (Akbari *et al.*, 2019). Similar to our results, some studies also reported Leydig cell hypoplasia (Khayyat, 2011 and Kumar, 2014), decrease in the level of serum testosterone (Ozguner *et al.*, 2005; Derias *et al.*, 2006; Kesari *et al.*, 2010; Khayyat, 2011; Kumar, 2014 and Mugunthan *et al.*, 2014) and complete absence of



spermatozoa (Meo *et al.*, 2010; Adebayo *et al.*, 2019; Al-Mayyahi *et al.*, 2020) in different animal models at various radiofrequency range. However, Dasdag *et al.*, 1999, found no decrease in sperm count or any abnormal sperm formation in EMR-exposed rats. Interestingly signs of recovery were reported in both the recovery groups (Fig.1C, E), which are consistent to the findings of (Ragy, 2014), where biochemicals changes in male albino rats due to exposure of mobile phone (900 MHz, 1 h/day for 60 days) were recovered after withdrawal of 30 days. Similarly, Thirty days following magnetic exposure, the splenic tissues appeared almost normal and manifested a tendency towards recovery (Zaghloul, 2011).

### CONCLUSION

Based upon the above findings, we concluded that chronic exposure to latest generation cell phone RF-EMR cause Leydig cell hypoplasia, reduce the sperm quality, its formation and level of serum testosterone. The 4G cell phone RF-EMR have more profound effect than 3G cell phone. The above changes recovered significantly, when exposure was removed. So it is advisable to minimize the use of mobile phones by limiting their use for basic applications. However further research is required involving other tissues of the body and to specify the time for complete recovery of exposed tissues.

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**Conflict of Interest:** Authors report no conflict of interest.

### REFERENCES

- Adebayo, E.A., Adeeyo, A.O., Ogundiran, M.A. and Olabisi, O. 2019. Bio-physical effects of radio frequency electromagnetic radiation (RF-EMR) on blood parameters, spermatozoa, liver, kidney and heart of albino rats. *Journal of King Saud University-Science*. 31(4) : 813-821.
- Akbari, A., Jelodar, G., Nazifi, S., Afsar, T. and Nasiri, K. 2019. Oxidative Stress as the Underlying Biomechanism of Detrimental Outcomes of Ionizing and Non-Ionizing Radiation on Human Health: Antioxidant Protective Strategies. *Zahedan Journal of Research in Medical Sciences*. 21(4).
- Al-mayyahi, R.S., Wa'il, A. and Ahmed, Z.A. 2020. The Effects of X Band Radar Frequency Exposure on Mice Testis and Kidney. *Poll Res*. 39: S142-S148.
- Aydin, M., Turk, G., Yuksel, M., Cevik, A., Apaydin, A. and Yilmaz, S. 2007. Effect of electromagnetic field on the sperm characteristics and histopathological status of testis in rats. *Medycyna Weterynaryjna*. 63(2) : 178-183.
- Baker, J.R. 1945. Cytological technique. *The Principles and Practice of Methods used to Determine the Structure of the Metazoan Cell*. (2nd Edition).
- Black, D. R. and Heynick, L. N. 2003. Radiofrequency (RF) effects on Blood Cells, Cardiac, Endocrine, and Immunological Functions. *Bioelectromagnetics*. 24(6) : 187-195. doi: 10.1002/bem.10166.
- Christopher, B., Mary, Y. S., Khandaker, M. U., Bradley, D. A., Chew, M. T. and Jojo, P. J. 2020. Effects of mobile phone radiation on certain hematological parameters. *Radiation Physics and Chemistry*. 166: 108443.
- Dasdag, S., Ketani, M.A., Akdag, Z., Ersay, A.R., Sari, I., Demirtas, Ö.C. and Celik, M.S. 1999. Whole-body microwave exposure emitted by cellular phones and testicular function of rats. *Urological Research*. 27(3) : 219-223.
- Derias, E.M., Stefanis, P., Drakeley, A., Gazvani, R. and Lewis-Jones, D.I. 2006. Growing concern over the safety of using mobile phones and male fertility. *Archives of Andrology*. 52(1) : 9-14.
- Kang, G.H., Lee, C.H., Seo, J.W., Sung, R.H., Chung, Y.H., Lee, S.K., Suh, Y.H. and Chi, J.G. 1997. In-vivo study on the harmful effect of the extremely low frequency unipolar pulsating magnetic field in mice. *Korean Medical Science*. 12(2) : 128-134.
- Kesari, K.K., Kumar, S. and Behari, J. 2010. Mobile phone usage and male infertility in Wistar rats. *Ind J Exp Biol*. 48 : 987-992.
- Khayyat, L.I. 2011. The histopathological effects of an electromagnetic field on the kidney and testis of mice. *Eurasia J Biosci*. 5 : 103-9.
- Kumar, S., Nirala, J.P., Behari, J. and Paulraj, R. 2014. Effect of electromagnetic irradiation produced by 3G mobile phone on male rat reproductive system in a simulated scenario. *Indian Journal of Experimental Biology*. 52(9) : 890-897.
- Meo, S.A., Al-Drees, A.M., Husain, S., Khan, M.M. and Imran, M.B. 2010. Effects of mobile phone radiation on serum testosterone in Wistar albino rats. *Saudi Med J*. 31(8) : 869-873.
- Moradpour, R., Shokri, M., Abedian, S. and Amir, T. 2020. The protective effect of melatonin on liver damage induced by mobile phone radiation in mice model.

- International Journal of Radiation Research.* 18(1) : 133-41.
- Mugunthan, N., Anbalagan, J. and Meenachi, S. 2014. Effects of long term exposure to a 2G cell phone radiation (900-1900 MHz) on mouse testis. *International Journal of Science and Research.* 3(9): 523-29.
- Ongel, K., Gumral, N. and Ozguner, F. 2009. The potential effects of electromagnetic field: a review. *Cell Membranes and Free Radical Research.* 1(3) : 85-89.
- Ozguner, M., Koyu, A., Cesur, G., Ural, M., Ozguner, F., Gokcimen, A. and Delibas, N. 2005. Biological and morphological effects on the reproductive organ of rats after exposure to electromagnetic field. *Saudi Medical Journal.* 26(3) : 405.
- Özorak, A., Nazýrođlu, M., Çelik, Ö., Yüksel, M., Özçelik, D., Özkaya, M.O., Çetin, H., Kahya, M.C. and Kose, S.A. 2013. Wi-Fi (2.45 GHz)-and mobile phone (900 and 1800 MHz)-induced risks on oxidative stress and elements in kidney and testis of rats during pregnancy and the development of offspring. *Biological Trace Element Research.* 156(1-3) : 229.
- Pearse, A.G. 1968. Histochemistry, theoretical and applied. *J. and a. Churchill London.* 1-759.
- Ragy, M.M. 2015. Effect of exposure and withdrawal of 900-MHz-electromagnetic waves on brain, kidney and liver oxidative stress and some biochemical parameters in male rats. *Electromagnetic Biology and Medicine.* 34(4) : 279-284.
- Saunders, R.D. and Kowalczyk, C.I. 1981. The Effect of Acute Far Field Exposure at 2.45 GHz on the Mouse Testis. *International Journal of Radiation Biology and Related Studies in Physics. Chemistry and Medicine.* 39(6) : 587-596.
- Singh, H. and Bagai, U. 2013. Effect of electromagnetic field on red blood cells of adult male Swiss albino mice. *Int J Theor Appl Sci.* 5(1) : 175-82.
- Singh, H. and Sharma, M. 2020. Impact of Cell Phone Radiations in Reproduction-A Review. *Biological Forum - An International Journal.* 12(2) : 30-38.
- Singh, H., Kumar, C. and Bagai, U. 2012. Biological effect of electromagnetic field of VDU on immune cells of Balb/c mice. *Biological forum-An International Journal.* 4 (2) : 82-91.
- Wang, S.M., Wang, D.W., Peng, R.Y., Gao, Y.B., Yang, Y., Hu, W.H., Chen, H.Y., Zhang, Y.R. and Gao, Y. 2003. Effect of electromagnetic pulse irradiation on structure and function of Leydig cells in mice. *National Journal of Andrology.* 9 (5): 327-330.
- Zaghloul, M. S. 2011. Effects of Chronic Exposure to Static Electromagnetic Field on Certain Histological Aspects of the Spleen and Some Haematological Parameters in Albino Rats. *Journal of American Science.* 7 (8).
- Zare, S., Alivandi, S. and Ebadi, A.G. 2007. Histological studies of the low frequency electromagnetic fields effect on liver, testes and kidney in guinea pig. *World Applied Sciences Journal.* 2 (5) : 509-511.
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