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Ameliorative Effect of *Punica Granatum* on Histological Integrity of Sertoli Cells and Leydig Cells in Rats with Exposure to Radio Electromagnetic Radiation

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Abstract: Nowadays, infertility is a global problem; it affects 15% of couples worldwide. Male infertility can be attributed to an array of mobile phone (MP) radiofrequency electromagnetic radiation (RF-EMR). The purpose of this study is to explore the capability of pomegranate juice (PJ) extract in protecting rat testis against the impairment caused by RF-EMR. To evaluate the effect of Radiofrequency electromagnetic radiation and efficacy of Pomegranate juice on the structural integrity of Sertoli cells and interstitial cells of Leydig. Thirty Sprague Dawley rats were split into five groups (n = 6/group) such as control - I; RF-EMR - II; RF-EMR + PJ - III; RF-EMR Recovery - IV; Pomegranate juice - V. Histological integrity of Sertoli cells and Leydig cells were found in 4G MP RF-EMR exposed rats when compared to group I. Seminiferous tubules (STs) showed the winding of interstitial spaces and an overall reduction in their size. Sertoli cells were resting irregularly. Leydig cells are darkly stained nuclei, vascular nuclei, and acidophilic vacuolated cytoplasm containing tiny lipid droplets. The co-administration of pomegranate juice showed improvement in STs was normal in shape. Sertoli cells and spermatogenic cells rest on an intact basement membrane. Sertoli cells appeared as tall cells between spermatogenic cells with prominent nuclei compared to RF-EMR group II. Exposure to 4G MP RF-EMR causes Histo-morphological changes (damage) of Sertoli and Leydig cells. However, co-administration of pomegranate juice acts as an agent against the 4G MP RF-EMR induced alterations.

Keywords: mobile radioelectromagnetic radiation, pomegranate juice, histological integrity, male infertility.

石榴对无线电电磁辐射大鼠支持细胞和睾丸间质细胞组织学完整性的改善作用

摘要：如今，不孕不育是一个全球性问题。它影响全球15%的夫妇。男性不育症可归因于手机(国会议员)射频电磁辐射(射频电子病历)阵列。本研究的目的是探讨石榴汁(PJ)提取物保护大鼠睾丸免受射频电子病历损伤的能力。评估射频电磁辐射和石榴汁功效对睾丸间质细胞和间质细胞结构完整性的影响。将30只斯普拉格道利大鼠分成5组（n=6/组），例如对照组 -一世；射频-电子病历-II；射频电子病历+ PJ - III; 射频电子病历恢复-IV；石榴汁 - V. 与 I 组相比，在4G国会议员射频-

电子病历暴露的大鼠中发现支持细胞和莱迪格细胞的组织学完整性。生精小管(英石)显示间质空间的弯曲和其大小的整体减小。支持细胞不规律地休息。间质细胞是深色染色的细胞核、血管细胞核和含有微小脂滴的嗜酸空泡状细胞质。石榴汁的共同给药显示STs的改善在形状上是正常的。支持细胞和生精细胞位于完整的基底膜上。与射频电子病历组II相比，支持细胞在具有突出细胞核的生精细胞之间表现为高细胞。暴露于4G国会议员射频电子病历会导致支持细胞和睾丸间质细胞的组织形态学变化（损伤）。然而，石榴汁的共同给药可作为对抗4G国会议员射频电子病历诱导的改变的药剂。

关键词：移动无线电电磁辐射，石榴汁，组织学完整性，男性不育。

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1. Introduction

Mobile phone technology has spread rapidly around the world. In 2019, more than 5 billion people had 4G android mobile devices, and over half of these connections are android mobile phones. However, the fastest-growing 4G android mobile phone technology has not been equal across nations. Smartphones have become necessary in our daily life [1]. These studies suggest that infertility is mainly related to exposure to "non-ionizing radiation" emitted by android mobile phones, toxic chemicals, and other environmental nuisances [2]. As the use of the android mobile phone has increased, it has also increased the concern regarding its harmful effects on health. The World Health Organization established the International RF-EMR project in 1996 to protect public health and to assess the scientific evidence of possible health effects due to electromagnetic radiation. The Radio-Frequency (RF) waves and duration of radiation are the main factors that influence the effects of android MP radiation on the reproductive system [3, 4]. Inability to get pregnant for 1 year even after continuation of sexual intercourse without using any safety device is defined as infertility [5]. The internal factors for male infertility include 'testicular abnormalities' (cryptorchidism, genital tract obstructions, immunological conditions, and infections). External factors are exposure to environmental chemicals, such as heavy metals, electromagnetic wave radiations, high temperature, smoking, alcohol, stress, and obesity; all of them can disrupt spermatogenesis [6, 7]. Seminiferous tubules, Leydig cells, Sertoli cells, and spermatozoa are the main targets of possible damage caused by the RF-EMR. RF-EMR exposure may reduce hormonal testosterone levels, damage sperm DNA, and impairs spermatogenesis [8, 9].

Pomegranate fruit and peel extract have a marked antioxidant capacity [10], with a high polyphenols content in ellagitannins condensed anthocyanins and tannins [11]. The potential therapeutic properties of Pomegranate juice are helpful in the treatment and prevention of cardiovascular disease [12], cancer [13], treatment of acquired immune deficiency syndrome [14] and diabetes [15] in addition to its uses for oral hygiene [16] and as an adjunct therapy to increase 'bioavailability of radioactive' dyes during diagnostic imaging [17]. Furthermore, Pomegranate fruit is used in folk medicine and remedies many symptoms (eyesore, scurvy, diarrhea, and blood clots). PJ consumption significantly increases the spermatogenic cell density, sperm count, hormonal testosterone levels, and antioxidant activity in male rats [18]. In addition, PJ has been proposed as chemotherapeutic, anti-inflammatory, anti-atherosclerotic [19-21]. The present study aimed to investigate the effect of 4G MP RF-EMR exposure on the SD rats and determine the efficacy of PJ supplementation in protecting the histological integrity of Sertoli cells and Leydig cells.

2. Materials and Methods

After approval by the "Institutional Animal Ethical Committee" (Ref. No: IAEC/PHARMA/SDUMC/2018/12a), this study was carried out. The experimental study was conducted at the animal house, SDUMC, Kolar (Karnataka, India). The duration of the study was 6 months.

2.1. Animals and Grouping

Thirty male SD rats were present in the study (10-14 weeks old; 150±180 gms). The animals were kept in Central Animal House for 12 days for acclimatization. Rats were fed pellets (Champaka Foods and feeds, Bangalore) and provided water throughout the study. The Central animal house and the experimental room were well ventilated with a temperature range of 20-24˚C, constant humidity (45-50%), and constant 12 Light: 12 Dark cycles. The rats were grouped into five groups (6/each group); Control I, RF-EMR II, RF-EMR + pomegranate juice III, RF-EMR Recovery IV, and Pomegranate juice V respectively.

Control Group I: neither exposed 4G MP RF-EMR nor given to PJ.

RF-EMR Group II: rats were exposed to mobile phone RF-EMR emitted (800 - 2400 MHz) for 60 minutes/day for 3 months.

RF-EMR + PJ Group III: six rats were exposed to MP RF-EM for 60 minutes/day for 3 months, following 3 months supplemented with Pomegranate juice (1 ml/day) [22].

RF-EMR Recovery Group IV: six rats were exposed to 4G MP RF-EMR for 60 minutes/day/3 months and kept un-exposed for 3 months.

Pomegranate Juice Group V: six rats were supplemented with PJ 1 ml/day for 90 days.

2.2. Inclusion and Exclusion Criterion

When procured, male healthy and active Sprague-Dawley rats (10-14 weeks old; 180 ± 10 gms) were included in this study. Female Sprague-Dawley rats and lesser weight (< 150) rats were excluded.

2.3. Mobile Phone RF-EMR Exposure Technique

Group II, III, and IV (two rats/each case) rats are exposed to RF-EMR emitted by 4G MP (VIVO 1803) 60 minutes /day for 3 months, respectively. Keeping a (800-2400 MHz) mobile phone in talking mode is represented in Fig. 1).

The mobile phones were hung down in the center of

the cage at a distance of 5 cm between the MP to the cage floor, and the radiation they emitted during the exposure was quantified by an RF-EMR meter (Meco - G RF - EMR) which were kept in rat case [22].

2.4. Plant Material

Pomegranate fruit material (Botanical name: *Punica Granatum*. Voucher No.: 0320) was authenticated by Dr. Madhava Chetty K, Assistant Professor, Department of botany SVU, Tirupathi, A.P.

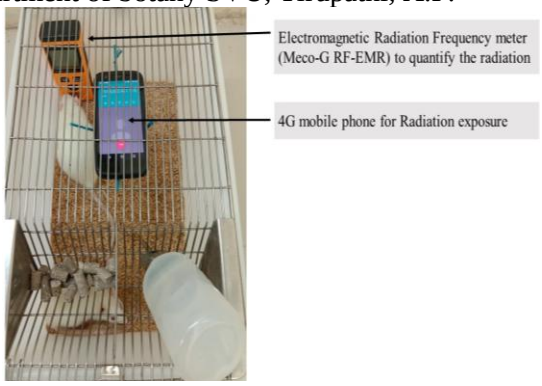

Fig. 1 The image shows the cage with rats and a mobile phone during RF-EMR exposure with a radiofrequency meter (Meco-G RF-EMR) to quantify the radiation

2.5. Extract Preparation

Fresh pomegranate fruits (Bhagwa variety) were purchased from the local market. Fruits were cleaned and free from evidence of insect infections and objectionable materials. Afterward, they were manually peeled without separating the seeds.

PJ was homogenized in the commercial blender (750W, Bajaj GX 11, India), filtrated with Labrador Buchner funnel, immediately poured into dark bottles, and stored at -20^{o} for no longer than one week. PJ was given through oral gavage daily 1ml/day for 3 months. This period is necessary to determine the effect of Pomegranate juice on "sperm production" because the animals need a period of 52 days [19].

2.6. Animal Sacrifice

After overnight fasting, animals were euthanized with ketamine (50 mg/kg, *i.p.*), and the testis was extracted immediately, fixed with 10% buffered formalin for histological integrity testis.

2.7. Tissue Processing

The fixed specimens (testis) were trimmed and subsequently dehydrated in ascending grades of absolute alcohol (60%, 80%, 90 %) and clean with xylene, impregnated in "paraffin wax" 60^{o}C, and embedded with the help of L-modes in paraffin for Histo-morphological assessments. Furthermore, blocks were cut into sections with a thickness of 5-6 µm and stained with H&E staining; sections were observed under a microscope [23].

3. Results

3.1. Assessment of Histological Integrity of Sertoli Cells and Leydig Cells (Hematoxylin and Eosin-Stained Sections)

In the present work, the Histological integrity of the testicular sections of the control group demonstrated a normal structure of seminiferous tubules with a regular shape. Sertoli and spermatogenic cells rest intact with the basement membrane (Fig. 2). Spermatogonia appeared as small cells and prominent rounded nuclei resting on the basement membrane. Sertoli cells appeared as tall cells between spermatogenic cells with prominent nuclei. In interstitial space between seminiferous tubules showed interstitial cells of Leydig, which appeared as large polygonal cells with vesicular nuclei. In group II, testicular sections are distorted; the histological architecture of some seminiferous tubules indicates a winding of interstitial spaces and a reduction in their size. Seminiferous tubules showed irregular basement membrane and detached, degenerated spermatogenic cells.

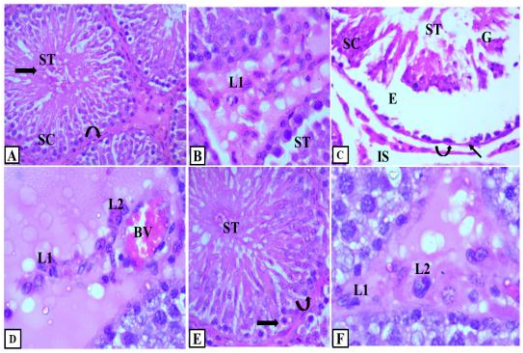

Fig. 2 Photomicrographs of testis tissue (Sertoli cells and Leydig cells) from S.D rats sections. Control group (A & B) showing: A - closely packed seminiferous tubules (ST) lined by Sertoli cells (curved arrow) and spermatogenic cells (SC), with sperms (long arrow) in their lumens; B - Leydig cells (L1) with vesicular round nuclei and acidophilic cytoplasm containing seminiferous tubules (ST) or observed (H & E stain, x1000); Mobile phone RF-EMR group (C & D) showing: C - Irregular basement membrane (long arrow), distorted seminiferous tubules (ST), wide interstitial space (IS), detached spermatogenic cells (SC), multinucleated giant cells (G), empty spaces (E) and Sertoli cell (curved arrow) resting on the irregular basement membrane; D - Interstitial space contains congested blood vessels (BV) and Leydig cells with darkly stained nuclei (L1), and the remaining cells (L1) are vesicular nuclei (H & E stain, x1000); Pomegranate juice group (E & F) showing: E - Packed seminiferous tubules (ST) lined by spermatogenic cells and Sertoli cell (curved arrow), apparently normal spermatogenic cells (thick arrow); F - Leydig cells (L2) shows acidophilic vacuolated cytoplasm and vesicular nuclei (L2) (H & E stain, x1000).

Sertoli cells showed a decrease in cytoplasm ground substance cytoplasm followed vacuolization, and Sertoli cells are entirely disturbed. Interstitial space increased, contained congested blood vessels, and Leydig cell (atrophy) appeared with small polygonal cells and darkly stained nuclei.

In group III, examination of Histological testicular sections showed that normal packed STs lined by layers of 'spermatogenic cells' and Sertoli cells are

resting on the fixed basement membrane. Few empty spaces are still seen among the spermatogenic epithelium. Interstitial space showed polygonal Leydig cells (darkly stained nuclei) with pale nuclei and acidophilic vacuolated cytoplasm.

In group IV, Histological testicular sections showed incomplete improvement with some packed STs and partial loss of spermatogenic cells. Some spermatogenic cells appeared with acidophilic cytoplasm either resting on a regular or irregular basement membrane. Other STs are still containing multinucleated giant cells. The interstitial spaces contained Leydig cells with pale dark nuclei, and congested blood vessels are present.

In group V, No Histological abnormalities were noted in the PJ group compared to the control group. However, STs were regular in the shape and arrangement of their cellular components. Sertoli cells and spermatogenic cells rest on the intact basement membrane. Sertoli cells appeared as tall cells between spermatogenic cells with prominent nuclei. In interstitial space between seminiferous tubules showed interstitial cells of Leydig, and cells appeared as "large polygonal" cells with vesicular nuclei.

4. Discussion

The 4G MP has become an essential part of daily life worldwide. In the covid-19 pandemic, the usage of MP was increased in adults, including adolescents. Although some studies have shown evidence regarding the harmful effects of RF-EMR on fertility, it is still controversial [24]. Several studies reported that usage of mobile phones (RF-EMR) led to decreased male fertility. Nonetheless, other studies showed no conclusive link between male infertility and mobile phone usage [25]. Therefore, the present study aimed to evaluate the consequences of increasing 4G MP (RF-EMR) usage on testes. The RF-EMR may affect biological systems and potential health risks by increasing free radicals, which enhance mainly lipid peroxidation, and by changing antioxidant defense systems of human tissues leading to OS [26]. There is general agreement that OS was implicated as one of the main culprits in male infertility [27] and that RF-EMR can intensify the generation of OS and cause an imbalance between the production of reactive oxygen species and their antioxidant defense system [28].

According to the available literature, the testicular Histological Integrity in S.D rats exposed to 4G MP RF-EMR and co-administration of PJ has not been studied yet. Histological integrity of the testes of rats exposed with 4G MP RF-EMR revealed degeneration in spermatogonia cells lining the seminiferous tubules. It is associated with incomplete spermatogenesis compared with the control group. The present study of the transmission of testicular Histo-morphological evaluation revealed degenerative changes mainly in the Sertoli cells and Leydig cells. The present study was focused on the protective effect of Pomegranate juice against 4G mobile phone RF-EMR exposed reproductive toxicity (Histo-morphology of Sertoli cells and Leydig cells) in male SD rats. Pomegranate juice has a protective effect due to its active ingredients like conjugated fatty acids [29], estrogenic flavonoids [30] phenolic acids [31], tannins [32, 33]; these compounds are found in substantial amounts in the Pomegranate juice and peels of the pomegranate fruit [34].

Kumar et al. [35] revealed that mobile phones working on 1910.5 MHz could cause significantly decreased sperm count, reduction in the diameter of the STs, testicular weight, and DNA damage in Wistar rats in 2 hours/day/60days of RF-EMR exposure. A study done by LatifaIshaq Khayyat [36] and Pradeep Kumar et al. [37] reported that RF-EMR of mobile phones induced Leydig cell hypoplasia, atrophied seminiferous tubules, wide interstitium, and decreased germ cell population, maturation arrest in the spermatogenesis, vacuolization in spermatogenic cells and pyknotic nuclei in a germ cell. They also observed detachment of Sertoli cells and spermatogonia from the basal lamina, residual cytoplasm, shrinkage, and debris of degenerating cells in the seminiferous tubules [36, 37]. Similarly, a previous study done by Turk G et al. reported that the daily consumption of pomegranate juice for 7 weeks caused a significant reduction in OS parameters and a marked increase in the level of spermatogenic cells and thickness of the germ layers [18]. According to Mahmoud and Solaiman et al., pomegranate juice groups revealed that histo-morphological examination of STs retained normal appearance. These results were confirmed Histo-morphometrically by the significant increase in the STs Diameter and germinal epithelial height [38]. Dare BJ et al. studied that 'Garcinia Kola and Vitamin E' had been seen to demonstrate hepatoprotective action against the oxygen free radical generated by the presence of Lead ions by maintaining the histological integrity of the testis of male Wistar rats [39].

Uluaydin et al. [40] supported that FSH causes Sertoli cells to produce androgen-binding protein, inhibin, and activating. These hormonal regulations by the hypothalamus and anterior pituitary are essential for male reproductive functions. RF-EMR emitted from mobile phones can cause thermal effects as manifested by temperature elevation and EMF strength value on the hypothalamus and pituitary gland after 4G MP RF-EMR exposure [40]. Seeham Ali Qasim et al. also mentioned that PJ prevents the decline in Leydig and Sertoli cells number and increases testosterone hormone level, prohibits reduction in several Sertoli cells Leydig cells and increases them that lead to increases in testosterone hormone [41].

5. Conclusion

This study indicated that exposure to isothermal

non-ionizing RF-EMR (800-2400MHz) emitted from 4G MP could produce Histological integrity effects on the testis of male S.D rats and these effects increased with these the time of exposure. However, the co-administration of PJ was protective of the 4G MP RF-EMR-induced alterations of Histological integrity changes due to its potent antioxidant effects. Hence, it can be said there is a positive effect of PJ consumption on male fertility. However, a realistic human exposure simulation remains challenging, and the extrapolation of experimental results to humans requires further studies.

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References

[1] ABDOLMALEKI A., SANGINABADI F., RAJABI A., and SABERI R. The Effect of electromagnetic waves exposure on blood parameters. *International Journal of Hematology-Oncology and Stem Cell Research*, 2013, 6: 13-16. https://ijhoscr.tums.ac.ir/index.php/ijhoscr/article/view/329/315

[2] BIN-MEFERIJ M. M., & EL-KOTT A. F. The radioprotective effects of Moringa oleifera against mobile phone electromagnetic radiation-induced infertility in rats. *International Journal of Clinical & Experimental Medicine*, 2015, 8: 12487–12497. https://pubmed.ncbi.nlm.nih.gov/26550159/

[3] KESARI K. K., KUMAR S., and BEHARI J. Effects of radiofrequency electromagnetic wave exposure from cellular phones on the reproductive pattern in male Wistar rats. *Applied Biochemistry and Biotechnology*, 2011, 164(4): 546-559. https://doi.org/10.1007/s12010-010-9156-0

[4] KUMAR S., KESARI K. K., and BEHARI J. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. *Indian Journal of Clinical & Experimental Biology*, 2010, 48(6): 586-592. https://pubmed.ncbi.nlm.nih.gov/20882761/

[5] WORLD HEALTH ORGANIZATION. *Laboratory manual for the examination and processing of human semen*. World Health Organization, Geneva, 2010.

[6] JEDRZEJOWSKA R. W., WOLSKI J. K., and HILCZER J. S. The role of oxidative stress and antioxidant in male fertility. *Central European Journal of Urology*, 2013, 66(1): 60–67. https://doi.org/10.5173/ceju.2013.01.art19

[7] ESTEVES S. C., MIYAOKA R., and AGARWAL A. An update on the clinical assessment of the infertile male. *Clinics*, 2011, 66(4): 691–700. https://doi.org/10.1590/S1807-59322011000400026

[8] LA VIGNERA S., CONDORELLI R. A., VICARI E., D'AGATA R., and CALOGERO A. E. Effects of the exposure to mobile phones on male reproduction: a review of the literature. *Journal of Andrology*, 2012, 33(3): 350-356. https://doi.org/10.2164/jandrol.111.014373

[9] DEEPINDER F., MAKKER K., and AGARWAL A. Cell phones and male infertility: dissecting the relationship. *Reproductive Biomedicine Online*, 2007, 15(3): 266-270. https://doi.org/10.1016/s1472-6483(10)60338-0

[10] KAUR G., JABBAR Z., ATHAR M., and ALAM M. S. Punica granatum (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. *Food and Chemical Toxicology*, 2006, 44(7): 984-993. https://doi.org/10.1016/j.fct.2005.12.001

[11] SEERAM N. P., ADAMS L. S., HENNING S. M., NIU Y., ZHANG Y., NAIR M. G., and HEBER D. In vitro antiproliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. *The Journal of Nutritional Biochemistry*, 2005, 16(6): 360–367. https://doi.org/10.1016/j.jnutbio.2005.01.006

[12] AVIRAM M., DORNFELD L., KAPLAN M., COLEMAN R., GAITINI D., NITECKI S., HOFMAN A., ROSENBLAT M., VOLKOVA N., PRESSER D., ATTIAS J., HAYEK T., and FUHRMAN B. Pomegranate juice flavonoids inhibit low-density lipoprotein oxidation and cardiovascular diseases: studies in atherosclerotic mice and in humans. *Drugs under Experimental and Clinical Research*, 2002, 28: 4962. https://pubmed.ncbi.nlm.nih.gov/12224378/

[13] LANSKY E. P., & NEWMAN, R. A. Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. *Journal of Ethnopharmacology*, 2007, 109: 177−206. https://doi.org/10.1016/j.jep.2006.09.006

[14] LEE J., & WATSON R. R. Pomegranate: a role in health promotion and AIDS? In: WATSON R. R. (Ed.), *Nutrients and Foods in AIDS*. CRC Press, Boca Raton, 1998: 179–192. https://arizona.pure.elsevier.com/en/publications/pomegranate-a-role-in-health-promotion-and-aids

[15] ROSENBLAT M., HAYEK T., and AVIRAM M. Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. *Atherosclerosis*, 2006, 187: 363−371. https://doi.org/10.1016/j.atherosclerosis.2005.09.006

[16] KIM M. M., & KIM S. Composition for improving oral hygiene containing Punica granatum L. extract. *Korean Patent*, 2002: 2002066042.

[17] AMORIM L. F., CATANHO M. T., TERRA D. A., BRANDAO K. C., HOLANDA C. M., JALES-JUNIOR L. H., BRITO L. M., GOMES M. L., DE MELO V. G., BERNARDO-FILHO M., and CAVALCANTI JALES R. L. Assessment of the effect of Punica granatum (pomegranate) on the bioavailability of the radiopharmaceutical sodiumpertechnetate (99mTc) in Wistar rats. *Cellular and Molecular Biology*, 2003, 49: 501–507. https://pubmed.ncbi.nlm.nih.gov/12899440/

[18] TURK G., SONMEZ M., AYDIN M., YUCE A., GUR S., YUKSEL M., AKSU E. H., and AKSOY H. Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. *Clinical Nutrition*, 2008, 27(2): 289-296. https://doi.org/10.1016/j.clnu.2007.12.006

[19] ROZENBERG O., HOWELL A., and AVIRAM M. Pomegranate juice sugar fraction reduces macrophage

oxidative state, whereas white grape juice sugar fraction increases it. *Atherosclerosis*, 2006, 188(1): 68–76. https://doi.org/10.1016/j.atherosclerosis.2005.10.027

[20] ADAMS L. S., SEERAM N. P., AGGARWAL B. B., TAKADA Y., SAND D., and HEBER D. Pomegranate juice, total pomegranate ellagitannins, and punicalagin suppress inflammatory cell signaling in colon cancer cells. *Journal of Agricultural and Food Chemistry*, 2006, 54(3): 980–985. https://doi.org/10.1021/jf052005r

[21] MALIK A., AFAQ F., SARFARAZ S., ADHAMI V. M., SYED D. N., and MUKHTAR H. Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. *Proceedings of the National Academy of Sciences of the United States of America*, 2005, 102(41): 14813–14818. https://doi.org/10.1073/pnas.0505870102

[22] BANAVATH A. N., & SRINIVASA S. N. Ameliorative effect of Punica granatum on sperm parameters in rats exposed to mobile radioelectromagnetic radiation. *Asian Pacific Journal of Reproduction*, 2021, 10(5): 225. https://doi.org/10.4103/2305-0500.326720

[23] PENNA-VIDEAU S., CERMENO-VIVAS J., MORENO-MARVAL M., and QUIROGA-NOTTI M. Histopathological changes in albino mouse testis induced by the administration of Tityus n. sp. venom. *Journal of Venomous Animals and Toxins*, 2000, 6(2): 167-179. https://doi.org/10.1590/S0104-79302000000200003

[24] SAROOKHANI M. R., ASIABANHA REZAEI M., SAFARI A., ZAROUSHANI V., and ZIAEIHA M. The influence of 950 MHz magnetic field (mobile phone radiation) on sex organ and adrenal functions of male rabbits. *African Journal of Biochemistry Research*, 2011, 5: 77-80. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.916.5265&rep=rep1&type=pdf

[25] AGARWAL A., SINGH A., HAMADA A., and KESARI K. Cell phones male infertility: a review of recent innovations in technology consequences. *International Brazilian Journal of Urology*, 2011, 37: 432-454. https://doi.org/10.1590/s1677-55382011000400002

[26] OZGUNER F., ALTINBAS A., OZAYDIN M., DOGAN A., VURAL H., KISIOGLU A. N., CESUR G., and YILDIRIM N. G. Mobile phone-induced myocardial oxidative stress: protection by a novel antioxidant agent caffeic acid phenethyl ester. *Toxicology and Industrial Health*, 2005, 21: 223-230. https://doi.org/10.1191/0748233705th228oa

[27] AGARWAL A., DEEPINDER F., SHARMA R. K., RANGA G., and LI J. Effect of cell phone usage on semen analysis in men attending infertility clinic: an observational study. *Fertility and Sterility*, 2008, 89: 124-128. https://doi.org/10.1016/j.fertnstert.2007.01.166

[28] BAHARARA J., ZAFAR-BALANEJAD S., KAMAREH E., and ASADI-SAMANI M. The effects of green tea extract on teratogenicity induced by low frequency electromagnetic field on bone marrow Balb/C miceembryo. *Journal of Herbmed Pharmacology*, 2014, 3: 47-51. http://herbmedpharmacol.com/Article/JHP_20150527124342

[29] SCHUBERT S. Y., LANSKY E. P., and NEEMAN I. Antioxidant and eicosanoid enzyme inhibition properties of pomegranate seed oil and fermented juice flavonoids. *Journal of Ethnopharmacology*, 1999, 66(1): 11–17. https://doi.org/10.1016/s0378-8741(98)00222-0

[30] VAN ELSWIJK D. A., SCHOBEL U. P., LANSKY E. P., IRTH H., and VAN DER GREEF J. Rapid dereplication of estrogenic compounds in pomegranate (Punica granatum) using on-line biochemical detection coupled to mass spectrometry. *Phytochemistry*, 2004, 65(2): 233–241. https://doi.org/10.1016/j.phytochem.2003.07.001

[31] LANSKY E. P., HARRISON G., FROOM P., and JIANG W. G. Pomegranate (Punica granatum) pure chemicals show possible synergistic inhibition of human PC-3 prostate cancer cell invasion across Matrigel. *Investigational New Drugs*, 2005, 23(2): 121–122. https://doi.org/10.1007/s10637-005-5856-7

[32] AMAKURA Y., OKADA M., TSUJI S., and TONOGAI Y. High-performance liquid chromatographic determination with photodiode array detection of ellagic acid in fresh and processed fruits. *Journal of Chromatography A*, 2000, 896 (1–2): 87–93. https://doi.org/10.1016/s0021-9673(00)00414-3

[33] YEHIA H. M., ELKHADRAGY M. F., and ABDEL MONEIM A. E. Antimicrobial activity of pomegranate rind peel extracts. *African Journal of Microbiology Research*, 2011, 4(22): 3664–3668. https://doi.org/10.5897/AJMR11.431

[34] ABDEL MONEIM A. E., & EL-KHADRAGY M. F. The potential effects of pomegranate (Punica granatum) juice on carbon tetrachloride-induced nephrotoxicity in rats. *Journal of Physiology and Biochemistry*, 2013, 69(3): 359–370. https://doi.org/10.1007/s13105-012-0218-3

[35] KUMAR S., NIRALA J. P., BEHARI J., and PAULRAJ R. Effect of electromagnetic irradiation produced by 3G mobile phone on male rat reproductive system in a simulated scenario. *Indian Journal of Experimental Biology*, 2014, 52: 890-897. http://nopr.niscair.res.in/bitstream/123456789/29335/1/IJEB%2052%289%29%20890-897.pdf

[36] KHAYYAT L. I. The histopathological effects of an electromagnetic field on the kidney and testis of mice. *EurAsian Journal of BioSciences*, 2011, 5: 103-109. https://doi.org/10.5053/ejobios.2011.5.0.12

[37] KUMAR P., & SHUKLA V. Ultrastructural changes in rat testicular tissue after whole body exposure to electromagnetic radiation emitted from mobile phones. *Journal of International Academic Research for Multidisciplinary*, 2014, 2(1): 518-526. https://www.semanticscholar.org/paper/ULTRASTRUCTURAL-CHANGES-IN-RAT-TESTICULAR-TISSUE-TO-Kumar-Shukla/2da274ddf9501533c7a2c0388fe820f53dfcd3e1

[38] MAHMOUD S. A., & SOLAIMAN A. A. Histological study of adult male rat seminiferous tubules following triclosan administration and the possible protective role of pomegranate juice. *Egyptian Journal of Histology*, 2014, 37(2): 233-247.

[39] DARE B. J., CHUKWU R. O., OYEWOPO A. O., MAKANJUOLA V. O., OLAYINKA P. O., AKINRINADE I. D., OLANIYAN T. O., OMOTOSO O. D., SHAFE M. O., OGUNDELE O. M., BEREMAKO O. O., and ADEH M. N. Histological integrity of the testis of adult Wistar rats (Rattus novergicus) Treated with Garcinia kola. *Reproductive System & Sexual Disorders*, 2012, 1(4): 1000113. https://doi.org/10.4172/2161-038X.1000113

[40] ULUAYDIN N. K., CEREZCI O., and SEKER S. S. Can Mobile Phone Usage Affect Hypothalamus-Pituitary-Adrenal Axis Response. Proceedings of the 10th Annual Computing and Communication Workshop and Conference, Las Vegas, 2020, pp. 780-783.

https://doi.org/10.1109/CCWC47524.2020.9031168
[41] QASIM S. A., & ALI A. S. Effect of Pomegranate Juice and Fresh Leaves of Eruca vesicaria on Testosterone Hormone Level in Blood Serum of Male Rabbits. *Systematic Reviews in Pharmacy*, 2020, 11(10): 1211-1214.

参考文:

[1] ABDOLMALEKI A., SANGINABADI F., RAJABI A., 和 SABERI R. 电磁波暴露对血液参数的影响。国际血液学-肿瘤学和干细胞研究杂志, 2013, 6: 13-16. https://ijhoscr.tums.ac.ir/index.php/ijhoscr/article/view/329/315
[2] BIN-MEFERIJ M. M., 和 EL-KOTT A. F. 辣木对手机电磁辐射致大鼠不育的辐射防护作用。国际临床与实验医学杂志, 2015, 8: 12487–12497. https://pubmed.ncbi.nlm.nih.gov/26550159/
[3] KESARI K. K., KUMAR S., 和 BEHARI J. 手机射频电磁波暴露对雄性维斯塔大鼠生殖模式的影响。应用生物化学和生物技术, 2011, 164(4): 546-559. https://doi.org/10.1007/s12010-010-9156-0
[4] KUMAR S., KESARI K. K., 和 BEHARI J. 评估微波暴露后雄性维斯塔大鼠的遗传毒性作用。印度临床与实验生物学杂志, 2010, 48(6): 586-592. https://pubmed.ncbi.nlm.nih.gov/20882761/
[5] 世界卫生组织. 人类精液检查和处理的实验室手册。世界卫生组织，日内瓦, 2010.
[6] JEDRZEJOWSKA R. W., WOLSKI J. K., 和 HILCZER J. S. 氧化应激和抗氧化剂在男性生育中的作用。中欧泌尿外科杂志, 2013, 66(1): 60–67. https://doi.org/10.5173/ceju.2013.01.art19
[7] ESTEVES S. C., MIYAOKA R., 和 AGARWAL A. 不育男性临床评估的更新。诊所, 2011, 66(4): 691–700. https://doi.org/10.1590/S1807-59322011000400026
[8] LA VIGNERA S., CONDORELLI R. A., VICARI E., D'AGATA R., 和 CALOGERO A. E. 接触手机对男性生殖的影响：文献综述。男科杂志, 2012, 33(3): 350-356. https://doi.org/10.2164/jandrol.111.014373
[9] DEEPINDER F., MAKKER K., 和 AGARWAL A. 手机与男性不育症：剖析关系。生殖生物医学在线, 2007, 15(3): 266-270. https://doi.org/10.1016/s1472-6483(10)60338-0
[10] KAUR G., JABBAR Z., ATHAR M., 和 ALAM M. S. 石榴花提取物具有有效的抗氧化活性，并消除了三乙酸铁对小鼠的肝毒性。食品和化学毒理学, 2006, 44(7): 984-993. https://doi.org/10.1016/j.fct.2005.12.001
[11] SEERAM N. P., ADAMS L. S., HENNING S. M., NIU Y., ZHANG Y., NAIR M. G., 和 HEBER D. 安石榴苷、鞣花酸和石榴单宁总提取物与石榴汁中发现的其他多酚结合，可增强体外抗增殖、凋亡和抗氧化活性。营养生物化学杂志, 2005, 16(6): 360–367. https://doi.org/10.1016/j.jnutbio.2005.01.006
[12] AVIRAM M., DORNFELD L., KAPLAN M., COLEMAN R., GAITINI D., NITECKI S., HOFMAN A., ROSENBLAT M., VOLKOVA N., PRESSER D., ATTIAS J., HAYEK T., 和 FUHRMAN B. 石榴汁黄酮类化合物抑制低密度脂蛋白氧化和心血管疾病：在动脉粥样硬化小鼠和人类中的研究。正在试验和临床研究的药物, 2002, 28: 4962. https://pubmed.ncbi.nlm.nih.gov/12224378/
[13] LANSKY E. P., 和 NEWMAN, R. A. 石榴及其预防和治疗炎症和癌症的潜力。民族药理学杂志, 2007, 109: 177−206. https://doi.org/10.1016/j.jep.2006.09.006
[14] LEE J., 和 WATSON R. R. 石榴：在促进健康和艾滋病方面的作用？在：WATSON R. R. (编辑)，艾滋病中的营养素和食物。化学橡胶公司出版社，博卡拉顿, 1998: 179–192. https://arizona.pure.elsevier.com/en/publications/pomegranate-a-role-in-health-promotion-and-aids
[15] ROSENBLAT M., HAYEK T., 和 AVIRAM M. 糖尿病患者食用石榴汁对血清和巨噬细胞的抗氧化作用。动脉粥样硬化, 2006, 187: 363−371. https://doi.org/10.1016/j.atherosclerosis.2005.09.006
[16] KIM M. M., 和 KIM S. 含有石榴提取物的改善口腔卫生的组合物。韩国专利, 2002: 2002066042.
[17] AMORIM L. F., CATANHO M. T., TERRA D. A., BRANDAO K. C., HOLANDA C. M., JALES-JUNIOR L. H., BRITO L. M., GOMES M. L., DE MELO V. G., BERNARDO-FILHO M., 和 CAVALCANTI JALES R. L. 评估石榴（石榴）对维斯塔大鼠放射性药物高锝酸钠 (99mTc) 的生物利用度的影响。细胞和分子生物学, 2003, 49: 501–507. https://pubmed.ncbi.nlm.nih.gov/12899440/
[18] TURK G., SONMEZ M., AYDIN M., YUCE A., GUR S., YUKSEL M., AKSU E. H., 和 AKSOY H. 石榴汁摄入对雄性大鼠精子质量、生精细胞密度、抗氧化活性和睾酮水平的影响。临床营养, 2008, 27(2): 289-296. https://doi.org/10.1016/j.clnu.2007.12.006
[19] ROZENBERG O., HOWELL A., 和 AVIRAM M. 石榴汁糖分降低巨噬细胞氧化状态，而白葡萄汁糖分增加它。动脉粥样硬化, 2006, 188(1): 68–76. https://doi.org/10.1016/j.atherosclerosis.2005.10.027
[20] ADAMS L. S., SEERAM N. P., AGGARWAL B. B., TAKADA Y., SAND D., 和 HEBER D. 石榴汁、总石榴鞣花单宁和安石榴苷可抑制结肠癌细胞中的炎症细胞信号传导。农业与食品化学杂志, 2006, 54(3): 980–985. https://doi.org/10.1021/jf052005r
[21] MALIK A., AFAQ F., SARFARAZ S., ADHAMI V. M., SYED D. N., 和 MUKHTAR H. 石榴汁用于前列腺癌的化学预防和化学治疗。美国国家科学院院刊, 2005, 102(41): 14813–14818. https://doi.org/10.1073/pnas.0505870102
[22] BANAVATH A. N., 和 SRINIVASA S. N. 石榴对暴露于移动无线电电磁辐射的大鼠精子参数的改善作用。亚太生殖杂志, 2021, 10(5): 225. https://doi.org/10.4103/2305-0500.326720
[23] PENNA-VIDEAU S., CERMENO-VIVAS J., MORENO-MARVAL M., 和 QUIROGA-NOTTI M. 施用提图斯诱导的白化病小鼠睾丸的组织病理学变化。sp。毒液。有毒动物和毒素杂志, 2000, 6(2): 167-179. https://doi.org/10.1590/S0104-79302000000200003

[24] SAROOKHANI M. R., ASIABANHA REZAEI M., SAFARI A., ZAROUSHANI V., 和 ZIAEIHA M. 950兆赫磁场（手机辐射）对雄性家兔性器官和肾上腺功能的影响。非洲生物化学研究杂志, 2011, 5: 77-80. http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.916.5265&rep=rep1&type=pdf

[25] AGARWAL A., SINGH A., HAMADA A., 和 KESARI K. 手机男性不育症：对近期技术创新成果的回顾。国际巴西泌尿外科杂志, 2011, 37: 432-454. https://doi.org/10.1590/s1677-55382011000400002

[26] OZGUNER F., ALTINBAS A., OZAYDIN M., DOGAN A., VURAL H., KISIOGLU A. N., CESUR G., 和 YILDIRIM N. G. 手机诱导的心肌氧化应激：新型抗氧化剂咖啡酸苯乙酯的保护作用。毒理学和工业健康, 2005, 21: 223-230. https://doi.org/10.1191/0748233705th228oa

[27] AGARWAL A., DEEPINDER F., SHARMA R. K., RANGA G., 和 LI J. 手机使用对不孕症诊所男性精液分析的影响：一项观察性研究。生育和不育, 2008, 89: 124-128. https://doi.org/10.1016/j.fertnstert.2007.01.166

[28] BAHARARA J., ZAFAR-BALANEJAD S., KAMAREH E., 和 ASADI-SAMANI M. 绿茶提取物对低频电磁场致骨髓巴尔布/C小鼠胚胎致畸的影响。草药药理学杂志, 2014, 3: 47-51. http://herbmedpharmacol.com/Article/JHP_20150527124342

[29] SCHUBERT S. Y., LANSKY E. P., 和 NEEMAN I. 石榴籽油和发酵汁类黄酮的抗氧化和类花生酸酶抑制特性。民族药理学杂志, 1999, 66(1): 11–17. https://doi.org/10.1016/s0378-8741(98)00222-0

[30] VAN ELSWIJK D. A., SCHOBEL U. P., LANSKY E. P., IRTH H., 和 VAN DER GREEF J. 使用质谱联用在线生化检测快速去除石榴（石榴）中的雌激素化合物。植物化学, 2004, 65(2): 233–241. https://doi.org/10.1016/j.phytochem.2003.07.001

[31] LANSKY E. P., HARRISON G., FROOM P., 和 JIANG W. G. 石榴纯化学品显示出可能对人前列腺癌3细胞通过基质胶的侵袭具有协同抑制作用。研究性新药, 2005, 23(2): 121–122. https://doi.org/10.1007/s10637-005-5856-7

[32] AMAKURA Y., OKADA M., TSUJI S., 和 TONOGAI Y. 采用光电二极管阵列检测的高效液相色谱法测定新鲜和加工水果中的鞣花酸。 色谱杂志一种, 2000, 896 (1–2): 87–93. https://doi.org/10.1016/s0021-9673(00)00414-3

[33] YEHIA H. M., ELKHADRAGY M. F., 和 ABDEL MONEIM A. E. 石榴皮提取物的抗菌活性。非洲微生物学研究杂志, 2011, 4(22): 3664–3668. https://doi.org/10.5897/AJMR11.431

[34] ABDEL MONEIM A. E., 和 EL-KHADRAGY M. F. 石榴（石榴）汁对四氯化碳诱导的大鼠肾毒性的潜在影响。生理与生物化学杂志, 2013, 69(3): 359–370. https://doi.org/10.1007/s13105-012-0218-3

[35] KUMAR S., NIRALA J. P., BEHARI J., 和 PAULRAJ R. 模拟场景下3G手机产生的电磁辐射对雄性大鼠生殖系统的影响。印度实验生物学杂志, 2014, 52: 890-897. http://nopr.niscair.res.in/bitstream/123456789/29335/1/IJEB%2052%289%29%20890-897.pdf

[36] KHAYYAT L. I. 电磁场对小鼠肾脏和睾丸的组织病理学影响。欧亚生物科学杂志, 2011, 5: 103-109. https://doi.org/10.5053/ejobios.2011.5.0.12

[37] KUMAR P., 和 SHUKLA V. 全身暴露于手机电磁辐射后大鼠睾丸组织的超微结构变化。多学科国际学术研究杂志, 2014, 2(1): 518-526. https://www.semanticscholar.org/paper/ULTRASTRUCTURAL-CHANGES-IN-RAT-TESTICULAR-TISSUE-TO-Kumar-Shukla/2da274ddf9501533c7a2c0388fe820f53dfcd3e1

[38] MAHMOUD S. A., 和 SOLAIMAN A. A. 三氯生给药后成年雄性大鼠曲细精管的组织学研究以及石榴汁可能的保护作用。埃及组织学杂志, 2014, 37(2): 233-247.

[39] DARE B. J., CHUKWU R. O., OYEWOPO A. O., MAKANJUOLA V. O., OLAYINKA P. O., AKINRINADE I. D., OLANIYAN T. O., OMOTOSO O. D., SHAFE M. O., OGUNDELE O. M., BEREMAKO O. O., 和 ADEH M. N. 用藤黄果处理的成年维斯塔大鼠(褐家鼠)睾丸的组织学完整性。生殖系统和性障碍, 2012, 1(4): 1000113. https://doi.org/10.4172/2161-038X.1000113

[40] ULUAYDIN N. K., CEREZCI O., 和 SEKER S. S. 手机使用会影响下丘脑-垂体-肾上腺轴反应吗？第10届年度计算与通信研讨会和会议论文集，拉斯维加斯，2020年，第 780-783 页. https://doi.org/10.1109/CCWC47524.2020.9031168

[41] QASIM S. A., 和 ALI A. S. 石榴汁和杜鹃鲜叶对雄性家兔血清睾酮激素水平的影响。药学系统评价, 2020, 11(10): 1211-1214.