AMELIORATIVE EFFECT OF PUNICA GRANATUM ON TESTIS IN SPRAGUE DAWLEY RATS EXPOSED TO MOBILE PHONE RADIO FREQUENCY ELECTROMAGNETIC RADIATION

THESIS SUBMITTED

TO

SRI DEVARAJ URS ACADEMY OF HIGHER EDUCATION AND RESEARCH

For Awarding the Degree as

DOCTOR OF PHILOSOPHY

IN MEDICAL ANATOMY

Under Faculty of Medicine

By

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April 2022

DECLARATION BY CANDIDATE

I, B. Anjaneyababu Naik, declare that thesis titled: **Ameliorative effect of Punica granatum on Testis in Sprague Dawley rats exposed to Mobile phone Radio-Frequency Electromagnetic Radiation,** is a original research work carried out by me for the award of **Doctor of Philosophy** in Medical Anatomy.

This study was carried under the supervision of **Dr. Sridevi N S,** Professor and Head, Department of Anatomy, Sri Devaraj Urs Medical College, A Constituent of Sri Devaraj Urs Academy of Higher Education and Research, Tamaka, Kolar, Karnataka.

No part of this has formed the basis for the award of any degree of fellowship previously elsewhere.

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Study was carried under the supervision of **Dr. Sridevi N S,** Professor and Head Department of Anatomy, Sri Devaraj Urs Medical College, A Constituent of Sri Devaraj Urs Academy of Higher Education and Research.

Any part of this thesis has not been submitted elsewhere for the award of any degree of fellowship previously.

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B.Anjaneyababu Naik

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1.13. KEY FACTS ABOUT THE TESTES

Function	Production of sperm and androgens (testosterone)
Scrotum	Superficial layer: skin Deep layer: dartos fascia (muscle fibers)
Protective layers of testes (superficial to deep)	External spermatic fascia Cremaster muscle Internal spermatic fascia Tunica vaginalis with parietal and visceral layers
Structure of the testes	External layer: tunica albuginea Internal structure: lobules with a tubular system
Histology	Spermatogenic cells: cells in all phases of spermatogenesis Leydig cells: secrete testosterone Sertoli cells: blood-testis barrier, support and nurture maturing spermatogenic cells
Duct system	Convoluted seminiferous tubules → straight seminiferous tubules → rete testis → efferent ductules → epididymis → ductus deferens → ejaculatory duct → prostatic urethra
Arterial supply	Testicular artery
Venous drainage	Pampiniform plexus and testicular vein
Lymphatic drainage	Pre-aortic lymph nodes
Innervation	Testicular plexus
Clinical relations	Cryptorchidism, atrophy, hematocele, torsion, hydrocele, chylocele and testicular tumors

1.14. Histology of testes

The testes are source of gametes and steroid sex hormones. It is encapsulated by the fibrous tunica albuginea and tunica vasculosa. Septa extending inwards from the tunica albuginea partition the gland into lobules. The bulk of the gland is composed of the seminiferous tubules, in which sperm develop.

1.14.1. Seminiferous tubules

Spermatozoa are produced in the germinal epithelium of the seminiferous tubules and released into the lumen of these ducts. The germinal epithelium contains both Sertoli cells and the developing spermatocytes. Sertoli cells extend from the basement membrane of the germinal epithelium to the lumen of the tubule. These cells envelope the developing sperm cells. They are joined to one another by junctional complexes and form the blood-testis barrier. These are the Leydig cells which produce and release testosterone. Myoid cells surround the tubules and generate rhythmic contractions to propel spermatozoa and fluid [24].

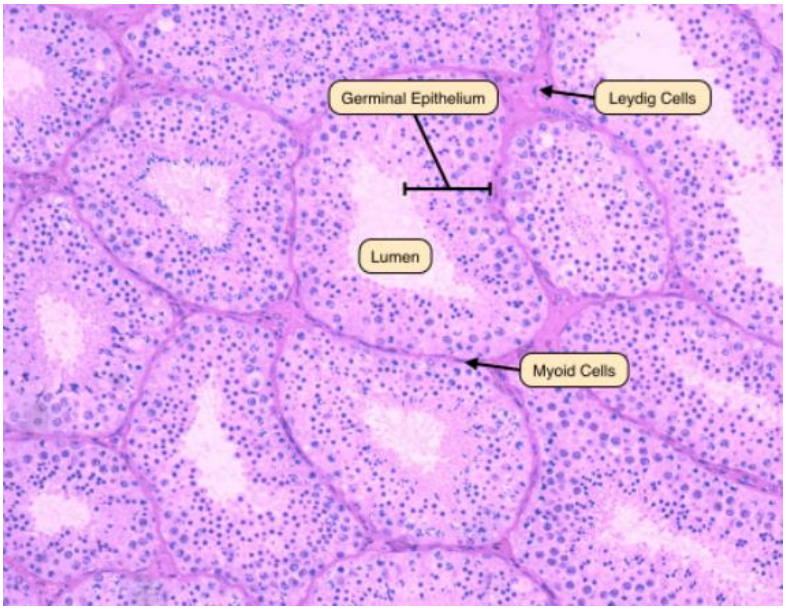

Figure 12: Image of seminiferous tubules (STs) section stained with H& E.

1.14.2. Spermatogenesis

The epithelium rests on a basement membrane and surrounds a lumen where sprematozoa are released. Identify the spermatogonia, located in the basal compartments of both membranes. These cells appear round and pale, with prominent nucleoli. Sertoli cells, with their characteristic oval-shaped nuclei, are also visible. These provide support to the developing primary spermatocytes, which have large, granulated nuclei that are preparing for the first meiotic division. Secondary spermatocytes, which contain 23 pairs of chromatids, are rarely visible [24].

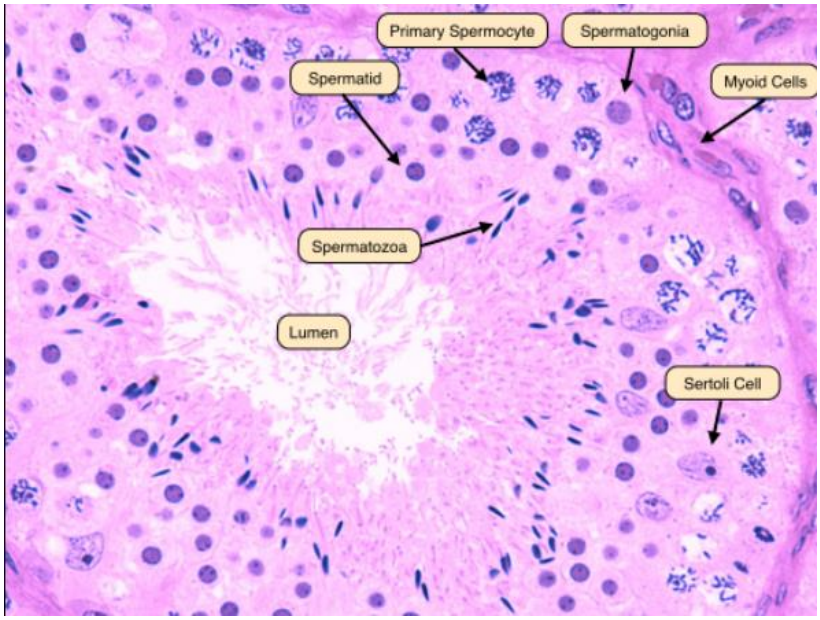

Figure 13: Image of spermatogenesis section stained with H& E.

1.14.3. Leydig cells

Interstitial or Leydig cells are located in the connective tissue surrounding the seminiferous tubules. They produce testosterone, the male sex hormone responsible for the growth and maintenance of the cells of the germinal epithelium and the development of secondary sex characteristics [18].

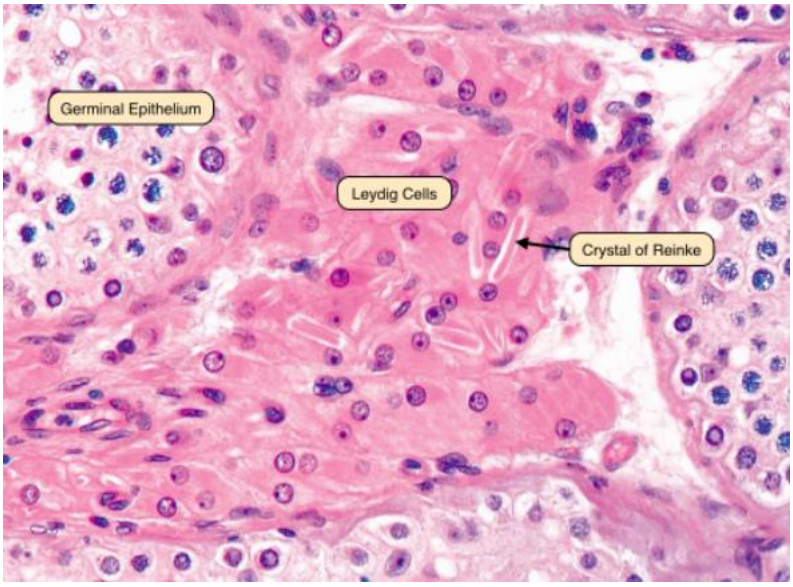

Figure 14: Image of Intestinal cells of Leydig testicular section stained with H& E.

1.14.4. Sertoli cells

Sertoli cells are located in the germinal epithelium and play a supportive role in the development of spermatozoa. These cells have abundant cytoplasm and extend from the basement membrane to the lumen. Sertoli cells have a characteristic oval nucleus with a dark nucleolus [24].

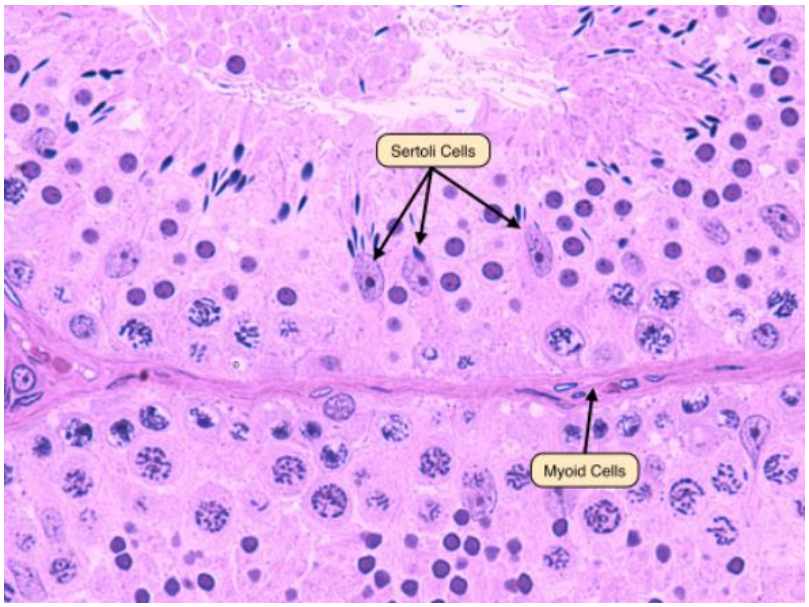

Figure 15: Image of Sertoli cells testicular section stained with H& E.

1.15. Pomegranates

The pomegranate (Punica granatum L), an ancient, mystical, and distinctive fruit, is the prominent member of the Punicaceae family, which consists of two species. Pomegranate fruit is used as an antiparasitic agent and to repair ulcers, and diarrhoea in Ayurvedic medicine. Pomegranate fruit is also used as a diabetic remedy in the Unani school of medicine, which is practised in the Middle East and India [19]. Pomegranate is also known for its cardioprotective, hepatoprotective, [20] anti-atherosclerotic, anti-diabetic, anti-obesity [21] anti-cancer [22] anti-arthritic [23] and anti-inflammatory [24] properties. Commercial whole fruit Pomegranate juice has much higher polyphenolic content and, as a result, antioxidant activity than aril juice.

Pomegranates have been shown to contain a total of 124 phytochemicals, which are classified as polyphenols and include tannins, flavonoids, alkaloids, and organic acids. Unsaturated fatty acids such as punicic acid, linoleic acid, oleic acid, palmitic acid, stearic acid, and linolenic acid are abundant in the seeds, accounting for 15.26% of the total weight of the seeds. The juice is primarily composed of straight chain fatty acids, the most prominent of which are citric acid and malic acid, which have concentrations of up to 4.85 and 1.75 g/L, respectively. Tartaric acid, oxalic acid, and succinic acid were also discovered in the juice. Punicalagin, the most abundant ellagitannin in pomegranates, is responsible for a wide range of health benefits. Since 1990, this antioxidant, which is now synonymous with pomegranate, has been the focus of a slew of medical studies. Pomegranates have a higher total polyphenolic content and antioxidant activity than other regular fruit juices, such as grape, cranberry, orange, and apple, as well as green tea and red wine. Punicalagins are thought to be the main component responsible for the anti-oxidant properties of pomegranates [19].

1.16. Bioactivities of pomegranate

Pomegranates have a variety of beneficial effects which are attributable to its most abundant ellagitannin, punicalagin. This antioxidant now synonymous with pomegranate has been the subject of numerous medical research studies since 1990. Pomegranates have higher total polyphenolic content and greater antioxidant activity than the other commonly consumed fruit juices including grapes, cranberry, orange and apple and for that matter even more than green tea and red wine. Punicalagins are considered to be the major component responsible for pomegranate's anti-oxidant property [25].

1.17. Antioxidant activity of Pomegranate juice

Antioxidant activity is the most important bioactivity of pomegranate and forms the basis of other activities such as cardioprotective, anti-atherosclerotic, anti-inflammatory, anti-diabetic, and anti-cancer, many of the papers published on it in the last decade have focused on it in vitro, ex vivo, and in vivo. Commercial whole fruit Pomegranate juice has much higher polyphenolic content and, as a result, antioxidant activity than aril juice. Pomegranate made up 62.8 percent of the overall phenolic content, whereas other hydrolysable tannins (HT) made up 16.8 percent, accounting for 78.5 percent of the antioxidant activity. Punicalagins have been shown to protect lipids, proteins, and DNA from oxidative damage through a variety of methods, including free radical scavenging, electron transfer to repair oxidatively damaged components, and metal ion chelation [19].

Peroxidation is a sort of oxidising agent that is particularly reactive. Although the production of ROS is a natural process in various organs, including the testis, alterations in their synthesis cause cellular oxidation and DNA damage. Unsaturated fatty acids abound in the plasma membranes of sperm. As a result, oxidative stress is a greater threat. In spermatozoa

cells, lipid peroxidation alters the liposome structure, which is linked to velocity loss and poor membrane integrity. Antioxidants, in theory, are chemicals that neutralise, scavenge, and reduce the generation of reactive oxygen species (ROS) and lipid peroxidation [26].

2. REVIEW OF LITERATURE

2.1. Review of literature about histological integrity of sertoli cells and leydig cells on RF-EMR

In 2011 S. Mustafa, et al, conducted a study to the goal of this research was to examine the effect of electrical waves at 2450 MHz on apoptosis and interstitial fibrosis in rat testicular tissue. The current study used male Wistar rats that were 12 weeks old. We randomly allocated eighteen rats to one of three groups: I, II, or III. The individuals were placed into three groups: cage control (group I), fake control and EMR at 2.45 GHz (group III) (group III). Group III was exposed to 2.45 GHz EMR for 60 minutes each day for 28 days at an absorption rate of 3.21 W/kg. When rats from group III were matched to rats from group II, I, the amount of Leydig cells in testis tissue was considerably lower (p 0.05). The Johnsen sperm biopsy score was used to estimate spermatogenesis and found a statistically significant difference between groups. caspase-3, and Bcl-2 levels were evaluated across groups, and no statistically significant difference was seen ($p < 0.05$). Electromagnetic fields have an effect on spermatogenesis and induce apoptosis in testis tissue as a result of heat and other stressors [27].

In 2011 Eman Abbas Farag, et al, conducted a study on the consequences of mobile phone usage on adult rats' testes, the progression of spontaneous recovery, and the protective effect of NG supplements against these effects. The research found that the mobile group had a degraded histological architecture of the testes, as well as biochemical and morphometric characteristics. However, group IV demonstrated retention of the testicular enable the creation and restoration of normal metabolic and morphometric parameters. But at the other hand, the support group had insufficient improvement, as shown by some testicular discomfort [28].

In 2003 Shui Ming, et al, conducted a study to investigate the impact of EMP irradiation has a detrimental influence on the system and functional properties of Leydig murine. A total of 155 male Pekin mice were randomly assigned to receive either irradiation or therapy. The former were subjected to five EMP pulses of 8 x 10(3), 2 x 10(4), or 6 x 10(4) V/m during a two-hour period. Light and scanning electron were used to investigate the pathogenic alterations in Leydig cells. 6 months, day, 4 days, seven days, fourteen days, and twenty-eight days after irradiation, serum testosterone (T), folliculogenesis protein (LH), and oestrogen (E2) levels were dynamically determined. Within 28 days of EMP radiation, the primary pathogenic alterations detected were edoema and vacuolation, increased cytoplasmic mitochondria, decreased lipid droplets, pale labeling of the predominance of lipid rafts, and partial or total rupture of vacuoles in Leydig cells. Serum T declined in all subjects to varying degrees during a 28-day period after EMP treatment at 8 x 10(3), 2 x 10(4), and 6 x 10(4) V/m, and notably at 6 h-14 d, 6 h-7 d, and 1 d-28. No measurable impact of EMP irradiation was seen on serum LH or E2. RF-EMR treatment mice may cause significant damage to the structure the function of LH stimulated cells in mice, affecting sexual function and sperm count in the long term [29].

2.2. Review of literature about histological integrity of sertoli cells and leydig cells on pomegranate juice

In 2015 Mohammed, et al, has studied the histologic approach, the protective effect of PJ versus streptozetocin induced diabetic on the structure of the rat testis. In diabetic rats, weight gain, testis weight, diameter of seminiferous tubules, and epithelial height were dramatically decreased. PJ increased the spermatozoa inside the seminiferous tubules. Depletion of all germinal cells were marked in untreated diabetic rats while the interstitial connective tissues were wide. Electron microscopic examination revealed vacuolations and degeneration in the

spermatogenic and Sertoli cells. Basement membrane was irregular and thickened with deposition of collagen fibers [30].

In 2017 Yassein, et al, the purpose of this study was to determine the impact of PJ on the testis of an old albino rat. PJ is a potent antioxidant that is used to treat a variety of medical conditions. The rats were randomly assigned to three groups of ten for the treatment group and fifteen for the other two groups. For 52 days, animals in (group I) and (group II) received pure water by gavage. Wherein [group III] animals received PJ through gavage daily for 52 days at a dosage of 10 ml/kg. The PJ group displayed images that were remarkably identical to those of the control group. Thus, it is seen that pomegranate juice has a favourable impact on testis in old rats, which may make this one of the most significant meals in the future[31].

2.3. Review of literature about seminal parameters [Sperm count, sperm motility, sperm morphology, sperm viability, sperm progressivity] on RF-EMR

In 2018 KK Kesari, et al, focused by examining the effect of exposure to uses RF-EMR on male fertility patterns, We will attempt to focus on energy emitted by MP, computers, Wi-Fi, and cookers, since these are the most suitable source of quasi cosmic rays that may help to infertility amplification. RF-EMF have been demonstrated to have a deleterious influence on sperm count [such as sperm count, appearance, and motility], disrupt the operation of signalling pathways in the mitochondria and endocrine system, and induce genotoxicity, chromosomal aberrations, and oxidative stress. This is followed by advice on future defences against these radiations. This result was obtained after in vitro and in vivo studies indicated that RF-EMF exposure had a deleterious impact on sperm quality [32].

In 2002 Mehardad D, et al, evaluated electromagnetic waves (EMW) emitted by mobile phones have an effect on sperm motility. The first and subsequent spermiogram parameters were compared. The number of rapidly progressing spermatozoa was significantly reduced in

the third spermiogram especially in comparison to the first. The decrease was from 32.3 percent (mean) to 26.1 percent. Additionally, there was an increase in progressive spermatozoa from a mean of 24.8 to 29.7 percent. All other spermiogram measures, such as semen volume, density, and morphology, were insignificantly different. The research revealed that electromagnetic waves from GSM-MP reduced the motility of fast progressing spermatozoa. These research results may have a bearing on how sub-fertile men are counselled [33].

In 2021 Darvish, et al, has studied the men's infertility was explored in relation to the influence of radiofrequency radiation. Final evaluations were conducted on a total of 14 papers that fulfilled the inclusion criteria. Radiofrequency waves were discovered to have an effect on motile sperm, seminal vitality, membrane permeability, appearance, volume, spermatozoa count, spermatozoa, and sperm fertility. The findings indicated that RF has a harmful impact on semen parameters, and given the present growth in RF wave usage and its involvement in male infertility, it is vital to educate men about the unfavourable consequences of RF-EMR. Additional research is required to develop less dangerous gadgets [34].

In 2017 Oyewopo, et al, the purpose of this study is to determine the impact of mobile phone radiation on testicular biologists and biochemical analysis. Histo-morphometry, biochemical analysis, and histological examinations were performed. Histo-morphometric measurements revealed, there was no significant change in the width of the germinal membrane between the experimental group and control group [p.05]. There was no significant difference in cross-sectional diameter between any of the experimental groups and the control group [p.05]. Group D rats showed a significant decrease ($p < .05$) in lumen diameter compared with group B rats. There was an uneven distribution of germinal epithelial cells in groups B, C and D. However, there was degeneration of the epithelia cells in group D when compared to the

control and group B rats. Also sera levels of gonadotropic hormones [FSH, LH and testosterone] significantly decreased ($p < .05$) in groups C and D compared with the control group [35].

2.4. Review of literature about seminal parameters [Sperm count, sperm motility, sperm morphology, sperm viability, sperm progressivity] on ameliorative effect of Pomegranate juice

In 2020 Mohsen, et al, determined how PJ reacts to aluminium-induced oxidative stress in male mice. Al lowered the weights of the body and organs, sexual behaviour, sperm concentration, normal sexual, total and progress sperm mobility, testosterone level, and seminiferous tube epithelial diameters. Additionally, the PJ decreased these effects and aluminium toxicity in male rats. In conclusion, PJ rescues male mice from aluminium exposure by enhancing sexual behaviour and fertility [36].

In 2021 Concetta Levine, et al, the purpose of this study was to determine ellagic acid's antigenotoxic activity. Ellagic acid is a naturally occurring polyphenolic molecule with potent antigenotoxic, antiinflammatory, and antiproliferative properties. By digesting normozoospermic human sperm tissues in benzene for 45, 60, and 90 minutes, an OS state was established in vitro. The findings demonstrate that flavonoid has a consistent protective impact on DNA repair, as well as sperm vitality and motility, via inhibiting intracellular ROS formation. The findings of this work reveal that ellagic acid is a viable chemical for protecting sperm DNA from oxidative damage, with potentially substantial translational implications for male infertility care [37].

In 2008 Narayanan SN, et al, has studied the spatial memory performance by using the morris water maze test in wistar rats exposed to 50 missed calls/day for 4 weeks from a GSM mobile phone (900-1800 MHZ) kept in the cage in a silent mode, stated that exposure affected the

acquisition of learning responses in wistar rats. This results in poor spatial navigation and object placed configuration in the mobile radiation exposed rats

In 2018 Okechukwu C E al, has studied the exposure system of electromagnetic field was constituted with a signal generator, external oscillator, power amplifier and antenna. An electromagnetic wave of 900 MHz was generated by a signal generator animals were divided into four groups (n = 5). The first group was the control group, the second group was exposed to mobile phone radiation for 6 h daily, the third group was subjected to swimming 3 times a week and more than 30 min each session or more than 90 min a week, and the fourth group containing five rats was exposed to mobile phone radiation for 6 h daily and was subjected to swimming for more than three times a week and more than 30 min each session or more than 90 min a week. This experiment lasted for 30 days. Short-term exposure of male Wistar rats to mobile phone radiation led to a statistically insignificant ($P > 0.05$) decrease in the serum testosterone levels and testicular weight, whereas exercise in male Wistar rats led to statistically nonsignificant ($P > 0.05$) increase in the testosterone levels and testicular weight [38].

2.5. Review of literature about testosterone hormonal levels on RF-EMR

In 20201 Ban Muhammed, et al, purpose of this study effects of RF-EMR emitted by MP and radio broadcasts on testes were examined. For 14 days, pets were treated to RF-EMR from a Samsung Note 9. Biochemical and histopathological analyses were carried out. Male sex hormone serum levels (follicle-stimulating hormone and testosterone) fell considerably (P0.05) in the 1/2 H and 1 H exposure groups compared to the sham-exposed group. The research demonstrates that persistent exposure to radiofrequency electromagnetic radiation from a mobile phone results in altered testicular function and a drop in the value of sexual chemicals. Sperm microscopy also revealed a reduction in the number of sperm, as well as

changes in their form and growth in the experimental groups. Additionally, altering histological parameters resulted in a change in the diameter of the cross-section, luminal, and bacterial epithelium in all experimental groups [39].

In 2013 Massood, et al, purpose of this study analysed the impact of keywords that are relevant the effect of FSH, LH, inhibin B, activin B, oxytocin, and testosterone levels in the blood were used to assess the effect of radiofrequency field [RF-EMF] therapy on the male rat reproductive system. Twenty male adult rats were divided into four groups and subjected to RF-EMF at a frequency of 900 MHz. At the trial's end, the continued exposure (LTE) group had significantly higher levels of FSH and LH than the pack of lies unit (p 0.05). Serum activing levels increased substantially in the LTE group after 30 days of RF-EMF exposure, whereas serum inhibin B levels declined substantially in the sham and brief moment exposed groups. Additionally, following 30 days of RF-EMF exposure, blood testosterone levels declined considerably in the LTE group compared to the short and medium time exposure groups. The findings suggest that exposure to RF-EMF affects reproductive hormone levels and may impair reproductive function. However, libido and inhibin B concentrations were significantly decreased as indications of fertility and spermatogenesis [40].

2.6. Review of literature about testosterone hormonal levels on ameliorative effect of pomegranate juice

In 2014 Olayan, et al, the purpose of this study was to determine the preventive effects of PJ versus oxidative stress and testes damage caused by carbon tetrachloride [CCl4] in adult Wistar rats. In compared to CCl4 injection, pomegranate juice substantially boosted the levels of testosterone, luteinizing hormone [LH], and follicular stimulated hormone [FSH] are determined. Pomegranate juice significantly leads to the activation of endogenous testicular

antioxidant enzyme oxidant, catalase (CAT), peroxidase, thioredoxin, and glutathione s - transferase, while lowering oxidative damage. Additionally, pomegranate juice treatment reversed germ and Leydig cell degeneration, as well as spermatogenesis defects induced by CCl4 injections [41].

In 2008 Turk, et al, investigated the effects of PJ intake on the quality of sperm, the density of spermatogenic cells, antioxidant activity, and testosterone level in male healthy rats were investigated. There was a substantial decrease in malondialdehyde (MDA) levels and a 'significant increase' in glut, peroxidase, cathode (CAT), Vit C levels in rats treated with PJ. When compared to control group, PJ ingestion enhanced epididymal sperm counts, motility, sperm cells cell number and size of tubule, and thickness of the germinal cell layer, while decreasing aberrant sperm rate [25].

2.7. Review of literature about androgen binding protein levels on RF-EMR

In 2019 Ling Guo, et al, evaluated the sperms by counting the amount of sperm cells, determining their abnormalities, and determining their survival rate. The morphology of the testis was determined by hematoxylin–eosin staining. The levels of expression of apoptosis-related protein in the testis were determined by Western blotting. In comparison to the group compared, the RF group had considerably lower sperm quality. After RF irradiation, the amounts of secreting components in SCs and the shape of the testis changed significantly. These results indicate that 220 MHz superheated plasma modulated RF media attention may impair male fertility in raccoons under the prevailing lab conditions, and that the disruption of Letter of credit secreting function and increased apoptosis of testis induced by the RF field may be a result of this detrimental effect [42].

2.8. Review of literature about androgen binding protein [ABP] levels on Pomegranate juice

In 2019 Yofa Sukmawat et al., determined the 30 adults male Wistar rats weighing between 200 and 300 gm, and aging 3 months were taken for this study and were divided into three groups: negative control (NC), positive control (PC), and treatment (T) groups. The PC and T groups were induced by allethrin 12 h per day for 31 days; however, only the T group was given vit E orally at 1 ml/gm body weight (BW) each day for 14 days. The paraffin block method was used to measure tubules' diameter, thickness of the seminiferous epithelial layer, and Sertoli cell number. The ABP levels were measured by enzyme-linked immunosorbent assay. vit E gave significant effect ($p < 0.05$) on tubular diameter at NC 123.67 ± 12.77, PC 147.16 ± 10.64, and T 130.08 ± 10.00; tubular epithelial thickness at NC 33.55 ± 3.21, PC 30.02 ± 1.53, and T 32.96 ± 2.81; Sertoli cells number at NC 55.48 ± 5.9, PC 43.84 ± 3.77, and T 53.44 ± 4.26; and ABP levels at NC 72.35 ± 39.06, PC 38, 48 ± 18.78, and T 86.10 ± 35.77, respectively [43].

In 2015 Rachna Kapoor, et al, determined the Consumption of pomegranate juice had no influence on blood The concentrations of estradiol, estrone, male, oestrogen, or high estrogen globulin (SHBG). 64 For three weeks, healthy postmenopausal ladies were randomly allocated to consume either 8 ounces of 100% fresh grapefruit juice (experimental) or apple juice (control). In general, women in the therapy group did not even see a significant decrease in serum hormonal changes or postprandial blood glucose levels when applied to females in the control group. Subgroup analysis of 38 regular girls found that individuals in the experimental group were significantly more likely to be obese. (pg/mL) and beta (pg/mL) levels than those in the control group [44].

NEED OF THE STUDY

Several ideological studies on effect of radio frequency-electromagnetic radiation (RF-EMR) from mobile phones (MP) on testicular functions (histological integrity of Sertoli cells and interstitial cells Leydig, Seminal parameters, testosterone levels and androgen binding protein levels) in rats, with is a short term (1 month) RF-EMR exposure with is 2G and 3G mobile phones.

There are several studies on ameliorative effect of Pomegranate juice on testicular functions (histological integrity of Sertoli cells and interstitial cells Leydig, Seminal parameters, testosterone hormonal levels and androgen binding protein levels) in rats. So the present study is focused on the combination of Ameliorative effect of Pomegranate juice on testicular Functions in male Sprague Dawley rats long term (3 months) exposed to 4G Mobile phone RF-EMR. This study may help in planning for treatment for infertility caused due to exposure to mobile phones.

AIM

- To study the Effect of Mobile Radio-Frequency Electro-magnetic Radiation (RF-EMR) on Structural Integrity of Sertoli cells, interstitial cells of Leydig, evaluate the expression of ABP levels and TH levels & Ameliorating Effect of Punica Granatum (PJ) in Male S.D Rats.

OBJECTIVES

- To evaluate the effect of Radio-frequency electromagnetic radiation and efficacy of Pomegranate juice on the structural integrity of sertoli cells and interstitial cells of Leydig.
- To evaluate the effect of Radio-frequency electromagnetic radiation and efficacy of Pomegranate juice on the seminal parameters.
- To determine impact of Radio-frequency electromagnetic radiation and efficacy of Pomegranate juice on the serum testosterone levels.
- To evaluate the effect of Radio-frequency electromagnetic radiation and an efficacy of Pomegranate juice on androgen binding protein.

RESEARCH QUESTION

- Is there a mobile RF-EMR effect on testicular functions and Ameliorating effect of pomegranate juice on mobile radio hazards
- Does Mobile RF-EMR affect the semen analysis, histological integrity of Sertoli cells, interstitial cells of leydig, Testosterone hormone levels and expression of Androgen binding protein levels?
- Does Punica Granatum reverse the effect of RF- EMR on semen analysis, histological integrity of Sertoli cells, interstitial cell of Leydig, Testosterone hormone levels and expression of Androgen binding protein levels?

6. 0. MATERIALS AND METHODS

6.1. Study design

This is a Preclinical study / Experimental study among all the parameters in methodology were compared between five groups (each group=6). The study was approved by the Institutional Animal Ethical Committee (IAEC /PHARMA/SDUMC/2018/12a), SDUAHER Tamaka, Kolar. The experiments were performed at 'Central Animal House' Laboratory, SDUMC, Tamaka, Kolar.

6.2. Animals

Thirty adult male Sprague Dawley rats (10-14 weeks old, weighing 150-180 g) were procured from Biogen Laboratory Animal Facility, Bangalore, India. The Sprague Dawley rats were housed in polypropylene cages, measuring 25 × 16 × 13 cms (L×B×H). The experimental rats were maintained under well-ventilated standard laboratory, where the room temperature of (24±2) ^{o}C, with 45% -50% constant humidity and kept in a "12 hours light/12-hour dark" cycle throughout this study. The S.D rats had free access to 'pellets and water' ad libitum. Before beginning of the experiment, the rats were allowed to adapt lab condition for12 days.

6.3. Diet

Rats were fed with standard food (pellets) and water ad libitum. The rat's pellets were procured from 'Champaka feeds & foods' Bengaluru. Standard pellet diet contains Crude protein: 18%, Calcium: 1%, Phosphorous: 0.5%, Crude fibre: 4%, Moisture: 10%, Crude fat: 4% and Nitrogen free extract (NFE): 60 %.

6.4. Bedding Material

Paddy husk were used for bedding material procured from S.N.S Rice mill, 25, KGF road, Bangarpet-563114. Paddy husk will be changed daily and care was taken in a humane manner while handling the rats.

6.5. Sample size

30 rats were included in this study.

6.8. Duration of study

3 years (2018-2021).

6.7. Sample size calculation

The sample size of study was estimated based on outcome variable on serum testosterone levels with minimum difference of 0.17 ng/ml [45], and Standard deviation of 0.145, with 90% statistical power at 5% level of significance.

The total sample size is calculated to be 30 (6/group).

n = 30
d = 0.145
Significance level = 0.05
Power = 90%

- Based on the above calculation, the present study required to enroll approximately 6 rats in each group (Total No 30).

6.8. Inclusion criteria

- Male healthy Sprague-Dawley rats with average weight of 150-180 grams (10-14 weeks old) when procured were included in this study.

6.9. Exclusion criteria

- Female Sprague-Dawley rats and lesser weight (<150) rats were excluded from this study.

6.10. Experimental design

After acclimatization, thirty male S.D rats were allocated into five groups (n=6/group) such as group I: the control group, group II: the mobile RF-EMR group, group III: the mobile RF-EMR + Pomegranate juice group, group IV: the mobile RF-EMR recovery group, and group V: the pomegranate juice group. Group I was neither exposed to RF-EMR nor given pomegranate juice, group II, III and IV were exposed to mobile emitted RF-EMR (800 MHz to 2 400 MHz) for 60 min/day for 90 days [46].

Groups	**Procedure/Technique**	**Mobile RF-EMR Exposure period**		**Recovery period**
		Duration (hour/day:6-7AM)	**No of Months**	**No of Months**
Group - I	Control	-	-	-
Group - II	RF-EMR	1	3	-
Group - III	RF-EMR+PJ	1	3	3
Group - IV	RF-EMR Rec	1	3	3
Group - V	PJ	-	3 (1ml/kg/BW)	-

Table 1: Experimental design (groups). RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, Rec: Recovery.

After 90 days of exposure to RF-EMR, the group III was supplemented with PJ for other 90 days (1 mL/kg/day) [25] and group IV was allowed to recover for 90 days without supplementation of pomegranate juice. Group V was supplemented with pomegranate juice for 90 days without exposure to mobile RF-EMR.

This period (90 days) was required to determine the effect of pomegranate juice on sperm production because the Sprague Dawley rats needed a time period of 2-3 months for the exact spermatogenic cycle including meiosis, spermiogenesis and spermatocytogenesis.

6.11. Mobile phone exposure technique

Mobile RF-EMR exposure technique The 4G mobile phone (Vivo 1803 mobile phone) with a specific absorption rate of 0.53 Watt/kg was hung down from the centre of the rat roof cage at a distance 5 cm between cage floor and mobile phone, and during the exposure period food and water was available ad libitum. Radiation that was emitted during the exposure was quantified by radiation frequency meter (Meco-GRF-EMR) which was kept at the periphery. The control rats were placed in a separate room where there was no exposure of mobile radiofrequency electromagnetic radiation [47].

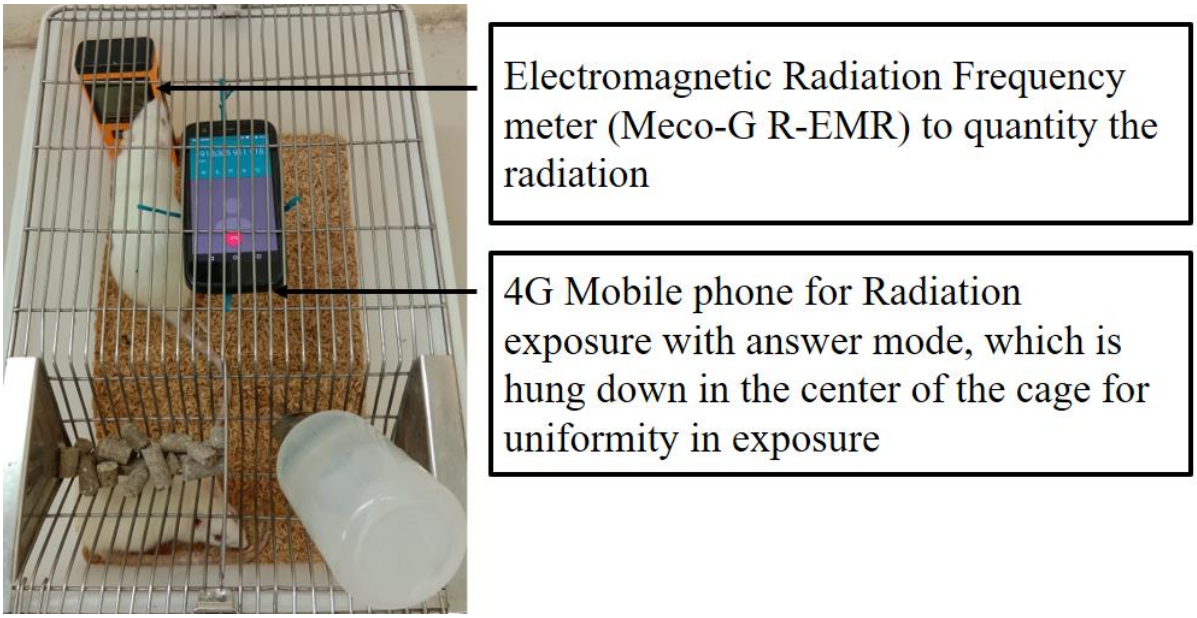

Figure 16: Image shows the cage with rats and mobile phone during RF-EMR exposure with radiofrequency meter (Meco-G RF-EMR) to quantify the radiation. Image captured in Central Animal House Laboratory, SDUMC, Tamaka, Kolar.

6.12. Plant material

Pomegranate fruit (bhagwa variety) was collected from local market in Kolar. The pomegranate fruit material (botanical name: *Punica granatum* L.; voucher number: 0320) was authenticated by Dr. Madhava Chetty K, Associate Professor, Dept. of Botany, Sri Venkateswara University, Tirupathi-517502, Andhra Pradesh, India.

6.13. Preparation of Pomegranate juice

Fresh and healthy pomegranate fruit was used in this study. Pomegranate fruit was washed under tap water and cut into four pieces and manually peeled in aseptic conditions. Pomegranate fruit juice was obtained by using mixer blender (Bajaj GX 11 750W), filtrated with a funnel and juice was immediately poured into dark - bottle wrapped with aluminum foil. Pomegranate juice was given to the mobile RF-EMR + Pomegranate juice group (III) and the pomegranate juice group (V) through oral gavage needle 1 mL/kg/BW/day [48].

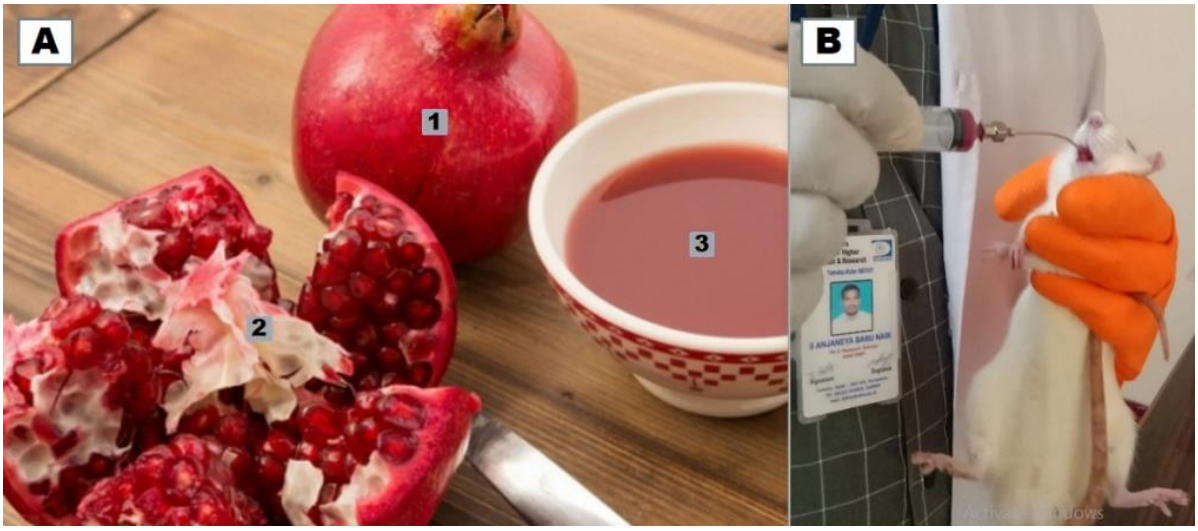

Figure 17: A (1): Pomegranate fruit, A (2): Pomegranate section, A (3): Pomegranate juice. (B) Oral dosing (Gavage) in adult Sprague Dawley rats- Image captured in Central Animal House Laboratory, SDUMC, Tamaka, Kolar.

6.14. Collection of sample

After the MP RF-EMR exposure and co–administration of PJ the rats were anesthetized using a combination of Xylazine (15 mg/kg BW) and ketamine (90 mg/kg, W/kg, IP) [49]. 2.5 ml of blood sample was collected through cardiac puncture and blood samples was collected at a fixed time (between 9 to 10 AM). The samples were allowed to coagulate for 20 minutes. The serum sample were collected by centrifugation at 3000 RPM for 15 minutes at room temperature. The obtained serum sample were stored at -20 $^{\circ}$C for testosterone hormonal analysis. Testes, epididymides, seminal vesicles was harvested *via* centre incisions in the scrotal sac. The cauda epididymis part was dissected and placed in a clean small petri dish for semen analysis. Left testis tissues was fixed in 10 % buffered formalin solution for histological examination and right testis was dissected and fixed in liquid nitrogen and stored at -80 $^{\circ}$C for androgen binding protein analysis.

6.15. Semen analysis methodology

6.15.1. Sperm count

A 1 cm incision was done at the epididymal cauda by using sterilized blade and all the semen was squeezed by using forceps into a petri dish containing 5 ml of 'phosphate buffered saline'. The glass petri dishes were closed and incubated at 34°c for 8-10 minutes to allow for sperm swim-up. The epididymal sperm was transferred to "Neubauer Hemocytometer". The sperm count was counted by using a light microscope (Labomed LX 200 LED) at 40 C magnification [50]. Sperm count = count in 5 squares X dilution factor 400X 06/mL [51].

6.15.2. Sperm motility

Caudal epididymis was mixed with few drops of normal saline. One drop sample was placed on a clean slide and covered with a cover slip. The slide was examined under microscope. The motile and non-motile sperm number was counted in 10 random fields and motility was expressed as a ratio of number of motile sperms to the total number of sperms [50].

6.15.3. Sperm viability

One drop of semen sample were placed on a clean glass slide and one drop of 0.5% eosin solution was added. After 2 min, the slides were observed under a light microscope (40X objective). Percentage of viable sperm (colourless) and non-viable sperm (coloured) was expressed. The relative sperm viability was calculated, total "viable sperms" (unstained) by the total "viable sperms" (stained and unstained) × 100 [52].

6.15.4. Sperm morphology

One drop of 'sperm suspension' was placed on a glass slide, a thin smear were done and few drops of 95% ethanol and smear were allowed to air dry overnight. The slides were stained with Papanicolaou staining technique. The slide was observed under microscope at 400X magnification, to detect the sperm morphological abnormalities which were as follows:

detached head, pyriform head, coiled tail, and multiple abnormalities. For each slide, the % of abnormal sperms was scored from at least 10 fields and 200 sperms [53, 54].

6.15.5. Sperm progressivity

The sperm progressivity was determined by subjecting grading system (grades): 4 or A: Excellent forward directional movement; 3 or B: Good forward direction movement; 2 or C: Fair forward directional movement; 1 or D: Poor forward directional movement, as described by the World Health Organization (2005) [55].

6.16. Histological Methodology

The Sprague dawley rats were decapitated, testis were carefully extracted out and tissues were fixed in 10 % 'buffered formalin solution' and further processed for paraffin blocks for histological examinations.

6.16.1. Principle

The principle behind the H & E stain is the chemical attraction b/w tissue and dye. **Hematoxylin is** a basic dye imparts blue, purple contrast on 'basophilic structures, primarily those containing nucleic acid moeties such as chromtatin, 'ribosomes and cytoplasmic' regions rich in RNA. An acidic **eosin** counterstains the 'basic elements' such as cytoplasm, RBCs, muscle and collagen' in varying intensities of 'pink, orange and red.

6.16.2. Tissue processing

- Processing of the tissue for paraffin blocks- Fixation - 10% Buffered formalin

  Dehydration - A. 50%, 70 %, 90% Ethyl Based Alcohol.

  Clearing - A. Xylene I -1 hour, amd II – 1 hour

  Impregnation - 56^0 C paraffin wax I – half an hour

6.16.3. Embedding/ Blocking

- The tissues samples were embedded with the help of melting wax and 'L-Moulds' allowed the 'warm liquid paraffin' wax (embedding medium) that solidifies into a firm block when it cools down to room temperature, labelled it for further sectioning.
- Embedding process provides support to the tissue to be cut on microtome.

Figure 18: Image represents the Rotary microtome- figure captured in research laboratory, Department of Anatomy, SDUMC, Tamaka, Kolar.

6.16.4. Section cutting

- Embedded tissues were sectioned using a microtome.
- Block were placed in the block holder. Setting the knife in the holder and screw tightly.
- Trimming or shaving the block and cutting the sections to the 4 micrometre thickness.
- 5-10 sections from each block was taken and mounted on a ‘gelatine coated’ slides and then slides were placed on a hot plate for 10-15 minutes [56].

Figure 19: Image represents section cutting- figure captured in research laboratory, Department of Anatomy, SDUMC, Tamaka, Kolar.

6.16.5. H&E Stain procedure

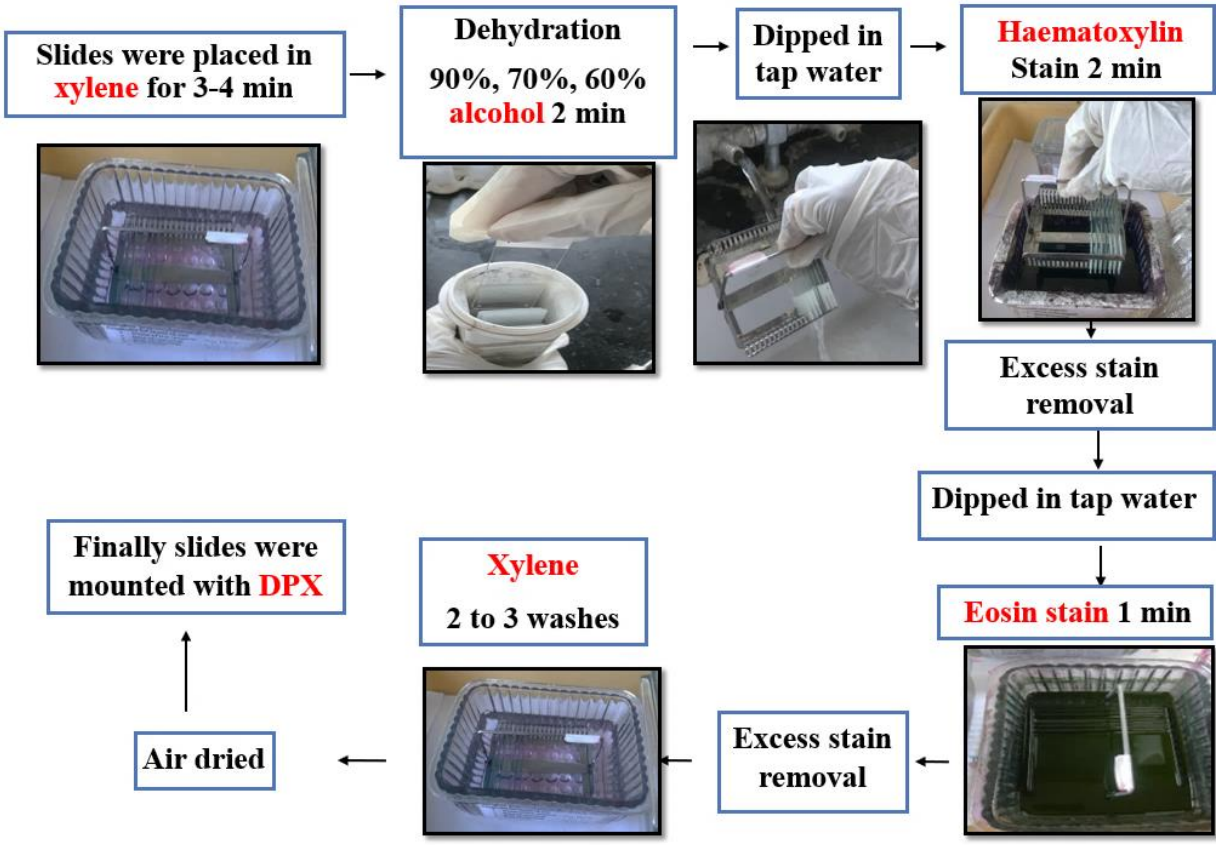

Figure 20: Image represents the H&E stain procedure - Image captured in research laboratory, Department of Anatomy, SDUMC, Tamaka, Kolar.

6.17. Measurement of Testosterone analysis in serum

End of the study period [6 months], the rats was anesthetized using a combination of Xylazine (15 mg/kg BW) and ketamine (90 mg/kg, BW, IP), blood samples was collected (2.5 ml) by cardiac puncture at a fixed time (between 9 to 10 AM). The samples were allowed to coagulate for 20 minutes. The serum sample was separated by centrifugation at

'3000 RPM for 15 minutes' at room temperature. Finally obtained serum sample was stored at -80 °C until testosterone hormonal analysis.

6.17.1. Principle

The Testosterone hormonal (TH) levels ELISA is 'sandwich enzyme-linked immunosorbent assay' to assay the level of Rat TH in samples. Standards are added to the Microtitre well which is pre - coated with Rat TH 'monoclonal Antibody'. Biotinylated Rat TH antibody is 'added to the microplate' to form a complex. Subsequently 'Streptavidin-HRP conjugate' is pipetted. After incubation (10 min) and a washing step TMB-Substrate A and B, are added. Blue - color develops on 'incubation and the reaction' is stopped with a 'Stop Solution' to form a yellow - color.

6.17.2. Reagents

- Standards
- Biotinylated Rat Testosterone Antibody
- 30 X Wash buffer
- Streptavidin HRP Conjugate
- TMB Substrate 'A'
- TMB Substrate 'B'
- Stop Solution

6.17.2. Preparation of Standard wells

Standard Concentration	Standard No	Dilution Particulars
3200 pg/mll	Standard, concentrated	Original Standard provided in the Kit
1600 pg/ml	Standard No.5	120 ul Original Standard + 120 ul Standard diluent
800 pg/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard diluent
400 pg/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard diluent
200 pg/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard diluent
100 pg/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard diluent

6.17.3. Assay Procedure

- Serum testosterone concentration was measured by ELISA method using a KineshDx Rat Testosterone hormonal (TH) ELISA kit (Cat No: K11-5126, 96 Well kit, Los Angeles, USA) as per the manufacturer instructions.
- Briefly, all the reagents, buffers, and samples were brought to the room temperature prior to use. 50 µl of standard solution and 40 µl of sample was added to the standard wells and sample wells respectively. Followed by 10 µl of Biotinylated Rat TH Antibody was added to the sample wells.
- Thereafter 50 µl of 'streptavidin-horseradish peroxidase (HRP) conjugate was added to both standard and sample wells.
- Covered the plate and incubated for 60 minutes at room temperature.
- After incubation the solution was discarded completely and wells were washed with a '1x washing' buffer solution four times.
- Added TMB Substrate A: 50 µl and TMB Substrate B: 50 µl to each well and incubated for 10 minutes in dark.

- Finally 50 µl of stop solution was added to each well and readings were taken in ELISA reader at 450 nm [57].

Figure 21: Image represents yellow color was measured at 450 nm. ELISA reader (Rayto, RT-6100 microplate reader)- figure captured in research laboratory, Department of Microbiology, SDUMC, Tamaka, Kolar.

The standard curved was prepared by obtained OD values. Based on standard curve the sample values were calculated.

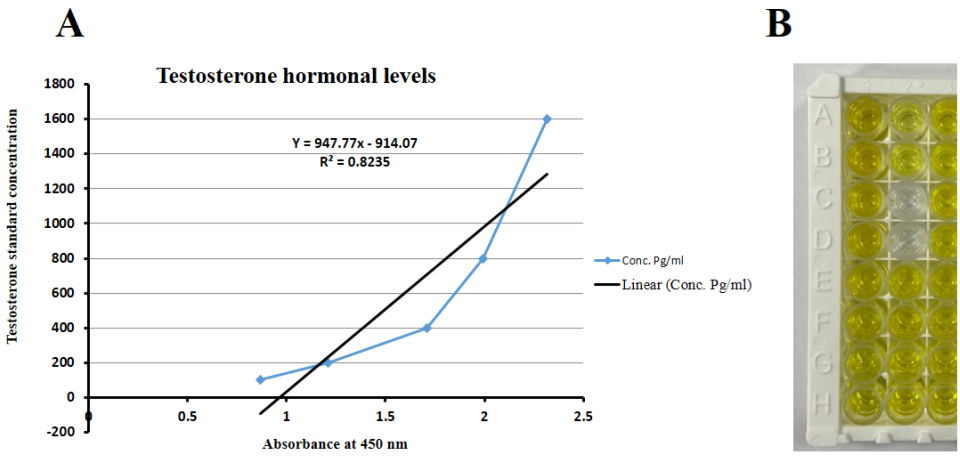

Figure 22: Representative image of (A) Testosterone curve and (B) Testosterone wells.

6.17.4. Calculation of sample concentrations

Formula = Y = 947.77*X - 914.07

$R^2 = 0.8235$

(Y- Unknown sample concentration)

(X-Sample O.D Value)

(R^2-Regression Line)

- Sample concentration was calculated by using above formula.

6.18. Androgen binding protein analysis

At the end of the study period [6 months], testis was harvested *via* centre incisions in the scrotal sac. Right testis tissue was dissected and fixed in liquid nitrogen and stored at -80 °C for analysis of androgen binding protein levels. Testis tissue was homogenized using 'Phosphate Buffer Saline' with a ratio of W: V (1:10). [58], the homogenate was centrifuged using 5,000 rpm for 15 minutes. After that supernatants were collected and stored at − 20 ◦C until ABP levels analysis. ABP levels were measured by ELISA method using a Kinesh Dx rat ABP ELISA kit (Cat No: KLR-1542, 96 Wells, Krishgen Biosystems) according to the kit

manufacturer instructions. The optical density can be measured with spectrophotometry wavelengths at 450 nm.

6.18.1. Principle

The method employs sandwich ELISA technique. Monoclonal antibodies are pre-coated onto micro-wells. Samples and standards are pipetted into micro-wells and Rat ABP, ABP present in the sample are bound by the antibodies. Biotin labelled antibody is added and followed by 'Streptavidin-HRP' is pipetted and incubated to form a complex. After washing 'microwells' in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Rat ABP, color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

6.18.2. Processing of Homogenatin

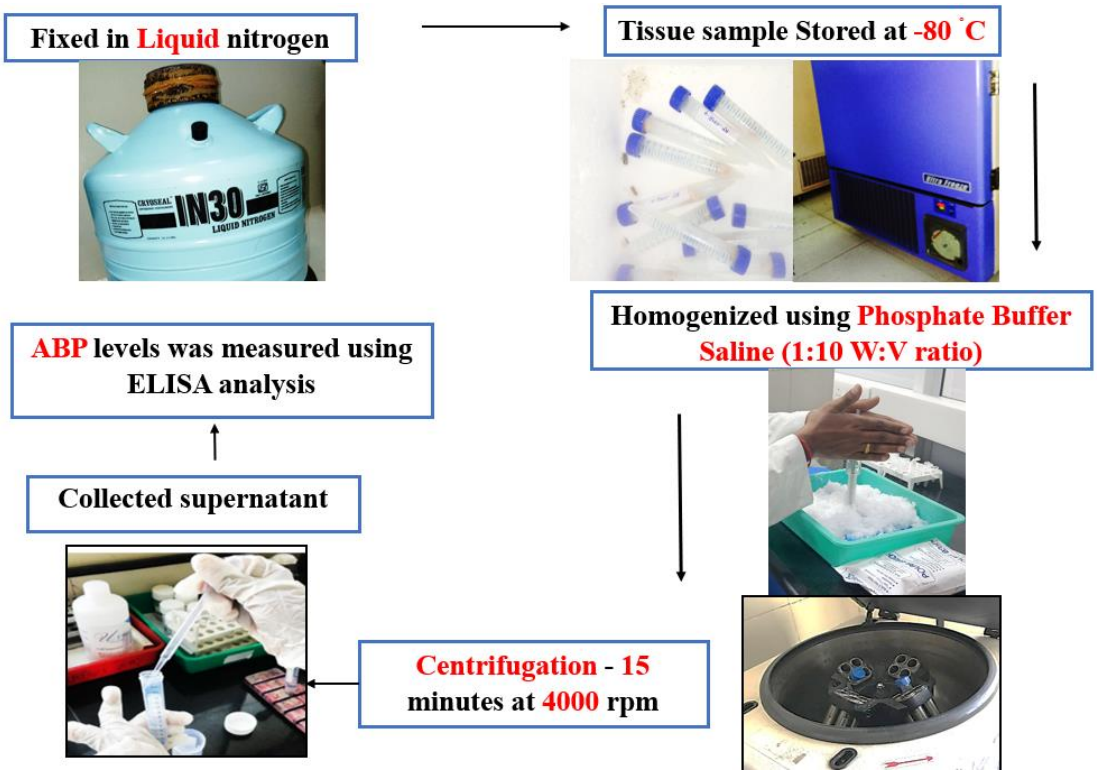

Figure 23: Image represents Processing of homogenatin- figure captured in research laboratory, Department of Cell biology and molecular genetics, SDUAHER, Tamaka, Kolar.

6.18.3. Reagents

- Standard
- Biotinylated ABP antibody
- 30X Wash buffer
- Streptavidin HRP Conjugate
- Substrate A
- Substrate B
- Stop Solution

6.17.2. Preparation of Standard wells

Standard Concentration	Standard Vial	Dilution Particulars
120 pmol/ml	Standard No.5	120 ul Standard Provided (240 pmol/ml) + 120 ul Standard Diluent
60 pmol/ml	Standard No.4	120 ul Standard No.5 + 120 ul Standard Diluent
30 pmol/ml	Standard No.3	120 ul Standard No.4 + 120 ul Standard Diluent
15 pmol/ml	Standard No.2	120 ul Standard No.3 + 120 ul Standard Diluent
7.5 pmol/ml	Standard No.1	120 ul Standard No.2 + 120 ul Standard Diluent

6.18.4. Assay Procedure

Androgen binding protein concentration was measured by ELISA method using a KineshDx Rat androgen binding protein (ABP) ELISA kit (Cat No: KLR-1542, 96 Well kit, Los Angeles, USA) as per the manufacturer instructions. Briefly, all the reagents, buffers, and samples were brought to the room temperature prior to use. 50 µl Standard solution and 40 µl of sample was added to the standard and sample wells respectively. Followed by 10 µl of Biotinylated Rat androgen binding protein Antibody was added to the sample wells. Thereafter 50 µl of 'streptavidin-horseradish peroxidase' conjugate was added to both standard and sample wells. Covered the plate and incubated for 60 minutes at room

temperature. After incubation the solution was discarded completely and wells were washed with a '1x washing' buffer solution four times. Added TMB Substrate A: 50 µl and TMB Substrate B: 50 µl to the each well and incubated for 10 minutes at in dark. Finally 50 µl of stop solution was added to each well and taken the reading in ELISA reader at 450 nm within 15 min [59].

The standard curved was prepared by obtained OD values. Based on standard curve the sample values were calculated.

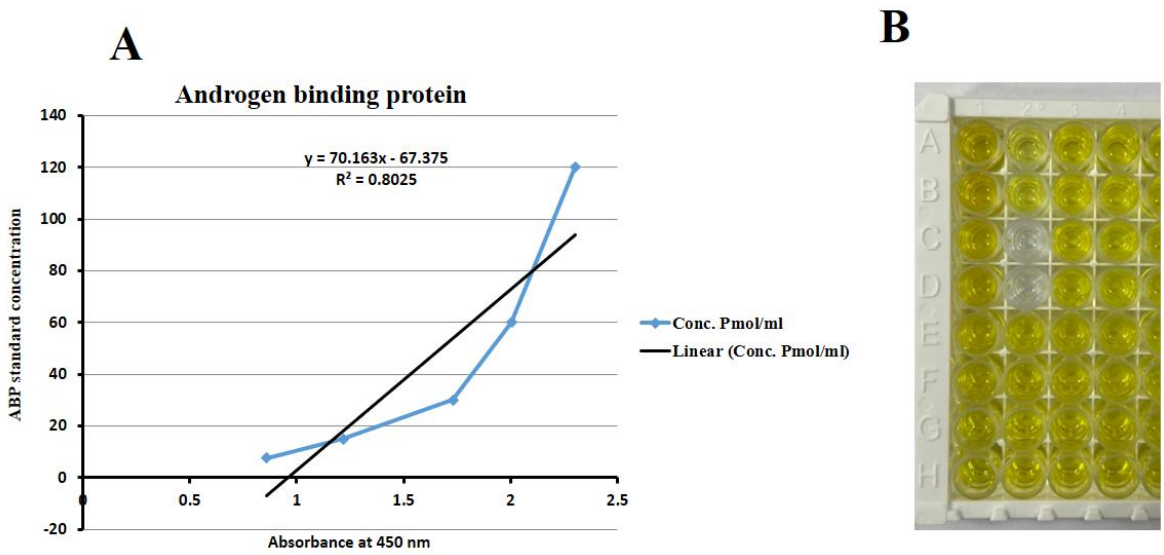

Figure 24: Representative image of (A) Androgen binding protein curve and (B) Androgen binding protein wells.

6.18.5. Calculation of sample concentrations

Formula = Y = 70.163*X - 67.375

R^2 = 0.8025

(Y- Unknown sample concentration)

(X-Sample O.D Value)

(R^2-Regression Line)

- Sample concentration was calculated by using above formula.

6.18.6. Statistical Analysis

The statistical analysis was carried out by using the SPSS (version 20; SPSS Inc) software program. Normal distribution of the data was presented as mean ± Standard deviation (mean ± SD). One-way analysis of variance and Bonferroni's post-hoc test were used to determine the significance among the multiple comparisons of 4G mobile phone RF-EMR and pomegranate juice treatment groups.

CHAPTER – 1

7.1. To evaluate the effect of Radio-frequency electromagnetic radiation and efficacy of Pomegranate juice on the structural integrity of sertoli cells and interstitial cells of Leydig.

7.1.1. Results

In the present work, the Histological integrity examination of the testicular sections of group I shows normal structure of seminiferous tubules with normal shape and arrangement of their cellular components. Sertoli cells and spermatogenic cells rest on intact basement membrane. Spermatogonia appeared as small cells and prominent rounded nuclei resting on basement membrane. Sertoli cells appeared as tall cells present in 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.

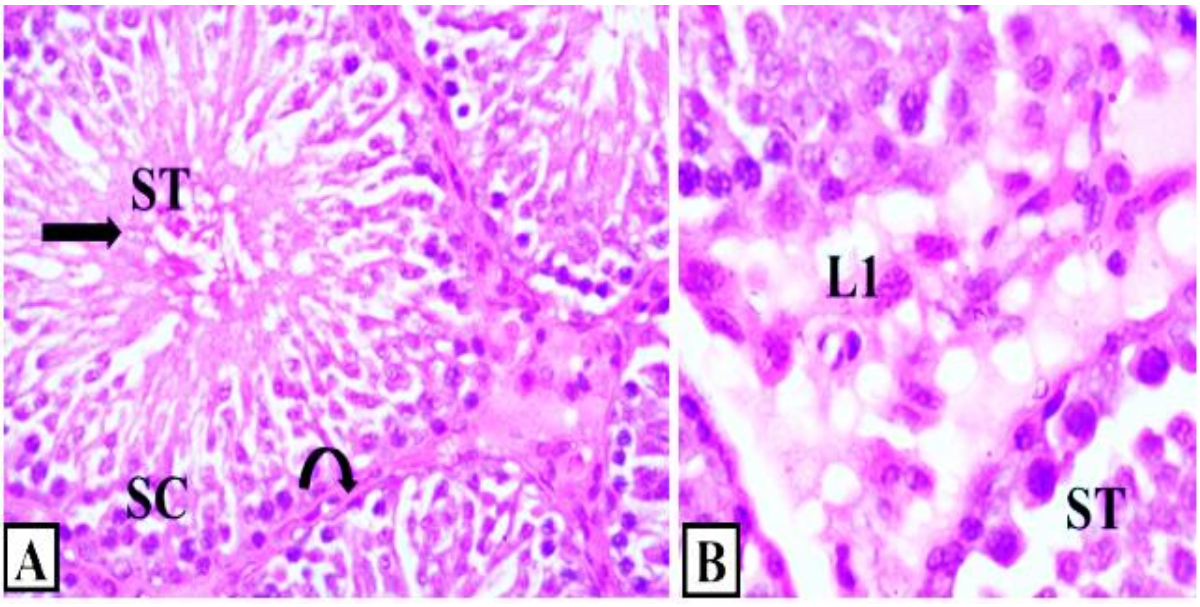

Figure 25: Photomicrographs of testis tissue (Sertoli cells and Leydig cells) from S.D rats sections showing. Control group (A & B) Showing: A: Closely packed Seminiferous Tubules (ST) lined by Myoid cells (curved arrow) and Sertoli cells (SC), with Sperms (long

arrow) in their lumens. B: Leydig cells (L1) with vesicular round nuclei and acidophilic cytoplasm containing Seminiferous Tubules (ST) or observed [H & E stain, X400].

In group II, testicular sections are distorted, Histological integrity architecture of some seminiferous tubules with winding of interstitial spaces and overall reduction in their size. Seminiferous tubules showed irregular basement membrane and detached, degenerated spermatogenic cells. Overall decrease in cytoplasmic ground substance in Sertoli cells, followed by vacuolization and cells are completely disturbed. Interstitial space increased, contained congested blood vessels and leydig cell (atrophy) appeared with small polygonal cells and darkly stained nuclei.

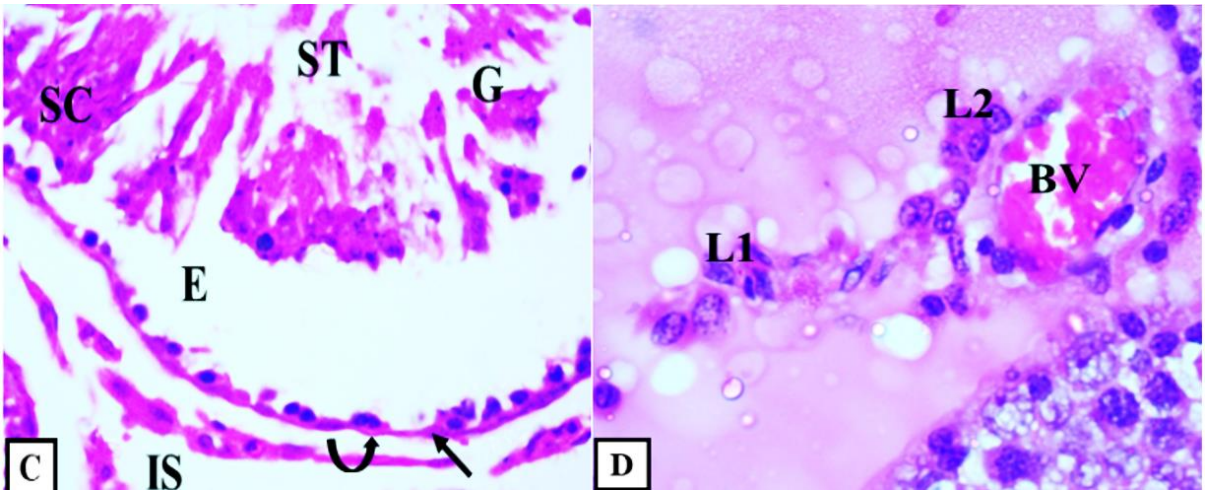

Figure 26: Photomicrographs of testis tissue (Sertoli cells and Leydig cells) from S.D rats sections showing. Mobile phone RF-EMR group (C & D) showing: C: Irregular basement membrane (long arrow), distorted Seminiferous Tubules (ST), wide Interstitial Space (IS), detached Sertoli cells (SC), multinucleated Giant cells (G) Empty spaces (E) and Myoid cells (curved arrow) resting on irregular basement membrane. D: Interstitial space contains congested Blood Vessels (BV) and Leydig cells with darkly stained nuclei (L1) and remaining cells (L1) are vesicular nuclei [H & E stain, X400].

In group III, examination of testicular Histological integrity sections showed that apparently normal packed STs lined by several layers of spermatogenic cells and sertoli cells are resting on the regular 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.

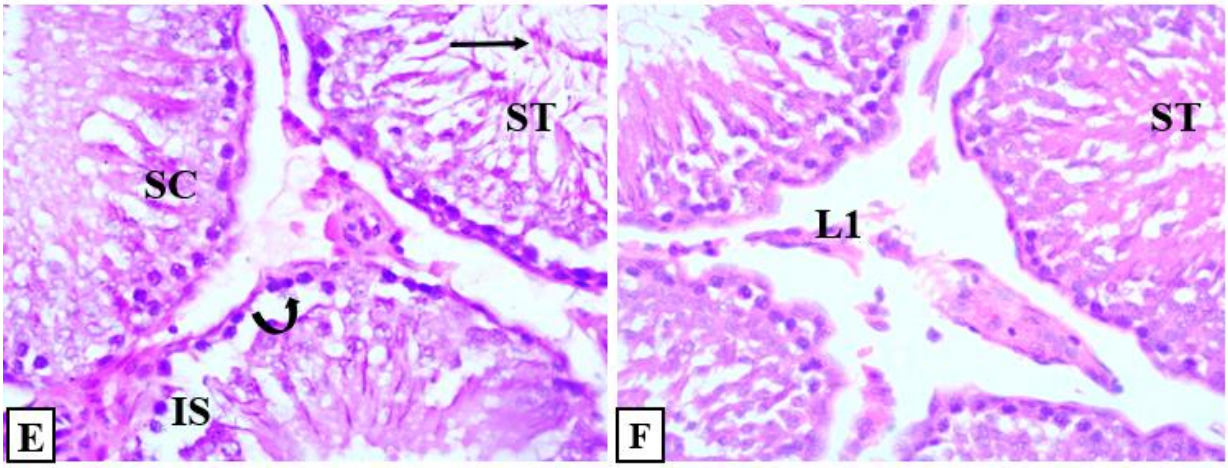

Figure 27: Photomicrographs of testis tissue (Sertoli cells and Leydig cells) from S.D rats sections showing. RF-EMR + PJ group (E & F) Showing: E : Improved testicular arrangement, Closely packed Seminiferous Tubules (ST) lined by Sertoli Cells (SC) and Spermatogenic cells (curved arrow), with Sperms (long arrow) in their lumens. F: Leydig cells (L1) with vesicular round nuclei and closely packed Seminiferous Tubules (ST) [H & E stain, X400].

In group IV, Histological integrity sections showed incomplete improvement where some apparently packed STs and partial loss of spermatogenic cells. Some spermatogenic cells appeared with acidophilic cytoplasm (pyknotic nuclei) either resting on 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.

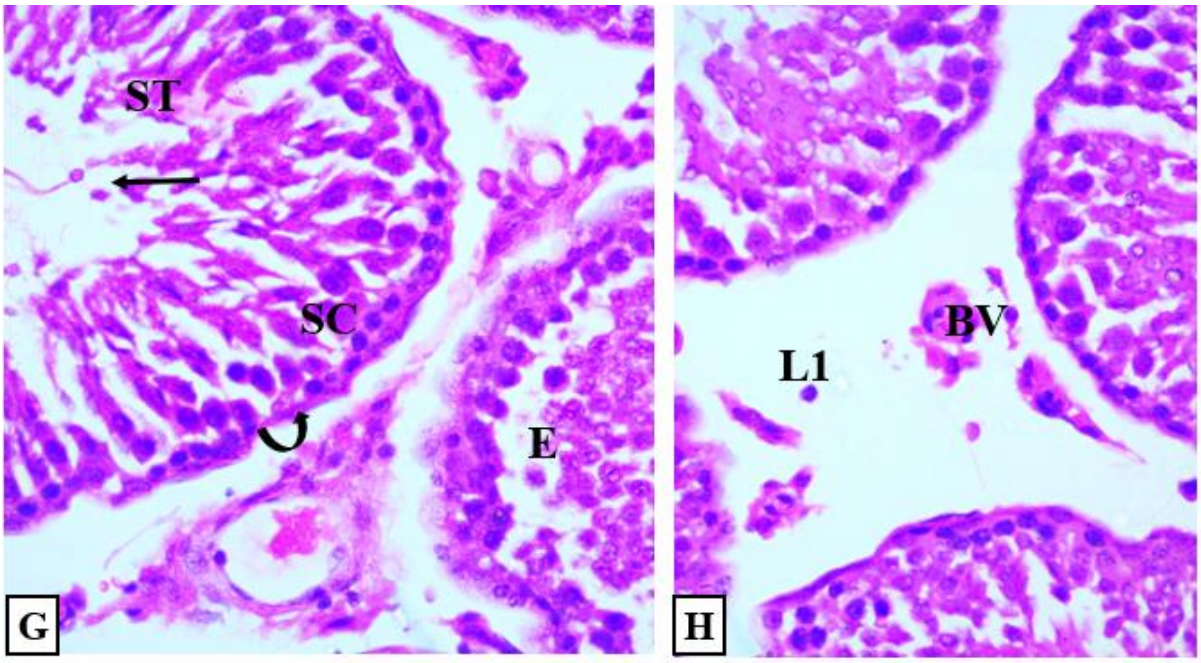

Figure 28: Photomicrographs of testis tissue (Sertoli cells and Leydig cells) from S.D rats sections showing. Mobile phone RF-EMR Recovery group (G & H) showing: G : distorted Seminiferous Tubules (ST), Myoid cells (curved arrow), Empty spaces (E) and Sertoli cell (SC) resting on irregular basement membrane. H: Interstitial space contains Blood Vessels (BV) and Leydig cells with darkly stained nuclei (L1) [H & E stain, X400].

In group V, there was no Histological integrity abnormalities was noted in the PJ group when compared to control group. However, STs was normal in shape and arrangement of their cellular components. Sertoli cells and spermatogenic cells rest on intact basement membrane. Sertoli cells appeared as tall cells present in 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.

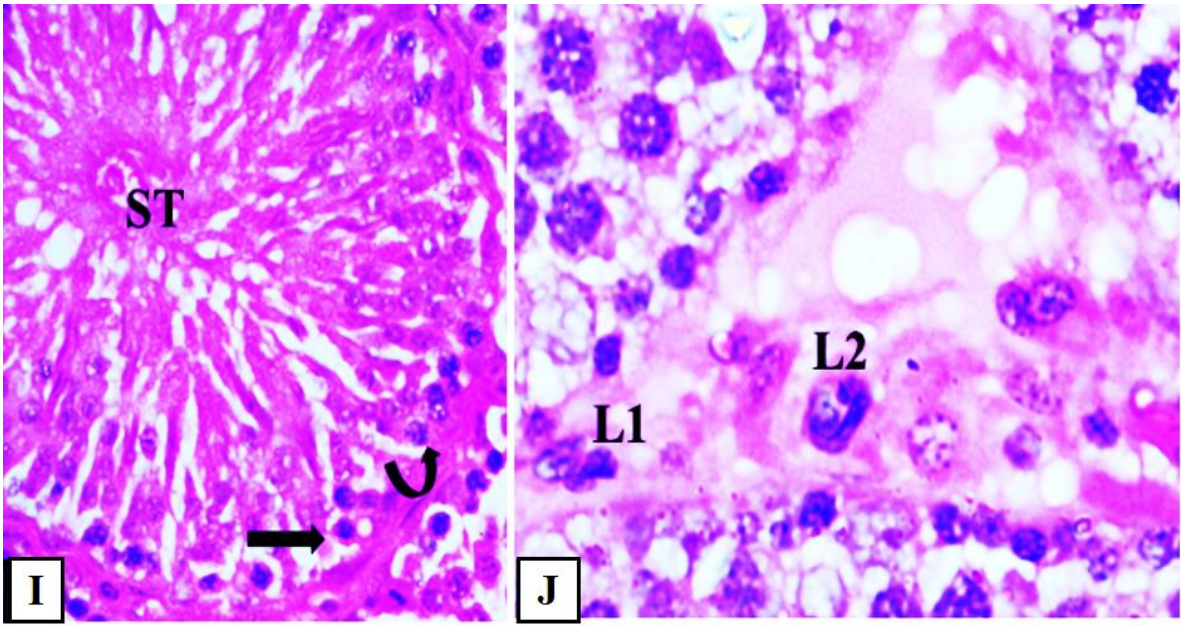

Figure 29: Photomicrographs of testis tissue (Sertoli cells and Leydig cells) from S.D rats Sections showing. Pomegranate juice (PJ) group (I & J) showing: I: Packed Seminiferous Tubules (ST) lined by Spermatogenic cells (curved arrow), apparently normal Sertoli cells (thick arrow). J: Leydig cells (L2) shows acidophilic vacuolated cytoplasm and vesicular nuclei (L2) [H & E stain, X400].

7.1.2. Discussion

The 4G MP have become an important part of daily life all over the world. In a view of '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, still it is controversial [60]. Several studies reported that mobile phone (RF-EMR) usage led to decreased male fertility. Nonetheless, other studies showed no conclusive link between male infertility and mobile phone usage [61]. Chronic exposure to MP RF-EMR decreased the diameter of the seminiferous tubules (STs) and also, increased number STs of per unit area of testis [62].

There general agreement that OS was implicated as one of the main culprit in male infertility [63], and that RF-EMR can intensify the generation of OS' and cause an imbalance between the production of 'reactive oxygen species' and their anti-oxidant defines system [64].

According to the available literature, the testicular Histological Integrity in SD rats exposed to RF-EMR and co-administration of PJ has not been studied yet. In the present study focus the testicular Histological integrity revealed that degenerative changes particularly in the "Sertoli cells, Leydig cells". In the present study, it was focused on the protective effect of Pomegranate juice, against 4G MP RF-EMR exposed reproductive toxicity (Histological integrity of sertoli cells and leydig cells) in male SD rats. Pomegranate juice has protective effect due to its active ingredients like conjugated fatty acids [65], estrogenic flavonoids [66], phenolic acids [67], tannins [68, 69], these compounds are found in substantial amounts in the Pomegranate juice and peels of the pomegranate fruit [70].

Kumar S et al., (2014) revealed that mobile phones working on 1910.5 MHz can cause significantly decreased sperm count, reduction in the diameter of the seminiferous tubules (STs), testicular weight and DNA damage in Wistar rats in 2 hour/day/60days of RF-EMR exposure [71]. LatifaIshaq Khayyat and Pradeep Kumar et al., reported that RF-EMR of mobile phone induced Leydig cell hypoplasia, atrophied seminiferous tubules, wide interstitial, and decreased germ cell population, maturation arrest in the spermatogenesis, vacuolisation in spermatogenic cells and pyknotic nuclei in 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 STs [72, 73]. Similarly, previous study done by Turk G et al., reported that the daily consumption of Pomegranate juice over a period of 7 weeks caused a significant reduce in OS parameters and marked increase in the level of spermatogenic cells and thickness of the germ layers [25]. Mahmoud and Solaiman et al.,

pomegranate juice groups revealed that Histological integrity examination of STs retained normal appearance. These results were confirmed Histological integrity by the significantly increase in the STs Diameter and germinal epithelial height [74].

7.1.3. Conclusion

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

CHAPTER-2

7.2. To evaluate the effect of Radio-frequency electromagnetic radiation and efficacy of Pomegranate juice on seminal parameters.

7.2.1. Result

Effect of MP emitted RF-EMR and ameliorative effect of PJ on sperm count

The sperm count were significantly reduced in the mobile RF-EMR group compared to the control and pomegranate juice groups ($p < 0.05$). However, the sperm count was significantly increased in the mobile RF-EMR + PJ group compared to the mobile RF-EMR group ($p < 0.05$). The sperm count was not significantly increased in the mobile RF-EMR recovery group compared to the mobile RF-EMR group ($p > 0.05$). The sperm count was increased in the pomegranate juice group compared to the control group ($p > 0.05$).

Effect of MP emitted RF-EMR and ameliorative effect of PJ on sperm motility

The sperm motility were significantly decreased in the MP RF-EMR group compared to the control group and PJ groups ($p < 0.05$). However, the sperm motility was significantly increased in the MP RF-EMR + PJ group compared to the MP RF-EMR group ($p < 0.05$). The sperm motility was significantly increased in the MP RF-EMR recovery compared to the 4G MP RF-EMR group ($p < 0.05$); however, it did not reach to the control and RF-EMR + PJ group levels. The sperm motility was not significantly increased in the PJ group compared to the control group ($p > 0.05$).

Effect of MP emitted RF-EMR and ameliorative effect of PJ on sperm viability

The sperm viability was significantly reduced in the MP RF-EMR group compared to the control and PJ groups ($p < 0.05$). However, the sperm viability were significantly increased in the MP RF-EMR + PJ group compared to the MP RF-EMR group rats ($p<0.05$). The sperm viability was significantly reduced in the MP RF-EMR recovery group compared to the MP RF-EMR group ($p < 0.05$). The sperm viability was not significantly different between the PJ group and the control group ($p > 0.05$).

Effect of MP emitted RF-EMR and ameliorative effect of PJ on sperm progressivity

The sperm progressivity was remarkably declined in the MP RF-EMR group (grade C) compared to control group (grade A) and the PJ group (grade A). However, the sperm progressivity was increased in the MP RF-EMR + PJ group (grade B) compared to the MP RF-EMR group (grade C). The sperm progressivity was not increased in the 4G MP RF-EMR recovery group (grade C) compared to 4G MP RF-EMR group (grade C).

Effect of MP emitted RF-EMR and ameliorative effect of PJ on abnormality sperm morphology

The abnormality of sperm morphology (detached head, pyriform head, coiled tail, and bent tail) was significantly increased in MP RF-EMR group compared to the control and pomegranate juice groups ($p < 0.05$). However, the abnormality of sperm morphology was significantly reduced in the MP RF-EMR + PJ group compared to the MP RF-EMR group ($p<0.05$). The abnormality of sperm morphology was comparable in between the MPRF-EMR recovery group and MP RF-EMR group. The abnormality of sperm morphology was decreased in the PJ group compared to control group; however, it was not statistically significant ($p>0.05$)

Table 2: Effects of mobile phone emitted RF-EMR and ameliorative effect pomegranate juice on sperm parameters.

Parameters	control	RF-EMR	RF-EMR+PJ	RF-EMR Rec	PJ
Sperm count(x10^6)	91.80±7.14	74.14±4.21[a]	87.10 ±2.01[b]	67.43±10.12[a]	93.15±8.01
Sperm motility (%)	92.16±1.72	49.66±2.94[a]	81.40±2.30[b]	69.28±3.11[ab]	91.28±9.25
Sperm viability (%)	90.66±1.21	69.00±2.44[a]	92.40±2.50[b]	54.18±2.48[ab]	87.83±2.31
Sperm progressivity	A	C	B	C	A

Data are expressed as Mean ± SD. n=6 in each group. One-way analysis of variance and Bonferroni's post-hoc test are used to determine the significance among the multiple comparisons of RF-EMR and PJ treatment groups. Sperm progressivity is graded as A: Excellent, B: Good, C: Fair, D: Poor, as described by the World Health Organization (2005). $p<0.05$ is considered statistically significant. a: compared to control group, $p<0.05$; b: compared to the RF-EMR group, $p<0.05$. RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, RF-EMR Rec: Radio frequency electromagnetic radiation Recovery.

Table 3: Effects of 4G mobile emitted RF-EMR and ameliorative effect of pomegranate juice on sperm morphology (percentage of abnormality).

Sperm abnormalities (%)	Control	RF-EMR	RF-EMR+PJ	RF-EMR Rec	PJ
Detached head	4.40 ± 0.35	8.90 ± 0.22[a]	5.60 ± 0.59[b]	8.80 ± 0.25	4.70 ± 0.56
Pyriform head	4.30 ± 0.12	7.70 ± 0.14[a]	5.80 ± 0.25[b]	7.50 ± 0.20	3.90 ± 0.09
Coiled tail	5.50 ± 0.43	7.10 ± 1.27[a]	5.90 ± 0.40	7.80 ± 1.99	5.20 ± 0.31
Bent tail	4.80 ± 0.15	7.20 ± 2.09[a]	5.10 ± 0.29[b]	8.90 ± 1.25	4.60 ± 0.33

Data are expressed as Mean ± SD. n=6 in each group. One-way analysis of variance and Bonferroni's post-hoc test are used to determine the significance among the multiple comparisons of RF-EMR and PJ treatment groups. $P < 0.05$ is considered statistically significant. a: compared to the control group, $p < 0.05$; b: compared to RF-EMR group, $p < 0.05$. RF-EMR: radio frequency electromagnetic radiation. RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, RF-EMR Rec: Radio frequency electromagnetic radiation Recovery.

7.2.2. Discussion

The mobile phones have become a part of daily life. In view of COVID-19 pandemic, the usage of smart phones was increased in adults including adolescents [75]. The usage of 4G mobile phones has been accompanied by parallel increase of the RF-EMR density in environment. On the other hand, the male infertility has increased in recent years [76, 77]. However, the epidemiological cause for male infertility has not been understood clearly.

Hence, we were interested to study the effect of long term exposure of MP emitted RF-EMR on male reproduction and efficacy of PJ on RF-EMR induced male infertility.

Our current study results showed that the chronic exposure [90 days (1 hour/day)] to RF-EMR emitted by MP leads to decrease of the sperm count, motility, viability and progressivity in rat semen. In addition, RF-EMR exposure also increased the abnormality of sperm morphology in rat semen. It indicates the chronic exposure of RF-EMR emitted by 4G mobile cause's male infertility in rats. Group III co-administration with PJ shows significant increase in spermatozoa and spermatids in the lumen. PJ increased sperm concentration, sperm motility, spermatogenic cell density and decreased abnormal sperm in male S.D rats [25].

Jong et al reported that the 30-day exposure (18 hours/day) to the mobile emitted RF-EMR leads to decrease of spermatogonia, germ cells and leydig cell count in rats [78]. Shokri et al reported that seven hours/day for 30 days exposure to the 2.45 GHz Wi-Fi EMR (high frequency radiation) induced a decrease in sperm parameters and increase of apoptosis positive cells and increased the caspase 3 activity in seminiferous tubules in rats [79]. Tas M et al reported that long-term exposure (3 hours/day for 365 days) of 900 MHz RF radiation altered male reproductive parameters in rats [80].

In contrast, studies reported that RF-EMR exposure did not decrease the sperm count in the experimental group compared to control animals [81, 82]. In contrast, our study reported that chronic exposure [90 days (1 hour/day)] to RF-EMR emitted by MP leads to decrease of the sperm count, motility, viability, and progressivity in rat semen including abnormality of sperm morphology. It might be due to the increase of 'oxidative stress' in rat testis exposure to the RF-EMR emitted by MP. Damegh et al stated that the 1 hour/day for 14 days exposure to the RF-EMR increases the oxidative stress in testis of rats [83].

The direct biological effects of mobile RF-EMR are divided into thermal effects by the RF-EMR energy absorption, stimulation function by the induced electric current and athermic action by the long term RF-EMR exposure. No clinical studies have evaluated the effect of mobile RF-EMR and co-administration of pomegranate juice on the sperm parameters, but some epidemiologic studies have shown the mobile phone use has negative effects of sperm parameters [84]. Punica granatum has protective effects due to its ingredients like phenolic acids [85], estrogenic flavonoids [86], tannins [87, 88] and conjugated fatty acids [89]. These compounds are found in substantial amounts in the seed oil, peels and juice of the Punica granatum fruits [90]**.**

Plant products such as leaves, seeds and fruits have natural antioxidants. Pomegranate juice has an ancient history of being used in several diseased conditions [91]. PJ is polyphenol rich juice with high antioxidant capacity. Pomegranate fruit has protective effects on cell due to its active ingredients like phenolic acids, tannins, estrogenic flavonoids and conjugated fatty acids [92]. Moreover, several studies have shown that the "pharmacological effects of flavonoids" are related to their antioxidant activity, which can be due to their ability to scavenge OH and O2, to chelate metal ions and to exert a synergistic effect of other anti-oxidant metabolites.

Our results in conjunction with the others mentioned above, suggest that flavonoids could constitute one of the active components of pomegranate [93, 94]. In addition, the abnormality of sperm morphology in rat semen was also decreased in the RF-EMR exposed rats supplemented with pomegranate juice compared to RF-EMR group rats. Turk et al [25], reported that 7-week supplementation of pomegranate juice increases the sperm quality, antioxidant capacity and decreases the abnormality of sperm in rats.

Yassien et al observed that 52 days of pomegranate juice supplementation increased the structural integrity of testis and TH levels in old rats compared to age-matched rats [95]. Mohsen et al stated that pomegranate juice supplementation increases the sperm count, progressive sperm motility, testosterone levels and epithelial diameters of the STs of the exposed male mice [96].

In line with reported studies, the current study reported that 90 days supplementation of pomegranate juice increases the sperm parameters and decreases the sperm abnormality in rats exposed to mobile RF-EMR. This could be due to rich antioxidant capacity of pomegranate juice, serving as protective mechanism against the oxidative free radicles generated by the MP RF-EMR. The limitation in the present study was that we did not analyse the oxidative status of testis and also the testosterone levels in rat, which can give us a better understanding of the role of pomegranate juice on reduced sperm parameters induced by the MP emitted RF-EMR.

7.2.3. Conclusion

In conclusion, the study results indicate that the mobile RF-EMR exposure reduces the sperm parameters and increases the sperm abnormality. However, the pomegranate juice supplementation reverses the 4G mobile RF-EMR induced damage on sperm quality in rats. It indicates that the pomegranate juice can be used as a nutritional supplement to improve the sperm quality.

CHAPTER – 3

7.3. To determine impact of Radio-frequency electromagnetic radiation and efficacy of Pomegranate juice on serum testosterone levels.

7.3.1. Result

The mean ± Standard Deviation (SD) of total serum testosterone hormonal (TH) levels in control group I was 2.45± 1.102 ng/ml and MP RF-EMR exposed group II was 0.92 ±0.22 ng/ml. In comparison to control group I, the mean total serum testosterone hormonal levels of 4G MP RF-EMR group II rats (60 min/day/3 months) was significantly lower ($p < 0.005$).

The mean ± SD serum testosterone levels was significantly higher in S.D rats that were exposed MP RF-EMR+ PJ group III (1.96 ±017 ng/ml) compared to RF-EMR group II (0.92 ± 0.22 ng/ml). However, there was no significant difference between MP RF-EMR group II (0.92 ± 0.22 ng/ml) the MP RF-RF-EMR Recovery group IV (1.07 ±013).

The mean ± SD testosterone hormonal levels (ng/ml) of obtained from control group I and PJ were 2.45 ± 1.102 and 2.93 ± 1.012ng/ml, respectively. Analysis of variance showed a significant increase in serum testosterone hormonal levels of control group I rats when compared to PJ group rats ($p<0.01$).

Table 4: Effects of Radio-frequency electromagnetic radiation generated by mobile phone (1hour/day/3 months) on serum TH level in control and Treatment group rats.

Parameters	Control	RF-EMR	RF-EMR+PJ	RF-EMR Rec	PJ
Serum testosterone	2.45 ± 1.102	0.92 ± 0.22	1.96 ±017[b]	1.07 ±013	2.93 ± 1.012[a]

P value statistically significant ($p < 0.05$). a: compared to the control group $p<0.05$. b: compared to the 4G MP RF-EMR group $p<0.05$. RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, RF-EMR Rec: Radio frequency electromagnetic radiation Recovery.

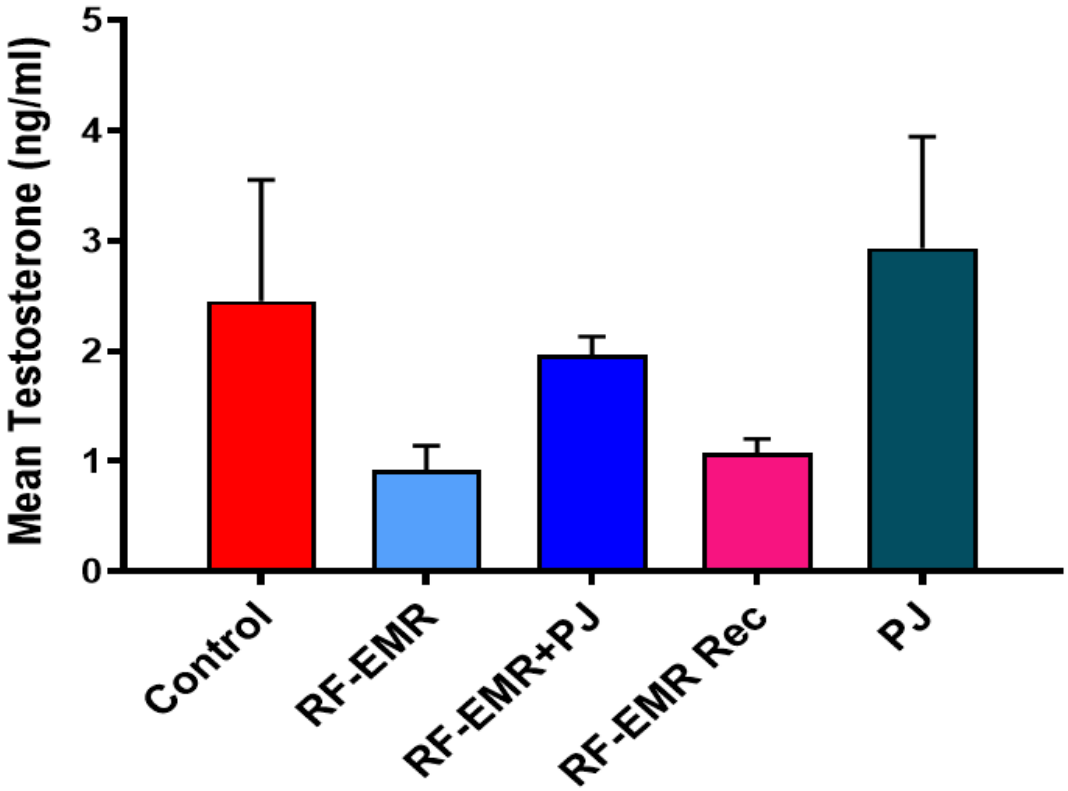

Figure 30: The serum testosterone hormonal levels of electromagnetic radiation generated by 4G mobile phone (1hour/day/3 months), Control and Treatment groups S.D rats. RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, RF-EMR Rec: Radio frequency electromagnetic radiation Recovery.

7.3.2. Discussion

The study revealed that the mean ± SD of total serum testosterone hormonal levels were decreased in the RF-EMR group compared to the controls. The similar results are seen in the M R Sarookhani et al who suggested that testosterone and FSH levels were disturbed as a result of mobile phone EMF exposure which can possibly affect reproductive functions [97]. Ali saeed Al Chalabi also reported in his study that there is a significant decrease in serum testosterone level in both exposure groups ($p \leq 0.05$) as compared with control group, with significant ($p \leq 0.05$) decrease in serum FSH level [98].

The present study also reported that the mean ± SEM serum testosterone levels was significantly higher in S.D rats that were exposed MP RF-EMR+ Pomegranate juice group compared to the RF-EMR group. Mohsen et al reported in their study that pomegranate juice exhibited a clear antioxidant effect to mitigate the disruption in the testes, especially by maintaining the testosterone concentration and sperm count and quality [99].

A mobile hazardous effect on male fertility may be manifested through variation in the serum TH levels. Testosterone hormone is a primary male gender hormone and plays a significant role in the spermatogenesis. The causative agents may be including RF-EMR. The effect of RF-EMR on living organisms depends on the frequency and intensity. These radiations may exert an effect on the state of polarization of the cellular membranes. An inadequate polarization of cellular membrane is responsible for the process of various abnormalities of testosterone synthesis, secretion which may impair spermatogenesis and ultimately become a cause of infertility. There are few animal studies that show that EMF radiation generated by mobile phones have a wide range of damaging effects on the male reproductive system and sperm parameters [100].

The similar results are also supported by Ebtesam et al, mentioned Pomegranate has a protective effect due to its active ingredients like tannins, phenolic acids, estrogenic flavonoids and conjugated fatty acids, these compounds are found in substantial amounts in the peels, juice and seed oil of the pomegranate fruits . It also showed the protective effect of pomegranate juice against CCl_4-induced reproductive toxicity in male rats and showed significant elevation in testosterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH) [101]**.**

Aminirad O et al also supported and reported in the study that pomegranate juice consumption increased sperm count in cases, the normal morphology of cases improved significantly and rate of fertility increased from 5.5 ± 3.3 to 10.0 ± 1.3 in case mice [102]..

7.3.3. Conclusion

Testosterone is a primary male gender hormone and any change in the normal levels may be devastating for reproductive and general health. The longer exposure duration of electromagnetic field decreased the spermatogenesis. MP might have deleterious effects on human reproductive health. This was evidenced by the biochemical (MDA, TH and sperm count) and histopathological changes of testis reflected as impairment of spermatogenesis. Thus, avoiding long-term and excessive use of MP is advisable to reduce the detrimental effect of RF-EMR.

Pomegranate juice consumption increases significantly sperm quality, spermatogenic cell density, antioxidant activity and testosterone hormonal levels in male rats. PJ consumption provided an increase in sperm motility, spermatogenic cell density and diameter of STs and germinal cell layer thickness and it decreased abnormal sperm count. PJ is able to improve the quality of sperm parameters, as well as fertility potential in mice. Probably, intake of this antioxidant by infertile men improves the quality of their sperm parameters.

CHAPTER - 4

7.4. To evaluate the effect of Radio-frequency electromagnetic radiation and an efficacy of Pomegranate juice on androgen binding protein.

7.4.1. Results

The S.D rats were exposed to MP RF-EMR group (19.87 $\pm$ 2.23) showed a significant decreased in the average of rat ABP levels when compared to Control group (27.66 $\pm$ 5.37). On the other hand, the S.D rats that were exposed to RF-EMR + co-administration of PJ group (23.36 $\pm$ 2.17) intake showed a significant increase in the average of ABP levels when compared to RF-EMR group (19.87 $\pm$ 2.22). Statistically, the result showed that no significant difference between RF-EMR group (19.87 $\pm$ 2.23) and RF-EMR Recovery group (19.98 $\pm$ 1.13) ($p < 0.05$).

In the same table, the 'results also found that co-administration of Pomegranate juice group (29.93 $\pm$ 4.12) showed a significant increase in the average of ABP levels when compared to Control group (27.66 $\pm$ 5.37). However, 1/day exposure to smart mobile phone RF-EMR can cause decrease Androgen binding protein levels, and co-administration of Pomegranate juice can help to elevate androgen binding protein levels in Sprague dawley rats.

Table 5: Effects of Radio-frequency electromagnetic radiation generated by mobile phone (1hour/day/3 months) on Androgen binding protein level in control and Treatment groups of S.D rats.

Parameters	Control	RF-EMR	RF-EMR+PJ	RF-EMR Rec	PJ
ABP (Pg/ml)	27.66 ± 5.37	19.87 ± 2.23	23.36 ± 2.17[b]	19.98 ± 1.13	29.93 ± 4.12[a]

P value statistically significant (< 0.05). a: compared to the control group $p < 0.05$. b: compared to the 4G MP RF-EMR group $p < 0.05$. RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, RF-EMR Rec: Radio frequency electromagnetic radiation Recovery.

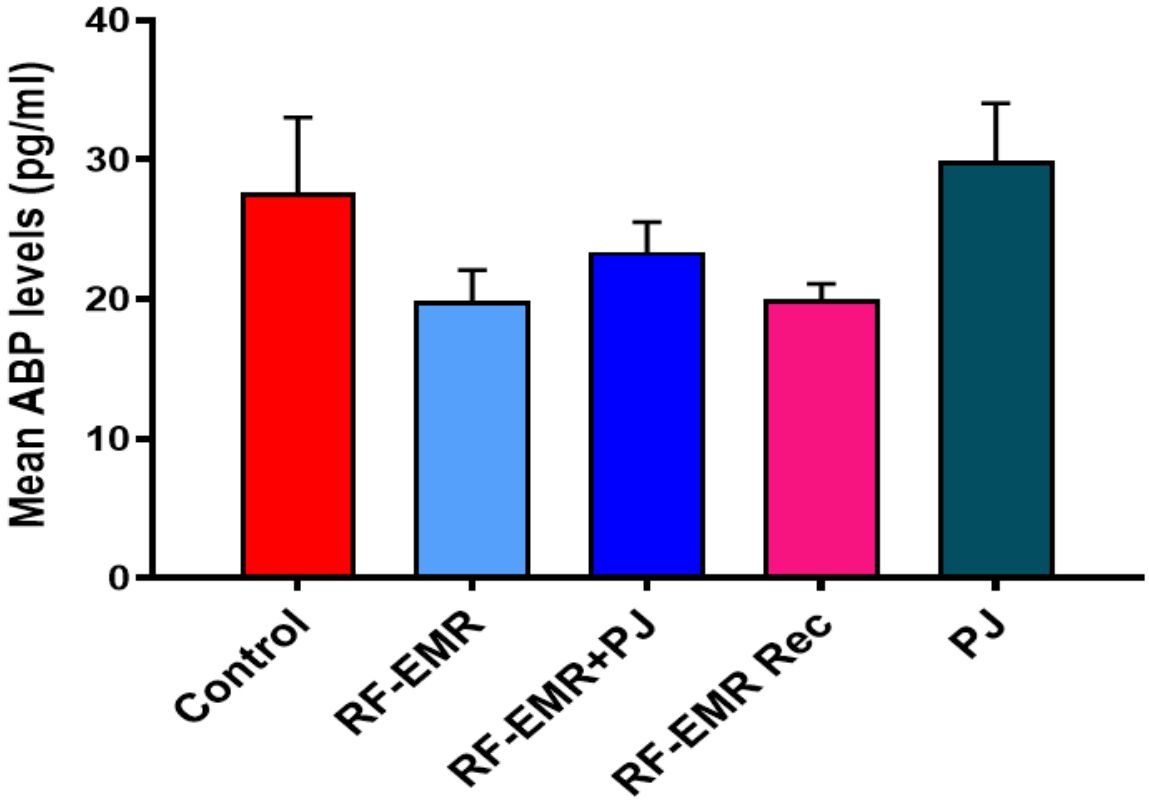

Figure 31: The ABP levels of electromagnetic radiation generated by 4G mobile phone (1hour/day/3 months) on Control and Treatment group of S.D rats. RF-EMR: Radio frequency electromagnetic radiation, PJ: Pomegranate juice, RF-EMR Rec: Radio frequency electromagnetic radiation Recovery.

7.4.2. Discussion

The study revealed that The S.D rats that were exposed to 4G MP RF-EMR group showed a significant decrease in the average of rat Androgen binding protein (ABP) levels when compared to Control group. The similar results are seen in the study by Wang et al mentioned that RF-EMR-induced changes in Leydig cell histology and impairment could be explained by oxidative stress and direct RF-EMR effects, which cause changes in protein kinase C in Leydig cells [102]. 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 the elevation of temperature and EMF strength value on the hypothalamus and pituitary gland after mobile phone exposure [103].

The present study also reported that RF-EMR +Pomegranate juice group showed a significant increase ABP levels when compared to RF-EMR group. Turk et al supported the results and found a significant decrease in malondialdehyde level and marked increases in glutathione (GSH), glutathione peroxidase and activities, and vitamin C level was observed in rats treated with different doses of Pomegranate juice [104].

Pomegranate juice consumption provided an increase in spermatogenic cell density and diameter of seminiferous tubules and germinal cell layer thickness, epididymal sperm count, sperm motility, and it decreased abnormal sperm rate when compared to control group [25].

Sertoli cells undergo degeneration of vacuoles in tubules exposed to pyrethroids so that the disruption of the function of the Sertoli cells can cause a decrease or even the absence of sperm cells caused by a decrease in androgen binding protein levels. Decreased androgen binding protein levels also indicate that the supply of testicular androgen (testosterone levels) for the epididymis can reduce the quality and quantity of sperm [105].

Mandana Beigi Boroujeni, et al showed the statistical comparison between sperm count and their viability and testosterone hormone amount showed a significant difference between control and experimental groups and the results showed an improvement of morphological condition of seminiferous tubules who got pomegranate extract peels [106].

7.4.3. Conclusion

Exposure to MP RF-EMR from various wireless devices has increased dramatically with the advancement of technology. One of the most vulnerable organs to the RF-EMR is the testes. This is due to the fact that testicular tissues are more susceptible to oxidative stress due to a high rate of cell division and mitochondrial oxygen consumption. As a result of extensive cell proliferation, replication errors occur, resulting in DNA fragmentation in the sperm. It could be used safely for treatment of sexual dysfunction due to the decrease in serum testosterone level, also Studies should be carried out to identify and isolate the active ingredients in those plants and to be extracted and used as a treatment of infertility

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The effect of 4G mobile phon

B. Anjaneyababu Naik

gave an oral presentation during International Virtual Anatomy Conference, organized by the Anatomical Society, King George's Medical University UP, Lucknow, from 20-22 February 2021

entitled

- 2400MHz) on sperm parameters and ameliorating effect of Punica granatum.

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Gave a Paper Presentation during E – National Conference AVEOCON - 2021

Organized by

Department of Anatomy, Teerthanker Mahaveer Medical College & Research Centre, Moradabad Uttar Pradesh

under aegis of Anatomical Society of India (UP Chapter) from 9-10th July 2021, entitled

"Ameliorative effect of Punica granatum on Histo-morphology of sertoli and leydig cell in rats with exposure to 4G mobile radio electromagnetic radiation"

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No. DMC/KLR/IEC/ 592 /2021-22 **Date: 08-01-2022**

CERTIFICATE

This is to certify that, the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Article entitled **"The effect of 800-2400 MHz 4G mobile phone radiation on sperm parameters and ameliorating effect of *Punica granatum*"** for **Publication** authored by **Mr. Anjaneyababu Naik** & Dr. Sridevi N S (Corresponding Author) in the Department of Anatomy at Sri Devaraj Urs Medical College, Tamaka, Kolar.

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CERTIFICATE

This is to certify that, the Institutional Ethics Committee of Sri Devaraj Urs Medical College, Tamaka, Kolar has examined and unanimously approved the Article topic entitled **"Ameliorative effect of *Punica grantum* on Histological Integrity of sertoli cells and leydig cells in rats with exposure to radio electromagnetic radiation"** for **Publication** authored by **Mr. Anjaneyababu Naik** & Dr.Sridevi N S (Corresponding Author) in the Department of Anatomy at Sri Devaraj Urs Medical College, Tamaka, Kolar.

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AUTHENTICATION CERTIFICATE

This is to certify that the following plant species for pharmacognostical / pharmaceutical / pharmacological / phytochemical investigation research work is identified and their botanical name and family name is given.

Botanical Name	Voucher number	Family
Punica granatum L.	0320	Punicaceae

Authenticated by
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SUMMARY

- Recent advancements in mobile phone technology is one of the leading causes of male infertility. This research aided in planning of treatments for infertility induced by cell phone use.
- Due to its powerful antioxidant effects, the incorporation of pomegranate juice altered the structural integrity of Sertoli cells and interstitial cells of Leydig, increased the sperm quality, spermatogenic cell density, antioxidant activity and testosterone hormonal level, Androgen binding protein levels in male rats and was found to be protective against exposure to 4G mobile phone radio-frequency electromagnetic radiation.
- It is advised to be precautious and not to expose continuously to longer durations (3 months) of mobile radiation and other sources of radiation.
- Further studies are required to correlate the same with human population.