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Impact of mobile phone electromagnetic radiations on sperm parameters and histo-architecture of rat testis and protective effect of pollen and propolis in them

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ABSTRACT: Mobile phones are the reality of modern world and the emission of electromagnetic radiations by them is a fact acknowledged worldwide and the effect of EMR on humans is concerning. Findings from literature suggest that EMR from mobile phones and various other sources affect male fertility by causing negative impact on histo-architecture of testis and sperm parameters. Bee pollen and propolis are one of the natural products renowned for their medicinal properties. This study investigated the ameliorative effect of bee pollen and propolis on the adverse changes caused by EMR from mobile phone in the form of decrease in sperm count and motility and histo-distortion in testis and the observations revealed that pollen and propolis showed protection against EMR by mitigating the negative impacts caused by them.

KEYWORDS: fertility, oxidative stress, histo-architecture, exposure, sperm parameters

INTRODUCTION: Electromagnetic radiation (EMR) consists of synchronized oscillations of electric and magnetic fields travelling at the speed of light. There are a number of sources of such radiations for example radio, television, electrical appliances, mobile phones, mobile phone towers etc. and the possible effect of EMR on living organisms is a topic of concern throughout the world. Mobile phones are an integral part of modern world telecommunication system. With a subscriber base of approximately 1177 million (as of July, 2023)[1], the Indian telecom

subscription ranks among the top ones in the world. Studies on the effect of mobile phone EMR on male fertilityare conflicting and their on the reproductive system is currently under active debate[2-6]. Inspite of this, the literature supports various studies suggesting the negative impact of EMR from various sources on sperm parameters, histology of testis and consequently affecting fertility. Some of the common findings includesignificant injury in structure and function of Leydig cells in mice, whose earlier and continuous effect is bound to affect sexual function and sperm production[7], higher incidence of sperm cell death and abnormal clumping of sperm cells[8], drop in sperm concentration, motility and a significant decrease in the diameter of seminiferous tubules[9].Various natural products prove useful in mitigating such effects and in improving reproductive health.Certain products which have been used for protection against EMR exposure include*Tinosporacordifolia* root extract[10], *Moringa oleifera* leaf extract[11] and Naringenin [12].

Since ages, honey bee products have been used in traditional medicine in treating a variety of diseases as they are known to possess a number of bioactive properties. Bee pollen and propolis are two such products. Honey bees collectpollen from flowering plants, store it in their hives and use it as the primary source of food for their hive whereas propolis is a resinous mixture collected by honey bees from various botanical sources and use it as a sealant for open spaces in their hive. The biological properties of pollen include its radio-protective potential[13], anti-chemo toxicity potential[14], nutritional potential [15], reproductive potential [16], anti-allergic potential [17], hepatoprotective potential [18], anti-mutagenic potential [19], free-radical scavenging activity[20], antioxidant potential [21], genotoxicity modulator [22], anti-neurotoxicity activity [23], immune-protector [24]and haemopoietic potential [25]. The biological properties of propolis include antimicrobial, antitumor, oxygen radical scavenging, antiviral, antifungal and antioxidant [26-32]. Therefore, pollen as well as propolis is a suitable candidate to be used as a potential candidate in minimizing the alterations caused by EMR from mobile phones in the testis of rats and the same was examined in this study.

MATERIALS AND METHODS: The study was conducted in the Department of Zoology, Panjab University, Chandigarh. For these experimental investigations, Sprague Dawley rats (weighing 150-200g) of male sex were procured from Central Animal House of Panjab University, Chandigarh (animal ethical clearance for this has been obtained from Panjab University ethical committee; Approval No.- PU/45/99CPCSEA/IAEC/2018/178). Animals were maintained in an environmentally controlled animal house with a temperature of 24±3°C in a 12 h light/dark schedule. The rats were housed in polypropylene cages bedded with sterilized rice husk and provided unlimited access to clean drinking water and standard animal pellet diet. Bee pollen and propolis were collected from the colonies maintained by Department of Zoology, Panjab University, Chandigarh. Aqueous extract of bee pollen was prepared by following the method of Yamaguchi *et al*., 2007[33]. The ethanolic extract was prepared by the method of Orsi*et al*., 2007[34]. All the doses were given orally by intra gastric gavage with the help of cannula fixed on a syringe, daily, for 15 days. Aqueous extract of pollen @100mg/kg body weight and ethanolic extract of propolis @200mg/kg body weight were given in respective treatments by dissolving in water. (The control group was given water using the gastric gavage). Rats were exposed to EMR by placing mobile phones (JIOPHONE with a 200mAh battery, head SAR=0.595W/kg and body SAR=1.102W/kg) over the polypropylene cages of rats, facing downwards, in call receiver mode, working over a frequency of Indian 4G band i.e. 850/1800/2300 MHz for two different time frames, 2 hrs and 5 hrs respectively (which were chosen on the basis of results of a questionnaire based survey conducted among 1000 college

going students). A pilot study was conducted prior to the main experiment in which one mobile phone was placed over the cage of control rats as well in no call mode and it was ensured that only the placement of mobile phone did not cause any changes in the studied parameters.

Experimental Design: The animals were divided into 9 groups having 6 rats each andthe experiment was carried out for a period of 15 days.

GROUP 1: (CONTROL) Standard diet + water
GROUP 2: (POLLEN) Standard diet + pollen extract
GROUP 3: (PROPOLIS) Standard diet + propolis extract
GROUP 4: (2 HR EMR) Standard diet + Continuous EMR exposure to rats for 2 hours per day
GROUP 5: (2 HR+P) Standard diet + Continuous EMR exposure to rats for 2 hours per day + pollen extract
GROUP 6: (2HR+PR) Standard diet + Continuous EMR exposure to rats for 2 hours per day + propolis extract
GROUP 7: (5 HR EMR) Standard diet + Continuous EMR exposure to rats for 5 hours per day
GROUP 8: (5 HR+P) Standard diet + Continuous EMR exposure to rats for 5 hours per day + pollen extract
GROUP 9: (5HR+PR) Standard diet + Continuous EMR exposure to rats for 5 hours per day + propolis extract

Parameters Studied:

- **Histopathology:** The histological studies of testis were undertaken using paraffin wax section preparation and Haematoxylin/Eosin staining by the method of Pearse (1968) [35].
- **Sperm Count and Sperm Motility:** After the animals were sacrificed, the cauda epididymis (both left and right) were taken out. The cauda epididymis was nicked in several places using a scalpel blade in such a way that it extended into but not through the lumen of the duct and blood vessels were avoided. The tubule segment was immersed in 10 mL of PBS buffer facilitating dispersion into the buffer which was maintained at 37 ºC. The segment was allowed to disperse for 40 min. After 40 min, 10 µL of sample was loaded onto a clean glass slide and smear was made. It was then dipped in methanol and was allowed to dry followed by H and E staining. Sperm morphology and motility were analyzed under light microscope. For sperm count, 2 mL of PBS was added to 0.5 mL of sample prepared as above and mixed gently, maintained at 37 ºC for 10 min. 10 µL of sample was taken and loaded in the hemo-cytometer and the spermatozoa were counted under a light microscope. The final sperm count calculation was done using the following formula:

$$\text{Total Count} = \frac{\text{Mean Count} \times \text{Dilution Factor}}{\text{Volume of one primary square}}$$

From this, the total number of sperms/g of epididymis was calculated[36]. In the similar manner, number of dead sperms was counted and their number was subtracted from total number of sperms to obtain the number of motile sperms and thereafter their percentage was calculated.

- **Statistical Analysis:**The data was expressed as mean ± standard deviation and the statistical analysis was done by one way analysis of variance (ANOVA) employing GraphPad Prism 8.0.2. Values of $p \leq 0.05$ were considered to indicate s significant difference between the groups.

RESULTS AND DISCUSSION:

Sperm Count and Motility (Figure 1&&2): Literature contains a number of studies supporting deleterious effects of electromagnetic radiations on sperm parameters like sperm count,

morphology and motility[37-41], semen quality[42], an increase in the percentage of sperm cells of abnormal morphology and decreased motility[43]. From the data obtained from total sperm count and percentage motility in sperms, it was observed that there was no significant difference in sperm count as well as motility among control, pollen and propolis groups, however both pollen and propolis supplementation caused an increase in sperm count as well as sperm motility but not to a significant extent. From the results, it was evident that as compared to the control group, exposure to mobile phone radiations caused a significant decrease in total sperm count as well as percentage of motile sperms. In 2 hours EMR exposed group, the total sperm count was 127.3×10^6 which was 19.33% lower as compared to the control group. Similarly the percentage motility in 2 hours EMR exposed group was 71.37% whereas in control group was 81.5%. In 5 hours EMR exposed group, the total sperm count was 94.50×10^6 which was 40.11% lower as compared to the control group and 25.77% lower as compared to the 2 hrs EMR exposed group. The percentage motility of sperms in 5 hrs EMR exposed group was 61.41% which was significantly lower as compared to the control group as well as 2hrs EMR exposed group. This not only showed the negative impact of mobile phone EMR on sperm count and their motility but also a time-dependent decrease caused by EMR. A few similar studies also reported time-dependent decrease in sperm parameters including sperm count, motility, viability and morphology caused by EMR [44,45]. Pollen extract supplementation improved the status of total sperms as well as sperm motility in both 2hrs+pollen group ($SC=160.7\times10^6$; SMP=81.83%) and 5hrs+pollen group ($SC=183\times10^6$; SM=78.37%). Similar improvements were shown by treatment with propolis extract in both 2hrs+propolis group ($SC=166.2\times10^6$; SM=81.65%)and 5hrs+propolis group ($SC=177.2\times10^6$; SM=78.37%).

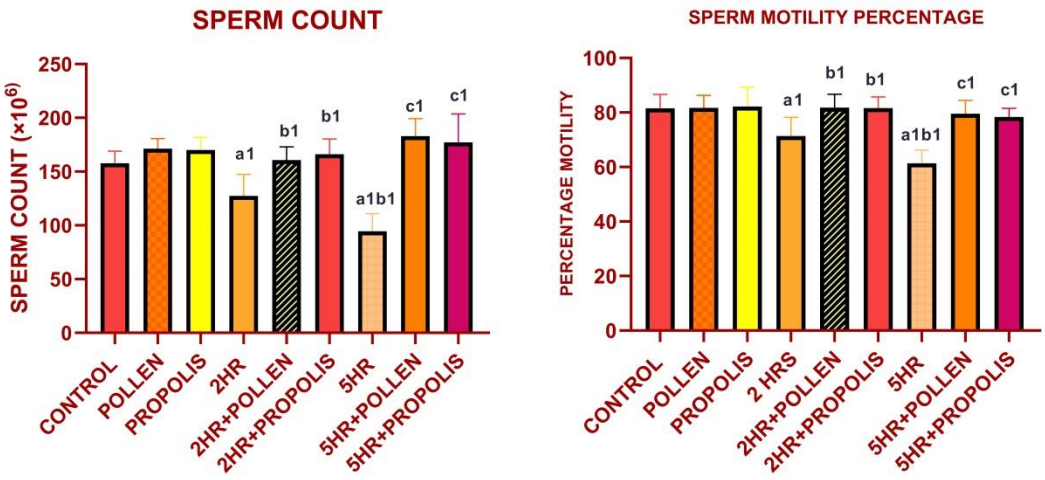
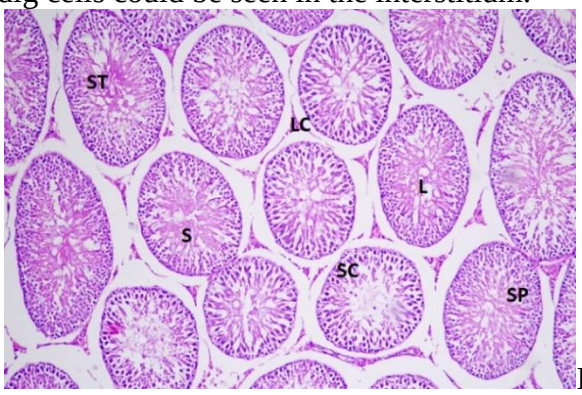
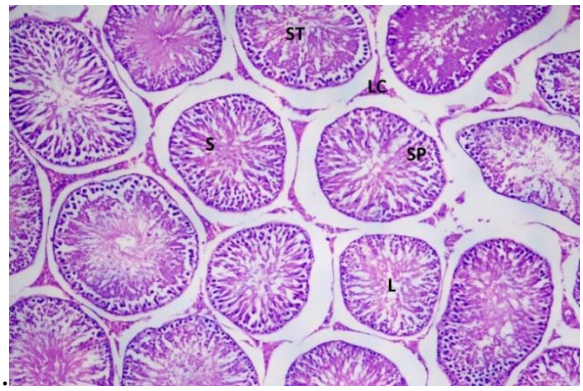


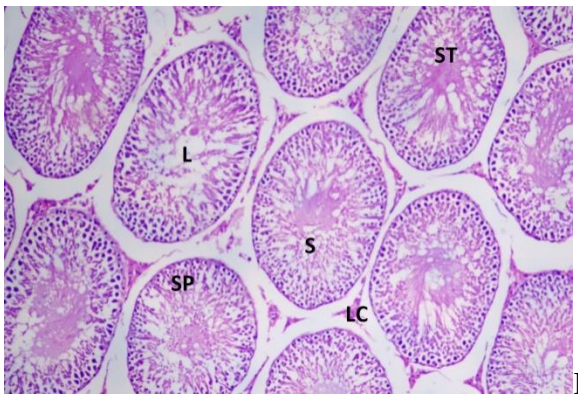
Figure 1&2. Graphical Representation of Sperm Count and Percentage of motile sperms
All the values are expressed as Mean ± S.D. (n=6)
a1 $p\leq0.05$ statistically significant difference w.r.t. control group
b1 $p\leq0.05$ statistically significant difference w.r.t. 2 hrs EMR-exposed group
c1 $p\leq0.05$ statistically significant difference w.r.t. 5 hrs EMR-exposed group

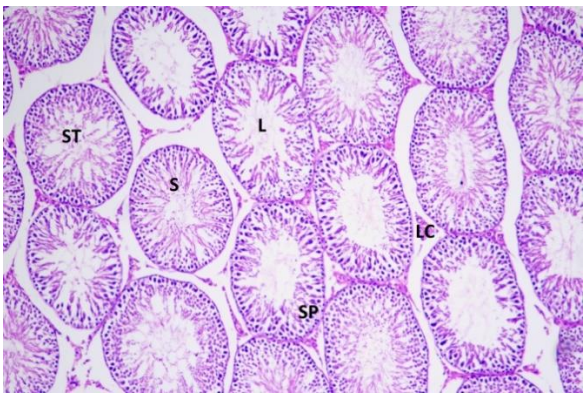
Histopathology (Figure 3): Microscopic examination of hematoxylin& eosin stained sections of testis tissue in control group showed normal architecture of seminiferous tubules. Easy identification of the spermatogonia, spermatocytes, spermatozoa, Sertoli cells and Leydig cells could be done. A large number of spermatozoa filled the lumen of seminiferous tubules. No significant difference in comparison to the normal architecture of testis was seen on

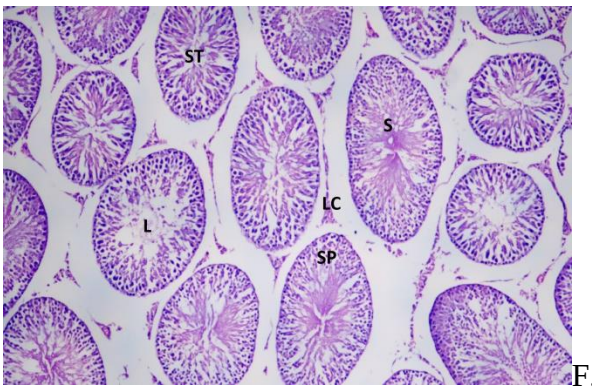
supplementation with pollen and propolis. In both these groups, the germinal epithelium was of normal thickness having adequate number of spermatozoa. They were closely connected with Sertoli cells of the epithelium. However, when rats were exposed to 2hours EMR from mobile phone, somewhat of a distorted morphology in comparison to the control group was observed. There was a decrease in the number of spermatogonia and spermatocytes which had condensed nuclei and a wider lumen as compared to control group was observed. The germinal epithelium was observed to be eroded at various places. In rats exposed to mobile phone EMR for 5 hours, it was observed that the seminiferous tubules had wavy outlines and wider lumen with lesser concentration of germ cells as compared to control group as well as 2 hours EMR exposed group. The germinal epithelium of some tubules were observed to be somewhat disrupted and showed shedding of germ cells into the lumina. A decreased volume of mature spermatozoa was seen. Debris of dead cells was observed in lumen of some of the seminiferous tubules. Deranged Leydig cells could be seen in the interstitium.

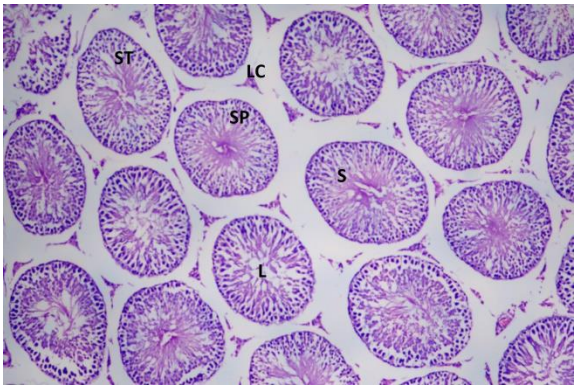
A. 

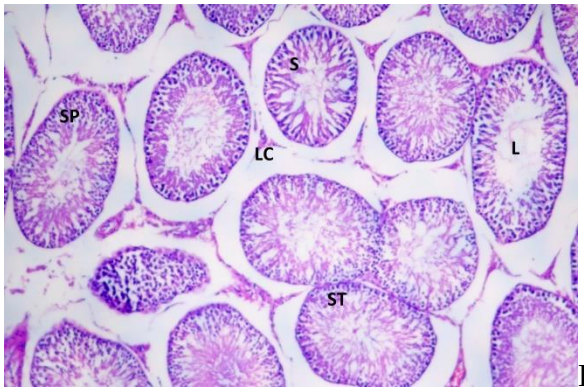
B. 

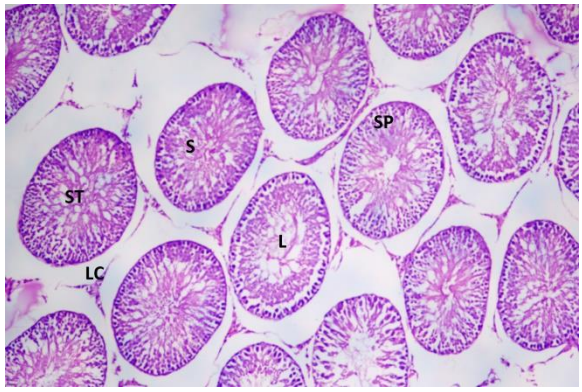
C. 

D. 

E. 

F. 

G. 

H. 

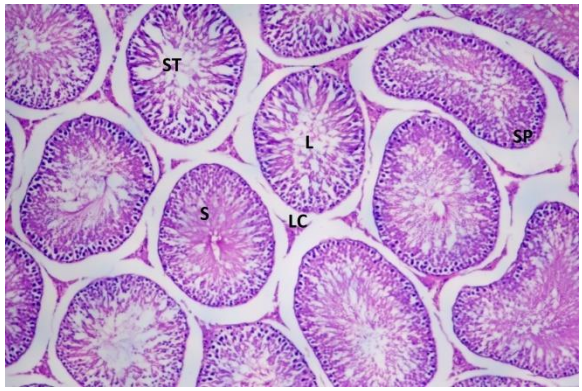
I. 

Figure 3. Light Micrograph of transverse section of testis of SD rats (40X)

A-Control; B-Pollen; C-Propolis; D-2hrs EMR; E-2hrs+pollen; F-2hrs+propolis; G-5hrs EMR; H-5hrs+pollen; I-5hrs+propolis; ST-Seminiferous Tubule; SP-Spermatocyte; S-Spermatozoa; LC-Leydig Cells; L-Lumen; SC-Sertoli Cells; GE-Germinal Epithelium

These results were supported by a study conducted in 2012 which reported that electromagnetic radiation from conventional cellular phone had a negative impact on testicular architecture and enzymatic activity. The study also indicated the possible role of vitamins C and E in mitigating the oxidative stress imposed on the testes and restoring normality to the testes[46].Various other studies from literaturereport a range of testicular maladies caused by electromagnetic radiations including depletion of spermatocytes, extensive necrosis of the germinal epithelium[47], genotoxic effect on epididymal spermatozoa [48], decreased sperm viability and motility in rats and also decreased sperm total anti-oxidant capacity leading to oxidative stress [49], decrease in Leydig cell proliferation and reduction in the levels of cell cycle distribution, secretion capacity of testosterone and reduced levels of P450scc mRNA [50]. When aqueous extract of bee pollen was given along with EMR exposure from mobile phones for both 2 hours as well as 5 hours, profound improvement in the histo-architecture of testis was observed. In both 2hrs+pollen group and 5 hrs+pollen group, the thickness of germinal epithelium increased with recovery of germ cell population. The lining of seminiferous tubules was intact and increase in number of spermatozoa could easily be observed with a normal arrangement of Sertoli cells in the tubules and Leydig cells in the interstitium. Similarly, treatment with ethanolic extract of propolis along with EMR from mobile phones for 2 hours as well as 5 hours mitigated the negative alterations caused by EMR in testis histology. Improvement in terms of thickness of germinal epithelium, increased number of germ cells, healthy and organized arrangement of Sertoli and Leydig cells, lumen filled with spermatozoa and firm lining of seminiferous tubules was observed.

The mechanism behind the observed changes in the histo-architecture of testis and decreased sperm parameters caused by mobile phone EMR as suggested by literature is the generation of oxidative stress which in turn affects various parameters of testis and thus affects fertility. During a study, while investigating the formation of free radical due to mobile phone exposure and effect on fertility pattern in 70-day-old male Wistar rats significant increase in free radical generation was observed. The findings in this study on antioxidant, malondialdehyde, histone kinase, micronuclei, and sperm cell cycle were clear indications of an infertility pattern, initiated due to an overproduction of reactive oxygen species[51]. Another study documented the impacts of RF-EMR on the male reproductive system, after considering any common observations that could provide insights on a potential mechanism and envisaged a two-step mechanism whereby RF-EMR was able to induce mitochondrial dysfunction leading to elevated ROS production[52]. It was also reported that chronic 900 MHz exposure induced oxidative damage in the blood and led to alterations in reproductive parameters in rats [53].Various other studies also implied that the mechanism behind the impact of RF-EMR on male fertility was increased level of reactive oxygen species or say oxidative stress [54-56].

To mitigate the impact of oxidative stress on various fertility parameters in the testis of rats, strong anti-oxidants are required and the two bee products used in this study are widely known for their anti-oxidant potential. Thephenolics present in bee pollen are known to be responsible for the antioxidant activity of bee pollen which include pinocembrin, quercetin, kaempferol, galangin and isorhamnetin, chrysin and caffeic acid [57-58]. Bee pollen extract has been reported to show anti-oxidant, free radical scavenging and modulatory properties against oxidative stress induced by a variety of agents [59-63]. In the present study as well, bee pollen has been able to successfully revert the negative changes caused by mobile phone EMR. Propolis is also a strong antioxidant and is used in the preparation of various medicines and cosmetics since primitive times. It is said that the flavonoids present in propolis possess anti-oxidant properties. The same has been observed during various studies conducted in the past and are available in literature[64-71]. Propolis is also pro-reproductive in nature i.e. it enhances the reproductive abilities and also mitigates the reproductive toxicity caused by a number of agents [72-74]. Recent studies also show that propolis possesses radio-protective properties [75-78]. Standing up to its reputation, in the present study as well, propolis has shown its full potential in ameliorating the oxidative stress caused by EMR from mobile phones and thereby improving the histo-architecture of testis and enhancing the sperm count and their motility.

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DECLARATION OF INTEREST STATEMENT: The manuscript has been read and approved by all the authors, the requirements for authorship as stated earlier in this document have been met, and each author believes that the manuscript represents honest work. The authors declare no conflict of interest.

CRITERIA FOR INCLUSION IN THE AUTHORS' LIST: Experimental work, manuscript writing and editing.

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