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THE PROTECTIVE EFFECTS OF BEE POLLEN AGAINST MOBILE PHONE ELECTROMAGNETIC RADIATION-INDUCED OXIDATIVE STRESS IN TESTIS OF RATS

CHAUDHARY D^{1*}, BHARTI U², KUMAR N R³

- 1: Research Scholar, Department of Zoology, Panjab University, Chandigarh, India
- 2: Associate Professor, Department of Zoology, PGGCG, Sector-11, Chandigarh, India
- 3: Retired Professor, Department of Zoology, Panjab University, Chandigarh, India

*Corresponding Author: Ms. Deepti Chaudhary: E Mail: deepti4557@gmail.com

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ABSTRACT

Modern world is the era of mobile phones which are one of the most common sources of electromagnetic radiations (EMR). All living organisms are constantly exposed to these EMR and their potential effect on biological systems is a subject of concern worldwide. According to literature EMR from mobile phones is capable of causing maladies ranging from mild to severe, oxidative damage being one of them. Bee Pollen has excellent natural antibiotic, nutritive and immune boosting properties. It is known to possess anti-oxidative, radio-protective, hepatoprotective, anti-allergic, immune-protector, anti-inflammatory, and tissue-regenerating properties which make it a potential candidate to be used in treatment against negative alterations caused by EMR from mobile phones. The objective here is to analyze and discuss the reproductive health risks associated with exposure to EMR emitted by mobile phones and the modulatory effect of bee pollen in mitigating the negative alterations. This study involved exposure of rats to mobile phones electromagnetic radiations and treatment with pollen along with EMR exposure. Thereafter comparisons and analysis of biochemical parameters of testis were carried out. The results showed that exposure to mobile phone electromagnetic radiations led to a decrease in the activity of GSH as well as various antioxidant enzymes studied (GST, SOD, CAT, GR, GPx) and caused an increase in lipid peroxidation in the testis of rats indicating oxidative stress and negative impact on fertility. However,

supplementation with pollen led to significant mitigation of the oxidative stress which suggested that pollen showed anti-oxidative, radio-protective and pro-reproductive properties against mobile phone EMR.

Keywords: exposure, reproductive health, oxidative stress, free-radical scavenger, fertility

INTRODUCTION:

We live in a digital world, a world of electronic gadgets and artificial intelligence. Electricity, mobile phones, laptops, wi-fi, smart watches and other such electronic gadgets are the basic necessities of this world. According to a report by World Economic Forum dated Oct 27, 2022, there are approximately 8.6 billion active mobile subscriptions worldwide [1]. There is no denying the fact that the lifeline of this digital world is electromagnetic radiation. There are various organizations in the world which monitor Radio Frequency Research and issue safety guidelines but a study by International Commission on the Biological Effects of Electromagnetic Fields (ICBE-EMF) reported that 25 years of substantial research on Radio-Frequency Radiations demonstrated that the assumptions underlying the FCC's (Federal Communications Commission, Australia) and ICNIRP's (International Commission on Non-Ionizing Radiation Protection, Germany) exposure limits were invalid and continued to pose a risk to public health. Adverse effects which were observed at exposures below the presumed threshold SAR (specific absorption

rate) included the non-thermal induction of reactive oxygen species (ROS), DNA damage, cardiomyopathy, carcinogenicity, sperm damage, and neurological effects, including electromagnetic hypersensitivity [2]. An extensive survey of literature on EMR suggests that it affects fertility, various body tissues including brain, kidney, liver, pancreas, causes tinnitus, painful fingers, sleeplessness, addiction, behavioral changes, causes oxidative, tissue, optical, dental and osteal damage, has impact on haematology, causes antibiotic resistance and even speech problems in children. EMR is responsible for harmful prenatal effects and is even reported to be carcinogenic. It not only affects humans but also hampers plant growth, mortality of chick embryos, disorientation and navigational problems in birds, milk yield in cows and colony development in honey bees [3-14]. According to the literature, one of the most studied aspects of electromagnetic radiation from mobile phones is its negative impact on male reproductive health. Some of the observations are summarized in **Table 1**.

Table 1: Effect of EMR on male reproductive health

S. No.	AUTHOR	OBSERVATIONS
1.	Yan <i>et al.</i> (2007) [15]	High incidence of sperm cell death and abnormal clumping of sperm cells.
2.	Desai <i>et al.</i> (2009) [16]	DNA damage (by prolonged OS), which accelerated neuronal and spermatozoal cell death and promoted neurodegenerative processes as well as promoted brain and testicular carcinogenesis.
3.	De Iuliis. (2009) [17], Kesari <i>et al.</i> (2010) [18] and Agarwal <i>et al.</i> (2011) [19]	Studies on the impact of mobile radiation on male fertility are conflicting, and the effects of the radiofrequency electromagnetic radiation (RF-EMR) emitted by these devices on the reproductive systems are currently under active debate.
4.	Salama <i>et al.</i> (2010) [20]	Drop in the ejaculation frequency in rabbits, notable increase in biting/grasping against teasers and mounting latency in accumulated means from the first to the fourth teasers.
5.	Kumar <i>et al.</i> (2011) [21]	Increase in caspase and creatine kinase and significant decrease in testosterone and melatonin.
6.	Sharma <i>et al.</i> (2011) [22]	Significant decrease in body weight, tissue weight, testes- body weight ratio and tubular diameter up to 15 days of irradiation. Occurrence of cent percent mortality by day 17th in irradiated control. Radiation induced histological lesions in testicular architecture observed to be more severe in irradiated control than the experimental group.
7.	La Vignera <i>et al.</i> (2012) [23] and Agarwal <i>et al.</i> (2014) [24]	Decrease in sperm count and motility and increase in oxidative stress.
8.	Ghanbari <i>et al.</i> (2013) [25]	Decreased sperm viability and motility in rats and also decreased sperm total anti-oxidant capacity leading to oxidative stress.
9.	Bin-Meferji <i>et al.</i> (2015) [26]	Hypospermatogenesis and maturation arrest of spermatozoa in the testes of rats.
10.	Liu <i>et al.</i> , (2015) [27]	Increase in the ROS (Reactive Oxygen Species) level and decrease in TAC (Total Antioxidant Capacity) in rat sperm. Alteration in the expression levels of apoptosis-related genes due to excessive oxidative stress triggering sperm apoptosis through bcl-2, bax, cytochrome c and caspase-3 signaling pathways.
11.	Banerjee <i>et al.</i> (2016) [28]	Significant genotoxicity by mobile phone radiation even in the permissible range when used for longer duration.
12.	Sengupta, (2016) [29], The Guardian, (2017) [30] and CNN, (2017) [31]	A decline in male sperm quality.
13.	Farag <i>et al.</i> (2018) [32]	Deterioration of histological architecture of testes and biochemical and morphometric parameters.
14.	Kesari <i>et al.</i> (2018) [33]	Deleterious effects on sperm parameters like sperm count, morphology and motility. Impact on the role of kinases in cellular metabolism, the endocrine system, produced genotoxicity, genomic instability and oxidative stress ultimately giving way to infertility.

Bee pollen: Honey bees collect pollen from flowering plants and store it in their hives. It is used as the primary source of food for the hive. It mainly consists of protein, sugars, carbohydrates, enzymes, minerals, and vitamins (A, B1, B2, B3, B5, C and H). It is considered to be a very rich source of protein. Since ages, it has been used in traditional medicine in treating a variety of diseases as it is known to possess a number of bioactive properties like radio-protective potential [34], anti-chemo toxicity potential [35], nutritional

potential [36-37], reproductive potential^[38], anti-allergic potential [39], hepatoprotective potential [40], anti-mutagenic potential [41], free-radical scavenging activity [42], antioxidant potential [43], genotoxicity modulator [44], anti-neurotoxicity activity [45], immune-protector [46] and haemopoietic potential [47]. Therefore, bee pollen is a suitable candidate to be used as a potential candidate against the alterations caused by EMR from mobile phones 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 had 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 $24\pm 3^{\circ}\text{C}$ 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 was 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) [48]. 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 was 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 from last over the cage of control rats as well along with other previously described cages 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 6 groups having 6 rats each and the 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: (2 HR EMR) Standard diet + Continuous EMR exposure to rats for 2 hours per day

GROUP 4: (2 HR+P) Standard diet + Continuous EMR exposure to rats for 2 hours per day + pollen extract

GROUP 5: (5 HR EMR) Standard diet + Continuous EMR exposure to rats for 5 hours per day

GROUP 6: (5 HR+P) Standard diet + Continuous EMR exposure to rats for 5 hours per day + pollen extract

1. Oxidative Stress Parameters:

- **Glutathione (GSH):** Activity of this enzyme was determined by the procedure described by Sedlak and Lindsay (1968) [49].
- **Lipid peroxidation (LPO):** Lipid peroxidation was assayed by the method of Buege and Aust (1978) [50].
- **Glutathione S-transferase (GST):** Estimation of GST was performed by the method of Habig *et al.* (1974) [51].
- **Glutathione peroxidase (GP):** Glutathione peroxidase was assayed by the procedure given by Pagila and Velentine (1967) [52].
- **Glutathione reductase (GR):** Glutathione reductase activity was determined by the method of Carlberg and Mannervick (1975) [53].
- **Superoxide dismutase (SOD):** Activity of this enzyme was determined by the procedure described by Kono *et al.* (1978) [54].

- **Catalase (CAT):** Catalase activity was determined by the method of Luck (1974) [55].

2. Statistical Analysis: The data was expressed as mean \pm standard deviation and the statistical analysis was done by one way analysis of variance (ANOVA) employing GraphPad Prism 8 software. Values of $p \leq 0.05$ were considered to indicate significant difference between the groups.

RESULTS:

The graphical representation of the following observations is given in **Figure 1**.

LIPID PEROXIDATION: Lipid peroxidation simply means the oxidative degradation of lipids resulting in cell damage. Malondialdehyde (MDA), a product of decomposition of lipid hydroperoxides is used here as an indicator of oxidative damage to cells and tissues. In the present study it was observed that the level of MDA in testis homogenate of control rats was 0.329 ± 0.022 nmoles/mg protein. Administration of bee pollen at a dose of 100 mg/kg body weight did not produce any significant change in the levels of MDA. In 2hr and 5 hr EMR exposed groups, the MDA levels of testis were observed to be 0.408 ± 0.028 , and 0.627 ± 0.025 nmoles /mg protein respectively indicating a highly significant ($p \leq 0.05$) increase from the control group level. The MDA levels of 5 hrs

EMR exposed group were 53.676% higher as compared to the 2 hrs EMR exposed group indicating proportionality with exposure time. On pollen supplementation, the levels of MDA decreased significantly as compared to their EMR exposed counterparts. In 2hr+pollen treatment group, the MDA levels were observed to be 12.5% lower as compared to the 2 hrs EMR exposed group and in 5 hrs+pollen treatment group, the decrease was by 14.035% as compared to the 5 hrs EMR exposed group.

REDUCED GLUTATHIONE (GSH):

Oxidative stress is harmful to cells resulting in DNA damage. The main reason behind oxidative stress is either an increase in the levels of reactive oxygen species (ROS) or decrease in the levels of antioxidants including reduced glutathione (GSH) or both. Reduced Glutathione (GSH) is a tripeptide of amino acids-glutamic acid, cysteine, and glycine. It has many biological functions including protection against reactive oxygen species. Decreased concentration of glutathione is indicative of oxidative stress. In the present study, the levels of GSH in control group were observed to be 1.548 ± 0.070 $\mu\text{moles/g}$ protein. On giving pollen, there was no significant change in GSH levels. On exposing the rats to mobile phone EMR, the levels of GSH dropped significantly i.e. by

10.788% in 2 hrs EMR exposed group and by 30.103% in 5 hrs EMR exposed group where the GSH levels of 5 hrs EMR exposed group were 21.651% lower as compared to the 2 hrs EMR exposed group suggesting that longer exposure to EMR from mobile phone caused oxidative stress to a greater extent. On treatment with pollen, the GSH levels in both 2hrs+pollen group and 5hrs+pollen group went up by 10.572% and 27.264% respectively as compared to their EMR exposed counterparts.

SUPEROXIDE DISMUTASE (SOD):

Superoxide is produced as a by-product of oxygen metabolism and it is capable of causing cell damage if not regulated properly. Superoxide dismutase, as the name itself suggests is an enzyme which causes the dismutation of the superoxide (O_2^-) radical to either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Therefore, SOD forms a very important antioxidant defense in almost all living cells that are exposed to oxygen. In the present study, it was observed that activity of SOD in the testis of control rats was 4.085 ± 0.120 IU/mg protein. The SOD activity of bee pollen supplemented group was not significantly different when compared with control rats. On exposing the rats to mobile phone EMR, the levels of SOD dropped significantly i.e. by 7.148% in 2 hrs

EMR exposed group and by 18.653% in 5 hrs EMR exposed group where the SOD activity of 5 hrs EMR exposed group was 12.391% lower as compared to the 2 hrs EMR exposed group again suggesting the proportionality of oxidative damage with exposure time. On treatment with pollen along with EMR exposure, the activity of SOD got upregulated by 9.359% in 2hr+pollen group as compared to the 2 hrs EMR exposed group and by 10.953% in 5hr+pollen group as compared to the 5 hrs EMR exposed group.

CATALASE (CAT): Similar in function to Superoxide Dismutase, Catalase (CAT) is an enzyme responsible for the decomposition of hydrogen peroxide to water and oxygen, thus preventing oxidative damage. In the present study, it was observed that the activity of Catalase in the testis of control rats was 0.192 ± 0.015 μ moles of H₂O₂ decomposed/min/mg protein. Pollen supplementation maintained the activity of CAT near control. On exposure to EMR from mobile phone for 2hrs, the activity of CAT decreased significantly by 36.458% as compared to the control group whereas on exposure to EMR for 5 hrs, the activity of CAT decreased even more i.e. by 40.104% as compared to the control group. On treatment with pollen along with 2hrs EMR from mobile phone, the activity of CAT increased

significantly by 26.229% as compared to the 2 hrs EMR exposed group and on treatment with pollen along with 5hrs EMR, the activity of CAT again increased significantly by 24.347%

GLUTATHIONE S-TRANSFERASE

(GST): GST is a family of low molecular weight cytosolic enzymes which catalyzes the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates so as to quench the reactive molecules and serve the function of detoxification. In this study, the GST activity in the testis of control rats was found to be 0.952 ± 0.036 μ moles of CDNB conjugate/min/mg protein. Pollen supplementation caused no significant alteration in the activity of GST. When the rats were exposed to EMR from mobile phones for 2 hrs, the activity of GST in their testis significantly reduced to 0.860 ± 0.038 μ moles of CDNB conjugate/min/mg protein (-9.663% as compared to control) whereas on exposure to EMR for 5 hrs further reduced the activity of GST to 0.729 ± 0.033 μ moles of CDNB conjugate/min/mg protein (-23.424% as compared to control). Pollen treatment, however, led to a significant upregulation of GST activity in both the treatment groups i.e. by 9.302% in 2hrs+pollen group and by 12.757% in 5hrs+pollen group as compared to their EMR exposed counterparts.

GLUTATHIONE PEROXIDASE (GPx):

Glutathione Peroxidase (GPx) is also a cytosolic enzyme whose function is to catalyze the reduction of H_2O_2 to water and oxygen meanwhile catalyzing the reduction of peroxide radicals to alcohols and oxygen thereby protecting from oxidative damage. In this study, the GPx activity in the testis of control rats was observed to be 0.625 ± 0.037 μ moles of NADPH oxidized/min/mg protein. Pollen supplementation maintained the activity of GPx near normal with no significant difference. EMR exposure from mobile phone for 2 hrs caused a significant decrease in the activity of GPx i.e. by 14.72% and for 5 hrs caused an even further decrease in GPx activity i.e. by 30.08% as compared to the control group. It was also observed that the activity of GPx in 5hrs EMR exposed group was 18.011% lower as compared to the 2 hrs EMR exposed group suggesting the oxidative stress due to EMR exposure was time dependent. Pollen treatment significantly mitigated the effect of EMR exposure by causing a significant increase in the activity of GPx in both the treatment groups i.e. by 13.508% in 2hrs+pollen group and by 16.933% in 5hrs+pollen group as compared to 2hrs and 5hrs EMR exposed groups respectively.

GLUTATHIONE REDUCTASE (GR):

In simple words, the main function of Glutathione reductase is to maintain the supply of GSH which plays a key role in the cellular control of reactive oxygen species. The mechanism of its action involves the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) utilizing NADPH in the process. In the present study, the activity of GR in the testis of control and pollen supplemented groups were found to be 0.325 ± 0.017 μ moles of NADPH oxidised/min/mg protein and 0.342 ± 0.015 μ moles of NADPH oxidised/min/mg protein with no significant difference with each other. EMR exposure from mobile phone for 2 hrs significantly decreased the activity of GR by 10.153% as compared to the control group and exposure for 5 hrs also significantly reduced the activity of GR by 30.461% as compared to the control group and by 22.602% as compared to the 2hrs EMR exposed group. On treatment with pollen, the activity of GR increased significantly in both the treatment groups i.e. by 10.273% in 2hrs+pollen group as compared to the 2 hrs EMR exposed group and by 21.681% in 5hrs+pollen group as compared to the 5hrs EMR exposed group.

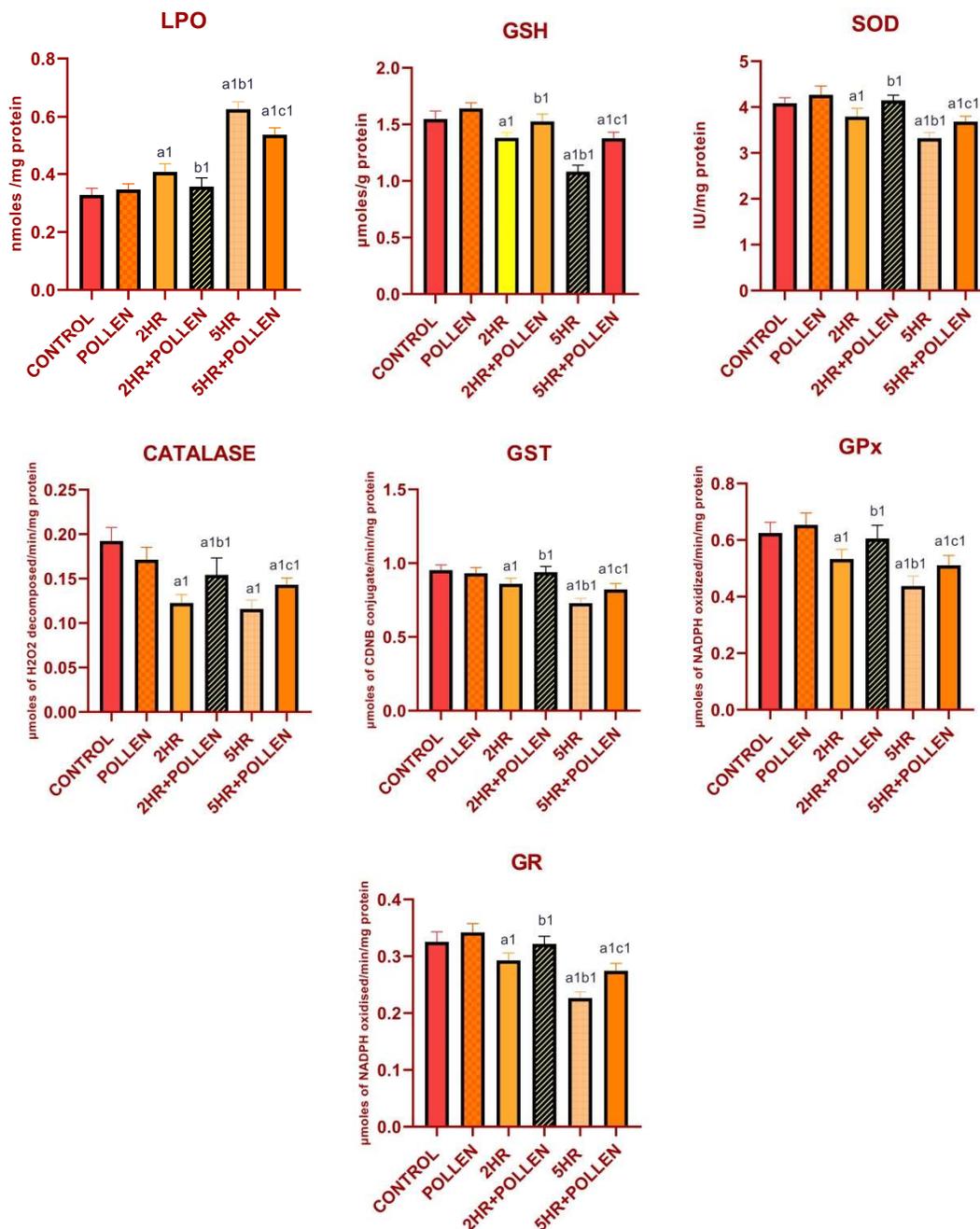


Figure 1: Graphical Representation of biochemical parameters of testis of SD rats
 All the values are expressed as Mean ± S.D. (n=6)
 a1 p<0.05 statistically significant difference w.r.t. control group
 b1 p<0.05 statistically significant difference w.r.t. 2 hrs EMR-exposed group
 c1 p<0.05 statistically significant difference w.r.t. 5 hrs EMR-exposed group

DISCUSSION:

Due to the widespread use of mobile phones throughout the world, its impact on human health is a major concern among researchers. Previously it was a notion that the negative impact caused by mobile phone electromagnetic radiation was due to heating of the device i.e. thermal effect. Another reason behind such an assumption was the non-ionizing nature of mobile phone electromagnetic radiations but there are always new aspects to human and technological research. Literature proves that the electromagnetic radiations emitted by mobile phones, irrespective of being non-ionizing, are capable of interfering with the oxidative repair mechanisms [56]. A study in 2016 reported that all type of man-made electromagnetic radiations including those generated by mobile phones were capable of inducing oxidative stress. The mechanism behind this involved the dysfunction caused in the voltage gated ion channels in the cell membranes [57]. It has been reported that polarized/coherent electromagnetic fields, even at very low field intensities in the ULF (ultra low frequency, 300Hz-3KHz) and ELF (extremely low frequency, 3-30 Hz) bands, could cause irregular gating of electro-sensitive ion channels or Voltage Gated Ion Channels on the cell membranes by the 'ion

forced-oscillation mechanism', consequently causing the disruption of the electrochemical balance of the cell [58-62]. In a review study conducted in 2017, it was stated that intracellular ROS levels depend upon the dynamic balance between ROS generation and elimination which gets influenced by magnetic fields. In most of the studies reviewed, magnetic fields increased ROS levels in various types of cells in humans, mice and rats. It also stated that multiple factors could cause such discrepancies, including but not limited to the type/intensity/frequency/exposure time of the magnetic fields [63].

While studying various markers of oxidative stress ((LPO, GSH, GST, SOD, CAT, GR, GPx) in this study, the common observation was the positive relation between EMR (from mobile phones) exposure and testicular oxidative stress. Exposure to EMR both for 2 hours as well as 5 hours led to an increase in the levels of lipid peroxidation whereas caused a decrease in the activity of various other antioxidant enzymes like GST, SOD, CAT, GR, GPx and also a decrease in the levels of reduced glutathione (GSH). These results were supported by various studies conducted in the past to evaluate the extent of oxidative stress caused by electromagnetic radiations from mobile phones. This was

supported by a study conducted in 2009, which reported that RF-EMR exposure resulted in a significant increase in lipid peroxidation and low GSH content in the testis and epididymis [64]. Another study reported a 3-5 fold decrease in the glutathione and GPx levels in the EMR exposed groups while investigating the possible effects of EMR from conventional cellular phone use on the oxidant and antioxidant status in rat blood and testicular tissue. Their observations included a significant increase in the diameter of seminiferous tubules [65]. Literature supports a number of studies conducted over a past few decades to evaluate the extent of oxidative stress caused by electromagnetic radiations from mobile phones as well as other sources using oxidative stress markers including MDA, LDH, NO, SOD, GPx, CAT, GR, urea, uric acid, creatinine, ALT (Alanine Transaminase), AST (Aspartate Aminotransferase), bilirubin, total antioxidative capacity, oxidative stress index, lipid hydroperoxide, total thiols, FRAP (ferric reducing ability of plasma) etc. Observations included similar findings involving an increase in the levels of MDA, lipid hydroperoxide and NO and decrease in the levels of total thiols, FRAP, urea, uric acid, creatinine, ALT, AST, bilirubin and decreased activity of enzymes like SOD, GR, GPx, CAT

leading to oxidative stress [66-72]. The observations also suggested that more the exposure time to EMR, more was the extent of oxidative damage i.e. exposure to EMR from mobile phone for 5 hours caused more significant negative alterations in the biochemical markers studied as compared to the 2 hours EMR exposure. Similar observations related to the time dependent impact of EMR were obtained during a study in which the authors observed a decrease in the sperm parameters in a time dependent pattern [73]. Bee pollen is a wonder product of honey bees and possesses anti-oxidant, free radical scavenging, radio-protective and pro-reproductive properties [74, 75]. Various studies in literature reported that bee pollen alleviated the negative changes in oxidative stress markers. Bee pollen supplementation has been reported to cause a significant decrease in the level of lipid peroxidation while elevating the activity of SOD, GR, GPx, GST, CAT and GSH representing reduced oxidative stress [76-78]. In the present study as well, supplementation with pollen along with exposure to EMR from mobile phone helped in ameliorating the oxidative stress caused by exposure alone by causing a decrease in the levels of lipid peroxidation and increase in the levels of GSH. Pollen supplementation along with exposure also

ensured an increase in the activity of all the anti-oxidant enzymes under study as compared to the non-pollen fed EMR exposed rats.

CONCLUSION:

The observations suggest that mobile phone EMR is capable of causing an oxidative disbalance in the testis of Sprague Dawley rats which can be mitigated to a significant extent on supplementation with pollen. This opens a door towards further research towards finding the mechanism behind the said biochemical alterations and modulatory effect of bee pollen on them.

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CRITERIA FOR INCLUSION IN THE

AUTHORS' LIST: Experimental work, manuscript writing and editing.

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