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### RESEARCH ARTICLE

## AMELIORATING ROLE OF MELATONIN AGAINST 2.45 GHZ MICROWAVE RADIATION INDUCED OXIDATIVE STRESS IN TESTIS OF SWISS ALBINO MICE.

\*C. L. Jonwal<sup>1</sup>, Rashmi Sisodia<sup>1</sup>, V. K. Saxena<sup>2</sup>, Archana Sharma<sup>1</sup> and Faiza Rifat<sup>1</sup>.

1. Department of Zoology, Rajasthan University, Jaipur, Rajasthan.

2. Department of Physics, Rajasthan University, Jaipur, Rajasthan.

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### Abstract

**Purpose:** Owing to growing demand of electronic appliances such as microwave oven and cell phones microwave (MW) radiations have been gradually increasing and it may adversely affect the reproductive pattern. The present study aimed to investigate the ameliorating role of melatonin against 2.45 GHz microwave radiations induced oxidative stress in testes of Swiss albino mice. **Materials and methods:** 6-8 weeks old male Swiss albino mice, weighing  $35 \pm 3$ gms were procured from inbred colony and were divided into four groups ( $n = 8/\text{group}$ ): Sham exposed, Melatonin (Mel) treated (2mg/kg), Microwave exposed with 2.45 GHz and Microwave + Melatonin (2mg/kg) treated. Microwave exposure was given in plexiglas cages for 2 hrs/day for 30 days. The power density was measured as  $0.25 \text{ mW/cm}^2$  and the specific absorption rate (SAR) was calculated to be  $0.09 \text{ W/kg}$ . **Results:** After completion of exposure period, mice were sacrificed and various stress related parameters: Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT), Malondialdehyde (MDA), Reactive oxygen species (ROS) and Protein carbonyl content were performed. Result shows that Melatonin prevented oxidative damage biochemically by significant increase ( $P < 0.001$ ) in SOD, GPx, protein carbonyl content, testicular weight and sperm count ( $P < 0.01$ ) and decreased ( $P < 0.001$ ) levels of MDA, CAT and ROS in MW + Mel group as compared to MW exposed group. **Conclusion:** Oxidative stress due to MW radiation caused adverse effects in testes of mice and melatonin supplementation proved to be a strong antioxidant and could improve the detrimental effects of microwave radiations.

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### Introduction:-

Humans in modern society are exposed to an ever-increasing number of electromagnetic fields (EMFs) generated from different sources of electromagnetic radiation (EMR) such as microwave ovens, civil and military radars, satellites, wireless communication, frequency modulation (FM). These electronic devices work on microwaves and are therefore constant source of microwave radiations. Microwaves (MWs) are a part of electromagnetic spectrum with frequency ranging from 300 MHz to 300 GHz and wavelength from 1mm to 1m. Widespread and haphazard usage of microwaves in industrial, scientific, medical, military and domestic applications has affected male

**Corresponding Author:- Rashmi Sisodia.**

Address:- Department of Zoology, Rajasthan University, Jaipur, Rajasthan.

reproductive system adversely. The leakage of EMF radiation into the environment is inescapable for human being and has posed a grave concern on human health (Crouzier et al., 2007; Wang et al., 2005). Reproductive cells or spermatozoa are of particular concern against exposure to microwave radiation as DNA integrity of spermatozoa is extremely important because they pass the genetic material to the next generation. The male reproductive system is highly vulnerable to physical factors and environmental assaults as they cause various reproductive disorders like testicular abnormalities, atypical sperm, chromosomal aberrations, infertility, testicular cancer and congenital defects in offspring (Havas, 2000; Ramadan et al., 2002; Kim et al., 2007). Chronic exposure to EMFs can enhance generation of ROS which in turn increases lipid peroxidation in addition to a decrease in antioxidant enzymes and vitamin A and E levels (Nazioglu et al., 2009; Kumar et al., 2011; Avendano et al., 2012; Atasoy et al., 2013; Aweda et al., 2003; Oksay et al., 2012). Paulraj and Behari (2004), suggested that EMFs cause non-thermal effects in tissues which in turn leads to various detrimental effects on male reproductive system. A plethora of studies have reported that male reproductive organs are highly sensitive to the extremely low frequency magnetic fields (Agarwal et al., 2003; Forgacs et al., 2006; Pasqualotto et al., 2000; Shen et al., 2000). MW radiations cause many adverse effects on reproductive system and may lead to infertility (Kesari et al., 2010, 2011; Nisbet et al., 2012). A few studies have suggested that increased oxidative stress leads to biochemically increased lipid peroxidation and free radical formation, (Kumar et al., 2011; Meral et al., 2007; Rao et al., 2008). MW radiations toxicity is mediated by oxidative stress and over production of ROS (De-Iullis et al., 2009; Oktem et al., 2005). Hence, a strong and effective anti-oxidant supplementation could be beneficial in preventing or reducing some complications of MW radiations in male reproductive system (Al- Damegh, 2012; Ilhan et al., 2004; Oral et al., 2006; Sokolovic et al., 2008). The cellular activities of spermatozoa are adversely affected by over production of free radicals due to microwave radiations (Balci et al., 2007; Kumar et al., 2010; Meral et al., 2007; Rao et al., 2008). Spermatozoa are susceptible to MW radiations because spermatozoa exhibit an inevitable lack of intrinsic oxidative enzymes like CAT, GPx, SOD as well as non-enzymatic molecules such as vitamins C or E or glutathione, which counteract the deleterious effects of oxidants in all aerobic cells. In light of the intrinsic lack of antioxidant protection with the extraordinary high lipid content of the plasma membrane, the spermatozoa are extremely vulnerable to oxidative stress (Henkel, 2011). Dasdag et al. (1999), observed abnormal changes in testicular function exposed to EMF through decreased sperm count along with narrower seminiferous tubules. Melatonin (*N*-acetyl-5-methoxytryptamine) is a hormone produced by the pineal gland and considered to be the most powerful endogenous antioxidant. It stimulates GPx and can play a role in the prevention of oxidative damage (Reiter., 1995). Recent studies have demonstrated that Melatonin is an antioxidant, which scavenges hydroxyl radicals generated *in vitro* by hydrogen peroxide exposed to ultraviolet light (Bayda et al., 2001; Reiter., 1999). It is an efficient scavenger of peroxy radicals than vitamin E (Pieri et al., 1994; Gulcin et al., 2003). The purpose of the present study was to evaluate the effects of MW radiations induced oxidative stress in testes and potential protective role of Melatonin against oxidative stress.

### Materials and Methods:-

The reagents and Melatonin were procured from Rahsiff scientific, New Delhi. The chemicals were of analytical grade (Merck and Sigma). All solutions were prepared on the day of experiment. Melatonin was administered orally to the mice (2 mg/kg body weight) following the work of Sokolovic et al. (2008).

### Experimental animals:-

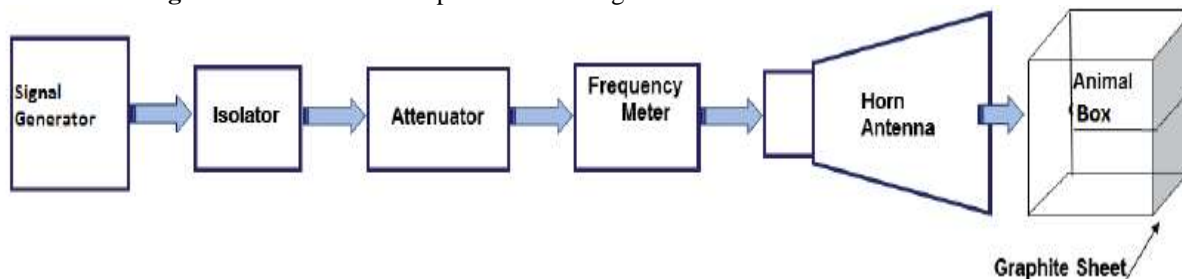
32 adult male Swiss albino mice, 6-8 weeks old, weighing  $35 \pm 3$  grams were used for the present study. Mice were maintained in the animal house as per the norms established by Institutional Animal Ethical Committee (IAEC). The mice were housed in clean plexiglas cages and maintained under controlled conditions of temperature with constant 12-h light and 12-h dark schedule and provided with standard mice feed and water *ad libitum*. The research was approved by the Departmental Ethical Committee (DEC) of Department of Zoology, University of Rajasthan for animal use.

### Exposure chamber:-

The experimental study was done using microwave radiation experimental bench (figure 1). The bench consists of signal generator, isolator, attenuator, frequency meter, horn antenna and a specially designed animal cage. A graphite sheet was used to minimize the reflection of scattered beam. A rectangular box made of plexiglas was used to house the mice. The box was properly partitioned and well ventilated with holes of 1 centimeter diameter. The dimensions of the box ( $4.5 \times 9 \times 9$  cm) were such that animals were comfortably placed, though they could not move much. Signal generator was capable of producing electromagnetic radiations up to 20 GHz frequencies and maximum power 1 watt. In this experiment it was set to generate microwave radiations of frequency 2.45 GHz and power density was measured to be  $0.25 \text{ mW/cm}^2$  at the center of the box by a power meter which is a peak sensitive

device ('RF power sensors 6900 series' and 'IFR 6960B RF power meter'; made of Aeroflex Inc., Wichita, KS, USA). The horn antenna was kept in H-plane configuration so that electric field of the waves was perpendicular to the ground surface. The electric field in the box was almost uniform because the dimensions of the box were of the order of one wavelength. Two experimental set-up were calibrated identically and were used for exposure to mice. Every day two mice were housed at a time in the box which was placed at the same location facing the horn antenna. No animal blocked the radiations falling on other animal. All the experiments were performed and repeated in a blind manner. The temperature in the chamber was maintained around 25-27°C throughout the experiment. The mice were exposed with 2.45 GHz MW radiations through the horn antenna for 2 hrs/day for 30 consecutive days. The whole-body specific absorption rate (SAR) was estimated to be 0.09 W/kg following the method of Durney et al. (1984).

**Figure 1:-** Sketch of the experimental arrangement of 2.45 GHz microwave radiations



#### **Experimental design:-**

32 mice were divided into four groups, each group consists of eight animals (n=8).

##### **Group I: Sham exposed (Control)**

Mice were kept in a plexiglas cage and placed in-front of the horn antenna aperture for 2 hrs/day for 30 days, without energizing the system. Mice were administered distilled water as control.

##### **Group II: Melatonin treated**

Mice were kept in a plexiglas cage and placed in-front of the horn antenna aperture for 2 hrs/day for 30 days, without energizing the system. Mice were administered Melatonin solution (2mg/kg body weight).

##### **Group III: Microwaves exposed**

Mice were kept in a plexiglas cage and placed in-front of the horn antenna aperture and exposed to 2.45 GHz microwaves for 2 hrs/day for 30 days. Mice were administered distilled water.

##### **Group IV: (MW exposed + Mel)**

Mice were supplemented with Melatonin 2mg/kg once daily 1 hr before exposure to 2.45 GHz for 2hrs/day for 30 consecutive days. Melatonin was administered at 08.00 AM daily to avoid its effects as neurotransmitter or neuromodulator (Drago et al., 2001). Other researchers used the same dose of melatonin in their studies (Meena et al., 2013; Koc et al., 2003; Sokolovic et al., 2008).

#### **Testicular weight:-**

The mice were sacrificed by cervical dislocation immediately after the completion of last exposure treatment. The testes were excised out, and adherent tissues were removed, and weighed by a Mettler analytical balance (BL-220H, Shimadzu Corporation, Kyoto, Japan).

#### **Sperm Count:-**

For sperm count caput and cauda were minced in 5 ml of PBS solution and left the solution for 10 minutes to release sperms and then the solution was taken in a test tube and centrifuged for a few seconds at 100 rpm so as to separate the sperms in the supernatant from the pellet. The supernatant for sperms count was diluted further and 10 µl of diluted sample was placed on the hemocytometer [Neubauer improved double ruling (Fein-Optik, Blankenburg, Germany)]. Sperms were counted in 5 squares of slide and then the average was used for calculations. Total sperms in four different groups were counted by following the equation given below.

$$\text{Sperm Count} = (\text{Dilution Factor}) (\text{Count in 5 squares}) (0.05 \times 10^6)$$

#### **Protein carbonyl content:-**

For this assay, Levine et al., (1994) protocol was used for detecting and quantifying oxidative modification of proteins owing to formation of carbonyl groups in proteins due to oxidative stress. Determination of carbonyl content is based on the reaction of carbonyl groups with 2,4-dinitrophenylhydrazine to form a 2,4-

dinitrophenylhydrazine. The assay was determined spectrophotometrically and reactive carbonyl derivatives were assessed by using DPNH molar extinction coefficient at 370 nm ( $22 \times 10^3$  L/mol/cm) and expressed as  $\mu\text{mole/mg}$  protein.

#### **Lipid peroxidation (LPO):-**

For LPO assay, the amount of Malondialdehyde (MDA) was assessed by reaction of TBARS (Thiobarbituric acid reactive substances) following protocol given by Buege and Aust (1978). A 10% tissue homogenate of testicular tissue (100 mg) was prepared in 9 ml of 1.15% KCl. Tissue homogenate (0.8 ml) was mixed with 1.2 ml solution of TCA (15% w/v), TBA (0.375% w/v) and HCl (0.25N) prepared in a 1:1:1 ratio. This final mixture was heated in a water bath for 30 min at  $90^\circ\text{C}$  and cooled. Then 2ml of freshly made NaOH solution was added to it. After centrifugation the absorbance was recorded at 532 nm using a UV-vis double beam spectrophotometer. A standard curve was prepared by using TMP. After comparison with a standard curve the LPO level was expressed in nmol MDA/gm tissue.

#### **Glutathione (GSH):-**

The GSH level was measured by the method of Moron et al., (1979). 100 mg testicular tissue was homogenized in the sodium phosphate-EDTA buffer then 0.6 ml DTNB solution was added to it. The optical density of the complex developed by the reaction of GSH and DTNB was measured at 412 nm using a UV spectrophotometer. The results were expressed as nmol GSH/100 mg of tissue.

#### **Superoxide Dismutase (SOD):-**

SOD was calculated following the work of Marklund and Marklund (1974). 100 mg testicular tissue was homogenized in 1ml of NaCl. Then it was centrifuged for 10 min. then 0.1 ml of supernatant was taken and mixed with 2.7ml of Tris buffer and then 0.1ml of pyragallol was mixed to this solution. Absorbance was taken at 420 nm. The results were expressed as  $\mu\text{mole/mg}$  tissue.

#### **Catalase (CAT):-**

Catalase enzyme was estimated by Aebi et al., (1984). Tissue homogenate was prepared in 5ml of phosphate buffer with 50mg of testicular tissue and centrifuged at  $4^\circ\text{C}$  for 10 minutes at 10,000 rpm. 0.1ml of supernatant was mixed with 1 ml PBS and 0.4 ml  $\text{H}_2\text{O}_2$ . Absorbance was measured at 420 nm. The results were expressed as nmole/ml.

#### **Reactive oxygen species (ROS):-**

ROS was measured by method described earlier ( Lee et al., 2006). Briefly 50 mg of testis tissue from each mouse was taken out and it was homogenized in 1 ml of PBS (0.1M  $\text{Na}_2\text{HPO}_4$ , 0.1M  $\text{KH}_2\text{PO}_4$ , 1.37M NaCl, 2.7mM KCl, pH 7.4) using homogenizer. It was passed through 100  $\mu$  pore sized cell strainer to get single cell suspension. Then 1 ml of RBC lysis buffer added with single cell suspension and kept for 10 minutes at RT. The suspension was centrifuged at 1200 rpm, for 10 minutes at  $4^\circ\text{C}$ . The pellet was resuspended in 1 ml of PBS and the cells were treated with 2' 7'- dichlorofluorescein diacetate (3.3 $\mu\text{M}$ ) (DCFH-DA; Molecular probes, Eugene, OR, USA). DCFH-DA is permeable to cell membrane it enters the cell and hydrolysed to DCFH by cell esterases. DCFH is non-fluorescent, impermeable to the cell membrane and readily reacts with intracellular hydrogen peroxide in the presence of cell peroxides and change into fluorescent- DCF, whose florescence can be measured using flow cytometry (Rastogi et al., 2010 ; Szejda et al., 1984). The cells were incubated at RT for 10 minutes in dark and washed using PBS the cells were examined using flow cytometry (Guava technologies, CA and USA) total 5000 cells were analysed in each sample to get the mean fluorescence intensity and were corrected for auto fluorescence of the unlabeled cells.

#### **Statistical analysis:-**

The values were expressed as mean  $\pm$  SD. Data were analyzed using One-way ANOVA with Bonferroni post-hoc test. *P* value at 0.05 was considered as the level of significance.

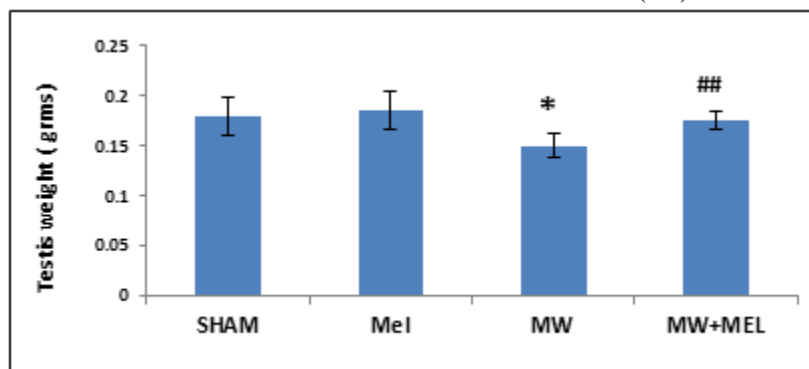
#### **Results:-**

##### **Testicular weight:-**

MW exposure resulted in significant reduction ( $P < 0.01$ ) in testicular weight in exposed group ( $0.15 \pm 0.012$ ) as compared to the control group ( $0.179 \pm 0.019$ ; Figure 2) and Mel group ( $0.185 \pm 0.017$ ). Melatonin administration prior to exposure resulted in highly significant increase ( $P < 0.001$ ) in testicular weight of MW + Mel Group ( $0.175$

$\pm 0.009$ ). Reduction in testicular weight indicates that chronic microwave radiation induces atrophy in testis and Melatonin treatment ameliorates detrimental effects of MWs.

**Figure 2:-** Ameliorating role of Melatonin on testicular weight in Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).

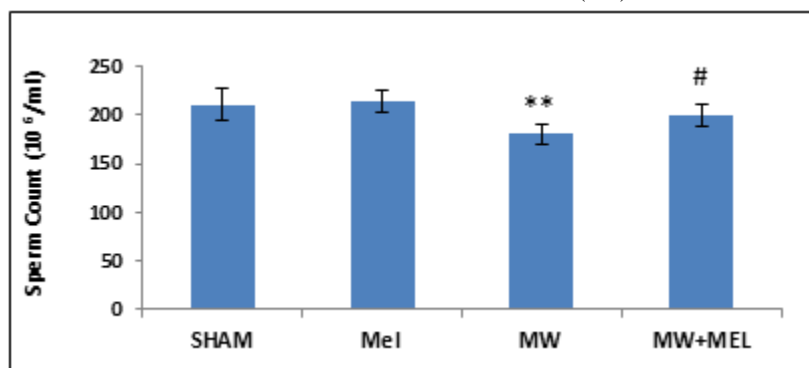


\* $p < 0.01$  (versus sham exposed and Mel), ##  $p < 0.001$  (versus MW)

#### Sperm Count:-

MW exposure resulted in highly significant reduction ( $P < 0.001$ ) in sperm count of exposed group ( $180.87 \pm 16.57$ ) as compared to control group ( $211.12 \pm 10.72$ ; Figure 3) and Mel group ( $213.5 \pm 11.5$ ). Melatonin administration prior to exposure resulted in significant increase ( $P < 0.01$ ) in sperm count of MW + Mel Group ( $199.87 \pm 11.67$ ).

**Figure 3:-** The effects of Melatonin on sperm count in Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).

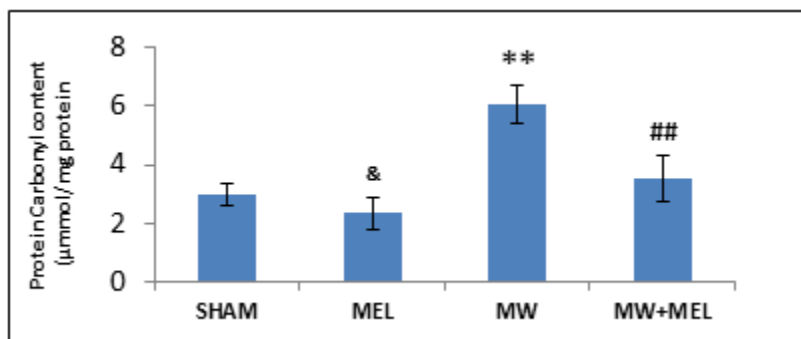


\*\* $p < 0.001$  (versus sham exposed and Mel), #  $p < 0.01$  (versus MW)

#### Protein carbonyl content:-

MW exposure led to significant higher ( $P < 0.001$ ) Protein carbonyl content in testicular tissue of exposed group ( $6.07 \pm 0.64$ ) as compared to sham exposed ( $2.98 \pm 0.37$ ; Figure 4) and Mel group ( $2.34 \pm 0.56$ ). Whereas, Melatonin treatment prior to irradiation prevented the protein oxidation and could bring Protein carbonyl content levels significantly low ( $P < 0.001$ ) in MWs + Mel group ( $3.52 \pm 0.77$ ).

**Figure 4:-** Concentration of carbonyl groups ( $\mu\text{mol}/\text{mg}$  protein) in testicular tissue of Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).

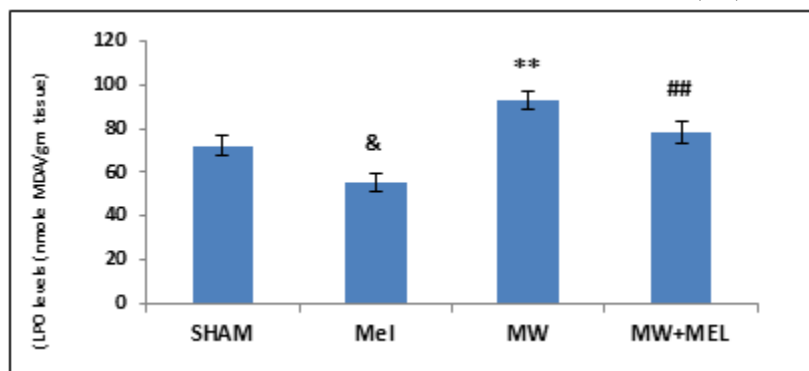


\*\* $p < 0.001$  (versus sham exposed and Mel), ## $p < 0.001$  (versus MW), &  $p < 0.01$  (versus sham exposed)

#### Lipid peroxidation (LPO):-

Analysis of LPO by thiobarbituric acid reaction showed a highly significant ( $P < 0.001$ ) increased levels of MDA in the MW exposed group ( $92.78 \pm 4.09$ ) as compared to sham exposed group ( $72.2 \pm 4.83$ ; Figure 5) and Mel group ( $55.6 \pm 3.9$ ). Whereas Melatonin treatment prior to irradiation reversed the adverse effects of MWs and resulted in highly significant decrease ( $P < 0.001$ ) in MW + Mel. Group ( $78.38 \pm 5.08$ ).

**Figure 5:-** Protective role of Melatonin on Malondialdehyde (MDA) levels in testis of Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).

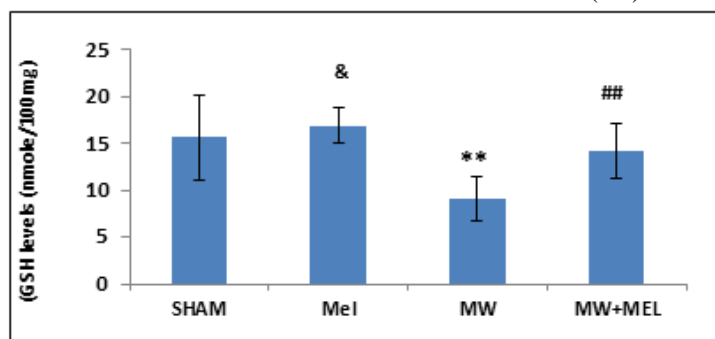


\*\* $p < 0.001$  (versus sham exposed and Mel), ## $p < 0.001$  (versus MW), &  $p < 0.01$  (versus sham exposed)

#### Glutathione (GSH):-

MW exposure resulted in significant decrease ( $P < 0.001$ ) in GSH in MW exposed group ( $9.1 \pm 2.34$ ) as compared to sham exposed group ( $15.62 \pm 4.60$ ; figure 6) and Mel group ( $16.89 \pm 1.9$ ). Meanwhile Melatonin treatment prior to irradiation resulted in highly significant increase ( $P < 0.001$ ) in MW + Mel. Group ( $14.2 \pm 2.96$ ).

**Figure 6:-** Protective role of Melatonin on GSH levels in testis of Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).

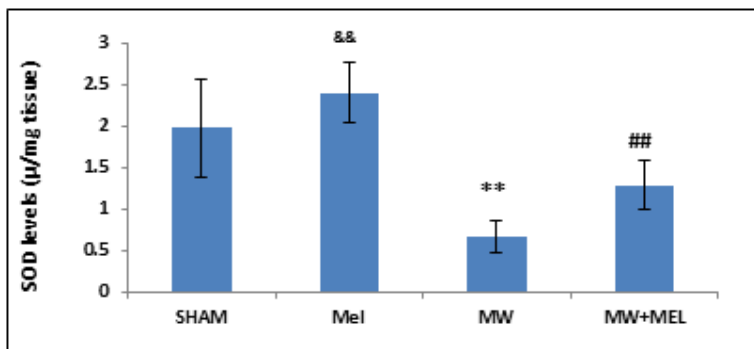


\*\* $p < 0.001$  (versus sham exposed and Mel), ##  $p < 0.001$  (versus MW), &  $p < 0.01$  (versus sham exposed)

#### Superoxide Dismutase (SOD):-

MW exposure resulted in highly significant ( $P < 0.001$ ) decrease in SOD in MW exposed group ( $0.67 \pm 0.198$ ) as compared to sham exposed group ( $1.98 \pm 0.59$ ; figure 7) and Mel group ( $2.4 \pm 0.36$ ). Meanwhile Melatonin treatment prior to irradiation resulted in highly significant increase ( $P < 0.001$ ) in MW + Mel. Group ( $1.29 \pm 0.29$ ).

**Figure 7:-** The effects of Melatonin on SOD levels in testis of Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).

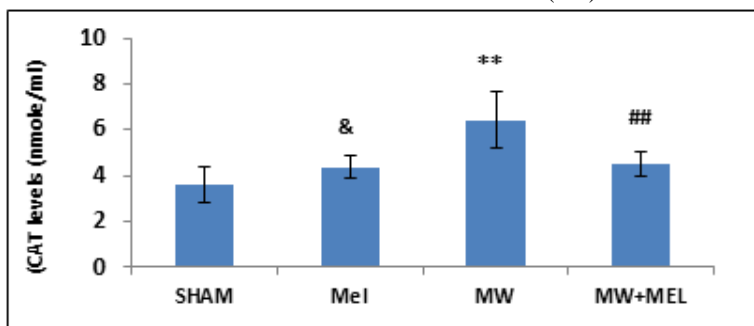


\*\* $p < 0.001$  (versus sham exposed and Mel), ##  $p < 0.001$  (versus MW), &&  $p < 0.001$  (versus sham exposed)

#### Catalase (CAT):-

MW exposure showed a highly significant ( $P < 0.001$ ) increase in CAT in the MW exposed group ( $6.43 \pm 1.21$ ) as compared to sham exposed group ( $3.61 \pm 0.76$ ; Figure 8) and Mel group ( $4.37 \pm 0.48$ ). Meanwhile Melatonin treatment prior to irradiation resulted in highly significant decrease ( $P < 0.001$ ) in MW + Mel. Group ( $4.51 \pm 0.51$ ).

**Figure 8:-** The effects of Melatonin on CAT levels in testis of Swiss albino mice exposed to 2.45 GHz microwave radiations. The values are means  $\pm$  (SD).



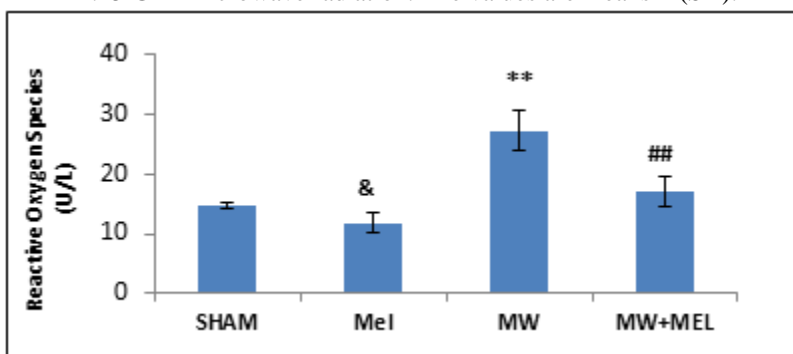
\*\* $p < 0.001$  (versus sham exposed and Mel), ##  $p < 0.001$  (versus MW), &  $p < 0.01$  (versus sham exposed)

#### Reactive oxygen species (ROS):-

MW exposure resulted in highly significant ( $P < 0.001$ ) increase in ROS in MW exposed group ( $27.23 \pm 3.39$ ) as compared to sham exposed group ( $14.7 \pm 0.61$ ; Figure 9) and Mel group ( $11.82 \pm 1.8$ ). Meanwhile melatonin treatment prior to irradiation resulted in highly significant decrease ( $P < 0.001$ ) in MW + Mel. Group ( $17.16 \pm 2.60$ ).



**Figure 9:-** The preventive effects of Melatonin on reactive oxygen species in testis of Swiss albino mice exposed to 2.45 GHz microwave radiation. The values are means  $\pm$  (SD).



\*\* $p < 0.001$  (versus sham exposed and Mel), ##  $p < 0.001$  (versus MW), &  $p < 0.01$  (versus sham exposed)

### Discussion:-

The findings of our experiments suggest that chronic MW radiation at 2.45 GHz increased the formation of MDA and badly affected the activities of antioxidants. The generation of ROS in the testicular cells consequent upon microwave exposure may be a possible interaction between EMFs radiation and biological system. ROS and free radicals are generated in the cells by energy transfer or by electron transfer reactions induced by EMF exposure. During this process a highly reactive singlet of oxygen atom is formed which results in the sequential reduction to various molecules of the cell such as superoxide, hydrogen peroxide, and hydroxyl radical (Georgiou, C. D. 2010). The various detrimental effects of 2.45 GHz EMFs on reproductive system were studied and EMFs were attributed to male infertility (Saygin et al., 2011; Kumar et al., 2011; Naziroglu et al., 2009; Hossmann et al., 2003). However each healthy cell is fortified with self-defense mechanism containing antioxidant enzymes: SOD, GPx and CAT so as to maintain a healthy balance of intracellular ROS. These antioxidants convert harmful substance into less harmful molecules. EMFs exposure to male reproductive system was reported to have developed testicular abnormalities, atypical sperm, chromosomal aberrations and congenital defects in offspring (Havas, 2000). The EMFs radiation emitted from various sources leads to the generation of ROS and causes male infertility (Kumar et al., 2010a,b). Free radicals cause various adverse changes in enzymes and structural proteins by introducing carbonyl groups (aldehydes and ketones) into proteins which leads to oxidative modification of proteins and may manifest in a variety of physiological and pathological processes (Levine et al., 1994). In support of above studies we have assessed the activities of antioxidant enzymes such as SOD, CAT, GPx, in mice testes and our results are in the line of earlier findings (Meena et al., 2013; Shang et al., 2004; Awad et al., 2006). Lipid peroxidation induces cellular injury to the spermatozoa due to leakage of sperm membrane fluidity (Aitken et al., 1994). The extent of microwave damage to the cell membrane of spermatozoa was monitored by measuring the amount of thiobarbituric acid reactive material (MDA) produced when polyunsaturated fatty acids in the membrane undergo peroxidation and the amount of solute leakage from cells. The increase in MDA level in our study seems to support the effect of electromagnetic field on sperms because mammalian sperm membranes contain highly unsaturated fatty acids and are also sensitive to oxygen-induced damage mediated by lipid peroxidation and free water-induced oxygen (Russo et al., 2006). In addition to this, since spermatozoa lack capacity for DNA repair on its own and therefore, would be removed from the germinal epithelium carrying the damages (Aitken and Koppers., 2011). Long-term exposure to EMF has adverse effects on the proliferation and differentiation of spermatogonia and this may be important in understanding the pathogenesis of EMFs induced male infertility proposed by Kim et al. (2007). EMF exposure causes abnormal changes in testicular function through decreased sperm count along with narrower seminiferous tubules (Dasdag et al., 1999). Lipid, Proteins, and DNA are the major categories of macromolecules that are damaged by free radicals and many studies have examined the role of Melatonin in protecting cell membrane lipids from free-radical destruction (Reiter et al., 1997; Reiter et al., 2000). Our findings show that Melatonin treatment in animals exposed to MW led to significant reduction in lipid peroxidation, protein oxidation and ROS. In vitro and in vivo studies have also shown that melatonin reduces the accumulation of the major products of lipid peroxidation when cell membranes are exposed to radical-generating agents. The mechanism for the inhibition of lipid peroxidation by Melatonin probably includes direct scavenging of the initiating radicals, especially  $^{\bullet}\text{OH}$  and  $\text{O}_2^{\bullet-}$ . Our results demonstrated that the activities of GSH were significantly different between the sham and the MW exposed groups because it has been studied that Melatonin functions in the recycling of GSH by promoting the activities of several enzymes, including GSH-Px, glutathione reductase and glucose-6-phosphate dehydrogenase



(Tan et al., 2000). Melatonin is also known to increase the tissue mRNA levels for SOD, the enzyme which dismutates  $O_2$  to  $H_2O_2$ , and catalase, which metabolically removes  $H_2O_2$  from the intracellular environment, thereby further reducing the  $H_2O_2$  levels and  $^*OH$  generation (Reiter et al., 2000). Based on our findings, it may be concluded that Melatonin treatment decreases the lipid peroxidation and stimulates the antioxidant enzyme activities in radiation induced testes and thus alleviates radiation toxicity to a large extent in the testes.

### Conclusion:-

It is concluded that 2.45 GHz MW radiation decreases the oxidative stress defense potential of male germ cells (spermatozoa) and it adversely affects the normal functions of testis. Reduction in GPx and SOD activity along with increased CAT, ROS, MDA and protein carbonyl content, observed in our study are clear indication of infertility pattern owing to overproduction of ROS under microwave field exposure. Melatonin has clear antioxidant properties and is likely to be a valuable drug for protection against detrimental effects of MW radiation and may be used as an antioxidant against oxidative stress. However, further experiments on this subject are needed to elucidate the mechanism of oxidative stress and protective role of Melatonin.

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### Declaration of interest:-

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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