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Research Article

EFFECTS OF MICROWAVE RADATIONS (2.45 GHz) ON BIOCHEMICAL ALTERATIONS IN BRAIN OF SWISS ALBINO MICE AND ITS AMELIORATION BY MELATONIN

Rajendra Jat*¹, Najendra Singh¹, Sanjay Singh¹, Rashmi Sisodia¹,
Deepak Bhatnagar² and Virender Saxena²

¹Neurobiology Laboratory, Centre for Advanced Studies, Department of Zoology,
University of Rajasthan, Jaipur

²Department of Physics, University of Rajasthan, Jaipur

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ABSTRACT

The widespread use of cellular telephones in recent years inevitably raises the question of the effects on brain function of the electromagnetic fields (EMF) emitted by such telephones. The aim of this work was to investigate the ameliorating role of melatonin against 2.45 GHz microwave radiation induced oxidative stress in brain of Swiss albino mice, changes in the biochemical parameter of the Swiss albino mice under the conditions of 2.45 GHz microwave radiation field exposition. 6-8 weeks old male Swiss albino mice, weighing 25±2gm were procured from inbred colony and were divided into four groups (n = 6/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 0.17 mW/cm² and the SAR was calculated 0.225 W/kg. After completion of exposure period, mice were sacrificed and various stress related parameters, such as Superoxide dismutase (SOD), Lipid peroxidation (LPO), GSH, Catalase (CAT) were performed. Result shows that melatonin prevented oxidative damage biochemically by significant increase ($P < 0.001$) in Catalase (CAT) and in Lipid per oxidation (LPO) was noted whereas significant decrease ($P < 0.001$) in Superoxide Dismutase (SOD), and Glutathione (GSH) in MW + Mel group compared to MW exposed group. It may be concluded that microwaves at 2.45 GHz frequency caused oxidative stress mediated cellular toxicity which leads to adverse and detrimental biochemical changes in brain of *Swiss albino* mice.

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INTRODUCTION

The electromagnetic field generated from the extremely low frequencies (30-300 Hz) to radiofrequency and microwaves (100 kHz-300 GHz) is usually recognized as non-ionizing radiation. Microwave radiation is a part of non-ionizing electromagnetic radiation present in the environment and this radiation has become a threat to human health since the introduction of wireless communication system and its increased usage (Croft RJ *et al.*, 2004). The environmental issues surrounding mobile phones are proving to be a sizeable challenge. The potential adverse effects of these non-ionizing radiations may depend on their accumulation over a long period of time. Public concerns about possible hazardous effects of exposure to these frequencies are increasing in our society due to increased usage of devices such as mobile phones, TV, Wi-Fi, etc. Microwave ovens operate by emitting a very high power signal in the 2.4 GHz band. Older devices

have poor shielding, and often emit a very "dirty" signal over the entire 2.4 GHz band. This can cause considerable difficulties to Wi-Fi and video transmission, resulting in reduced range or complete blocking of the signal.

The exposures to extremely low frequency magnetic field (ELF-MF) in the environment have increased considerably. Epidemiological studies suggest that there is a possible association between ELF-MF exposure and increased risks of cardiovascular disease, cancers and neurodegenerative disorders. There is evidence that microwaves may produce adverse biological effects in the nervous system (Kesari and Behari, 2009, Sharma *et al.*, 2014, Pall, 2015 and Deniz *et al.*, 2017), even at low levels of radiation power (Jauchem 2008).

The effect of microwave (MW) radiations on biological systems is primarily identified as due to an increase in temperature i.e. thermal though non thermal effects have also been identified (Paulraj and Behari 2004). Researchers have

*Corresponding author: **Rajendra Jat**

Neurobiology Laboratory, Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur

been done pointing enhancement of the presence of free radicals after electromagnetic field (EMF) exposure (Kumar *et al.* 2010, Yoshikawa *et al.* 2000) which lead to a wide varieties of clinical disorders and environmental stress (Houten *et al.* 2006). Microwave radiation exposure may alter the normal antioxidants level due to formation of free radicals (Kesari *et al.* 2010). Melatonin has direct free radical scavenging and indirect antioxidant activities, possibly from its ability to limit free radical generation at the mitochondrial level. Melatonin (N-acetyl-5-methoxytryptamin) is a hormonal product of the pineal gland. Its synthesis is higher at night than during the day in all vertebrates including man. Once melatonin is produced in the pineal gland it is quickly released into the vascular system. The rapid release of melatonin is generally believed to relate to its high lipophilicity which allows it to readily pass through the membrane of the pinealocytes and the endothelial cells which line the capillaries. The result of the nocturnal synthesis and secretion of melatonin is high blood levels at night. Melatonin is a very potent hydroxyl radical scavenger, free radicals and the hydroxyl radical in particular, because of its very high reactivity, can be extremely damaging to macromolecules in cells. Melatonin is a more powerful scavenger and affords protection of molecules, especially DNA, from oxidative damage. Whereas the free radical quenching activity of melatonin does not require a receptor, Reiter (1993) reported that it may be bound in the nucleus thereby providing on-site protection to DNA. Besides scavenging the highly toxic hydroxyl radical, melatonin also stimulates glutathione peroxidase activity which metabolizes the precursor of the hydroxyl radical, hydrogen peroxide, to water. Thus, melatonin has at least two means to protect the cell from oxidative damage, i.e., it breaks down hydrogen peroxide to harmless water and, in the event any hydroxyl radicals are formed, melatonin scavenges them. Melatonin may be the premier molecule to protect the organism from oxidative damage (Reiter RJ 1993).

MATERIALS AND METHODS

Experimental animals

Male Swiss albino mice (*Mus musculus*) 6-8 weeks old, weighing 25±5 grams were procured from an inbred colony. They were housed in clean polypropylene cages and maintained under controlled conditions of temperature (25 ±1.5°C) and light (12 hr light and 12 hr dark). They were provided with standard mice feed in form of pellets procured from Ashirwad Industries, Chandigarh and water *ad-libitum*. Only healthy mice were used for the experiments.

2.45 GHz exposure system, exposure conditions and dosimetry

Microwave radiation experimental bench (fig.1) was used for exposure to mice. The bench consists of signal generator (N5181A MXG Analog Signal Generator, Agilent Technologies, USA), isolator, attenuator, frequency meter, horn antenna (32.4×24.8 cm²) and a specially designed animal cage. A graphite sheet was also used to minimize the reflection of scattered beam. Eight mice were housed at a time in a rectangular partitioned cage made of plexiglass which was well ventilated with holes of 1 centimeter (cm) diameter. The dimensions of the cage (4.5×9×9cm) were such that animals were comfortably placed, though they could not move. The

horn antenna was kept in H (Magnetic field) plane configuration. Therefore electric field was perpendicular to the ground surface. Field was almost uniform because the dimension of the cage is of the order of wavelength. At near field distance from the horn antenna, it was found that the power density measured was 0.174 mW/cm² (milliwatt/centimeter square) which was maximum. The mice were exposed with 2.45 GHz MW radiation source through the antenna for 2 hours/ day for 30 days as shown in Fig.1. The whole microwave exposure system facility was provided by Department of Physics, University of Rajasthan, Jaipur.

Specific Absorption Rate (SAR)

The emitted power of microwaves was measured by a power meter (fig.1) which was a peak sensitive device (RF power sensors E4418 EPM Series Power Meters, made of Agilent Technologies, Inc. Headquarters: Santa Clara, California, USA). Every day, the cage was placed in the same position below the horn antenna. A similar experiment was performed with the sham-exposed animals without energizing the system. Power density was recorded (0.174 mW/cm²) and SAR was calculated following the work of Durney *et al.* (1984) as 0.368 W/kg (estimation was based per animal).

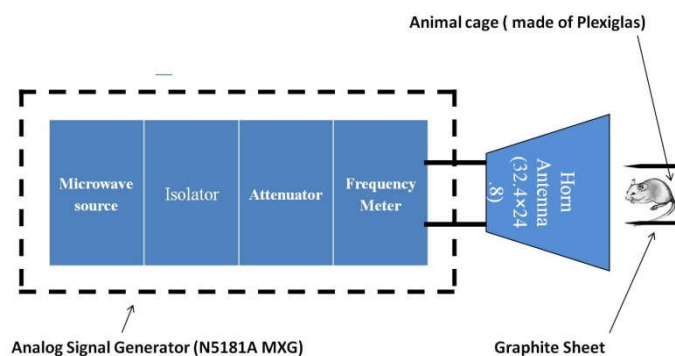


Figure 1 Diagrammatic view of 2.45 GHz microwave exposure setup (N5181A MXG Analog Signal Generator, Agilent Technologies, USA).

Drug (Melatonin)

Melatonin was procured from HiMedia Laboratories, Mumbai, India. Distilled water was used as the solvent. The concentration of melatonin in distilled water was made according to the individual body weight (2 mg per kg body weight) of mice as per earlier study (Sokolovic *et al.* 2008).

Removal of Brain Tissue

Six mice were sacrificed by cervical dislocation from each group for biochemical assays after completion of experiment. An incision was made at the sides of the jaws to separate the upper and the lower palates. The upper palate was cut in the middle and after having cleared of the surrounding tissue; brain was excised and separated from the spinal cord at the decussation of the pyramids. The intact whole brain was then removed carefully and homogenate was prepared and used for biochemical estimations of LPO (Lipid peroxidation), GSH (Glutathione), SOD (Superoxide dismutase) and Catalase

Experimental design

To study impact of microwave exposure on whole brain of Swiss albino mice and its amelioration by melatonin, mice were divided into four groups viz.-

Group I: Sham exposed (without energizing the system)

This group was sham exposed for 2hr/day for 30 days.

Group II: Melatonin treated (without energizing the system)

This group was Melatonin treated for 2hr/day for 30 days.

Group III: Microwaves exposed.

This group received 2.45 GHz microwaves for 2hr/day for 30 days.

Group IV: Melatonin + Microwaves

This group was supplemented with melatonin (2 mg/ kg body weight) one hour before whole body microwave exposure for 2 hr/day (2.54 GHz) for 30 days.

Mice in all the groups were autopsied by cervical dislocation immediately after completion of experiment. Brain was removed, weighed and then used for various biochemical studies

Biochemical Estimations in Brain

Lipid Peroxidation (LPO) Assay

LPO was measured by the method of Buege and Aust (1978). A 10% tissue homogenate of the brain (1 g) 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)- HCl (0.25N) prepared in a 1:1:1 ratio. This final mixture was heated in a water bath for 30 min at 80°C and cooled. After centrifugation the absorbance was recorded at 532 nm using a UV-vis double beam spectrophotometer 2203 (Systronics india Ltd. Naroda, Ahmedabad, Gujarat, India). A standard curve was prepared by using TMP. After comparison with a standard curve the LPO level was expressed in n mol gm/tissue.

1. **Glutathione (GSH) Assay-** The reduced GSH content of tissue samples was determined in brain by the method of Moron *et al.* (1979). A tissue sample was homogenized in the sodium phosphate-EDTA buffer. 0.6ml DTNB was added to it. The optical density of the yellow colored complex developed by the reaction of GSH and DTNB was measured at 412 nm using a UV-vis spectrophotometer. The results were expressed as nmol GSH/100 mg of tissue.
2. **Superoxide Dismutase Assay-** SOD was measured by the method of Marklund & Marklund (1974). 100mg of tissue was dissolved in 1 ml of NaCl for homogenate preparation, 0.1 ml of supernatant was taken for estimation. This tissue sample was then homogenised in 2.7 tris buffer and 0.1 ml of pyragallol was mixed to this solution. Absorbance was measured at 420 nm.
3. **Catalase Assay-** Estimation of catalase enzyme was based on the method proposed by Aebi *et al.* (1984). Homogenate was prepared by adding 5 ml of phosphate buffer in 0.5 gm of tissue which was centrifuged for 10 mins at 10,000 rpm. 0.1ml of supernatant was mixed with 1 ml PBS and 0.4 ml H₂O₂. Absorbance was read at 420 nm.

Statistical analysis

Data were analyzed using two-way ANOVA (Analysis of variance) followed by Student's t-test.

RESULTS

Lipid peroxidation

Lipid peroxidation product as reflected by thiobarbituric acid substance (TBARS) equivalent content was quantified in all four groups of mice after completion of experiment. LPO in Melatonin treated mice (Group II) declined significantly (p<0.001) compared to sham irradiated mice (group I). Microwave exposure (Group III) resulted in highly significant (p<0.001) increase in the level of lipid peroxidation in mice whole brain compared to sham irradiated mice. Oral supplementation of Melatonin prior to 2.45 GHz exposure (Group IV) decreased LPO significantly (p<0.001) in mice compared to Group III mice (Fig.2).

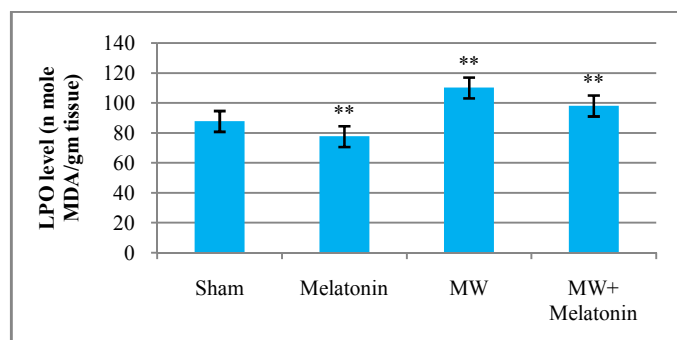


Fig 2 Variations in LPO levels (n mole MDA/gm tissue) ± SD in whole brain of Swiss albino mice after 30 days exposure to 2.45 GHz microwaves in the presence and absence of melatonin supplementation

Statistical comparison:

Sham vs Melatonin, Sham vs Microwaves, Microwaves vs Microwaves+Melatonin

**-. p<0.001- highly significant, *- p<0.05- significant, n- non- significant

Reduced Glutathione

Exposure to 2.45 GHz microwaves resulted in sharp deficit in GSH level estimated immediately after completion of exposure compared to sham irradiated mice. Melatonin supplementation before microwave exposure could significantly (p<0.001) ameliorate the GSH levels in mice compared to only microwaves exposed mice. Supplementation of only Melatonin to mice could maintain a significantly higher level of GSH content in mice whole brain (Fig.3).

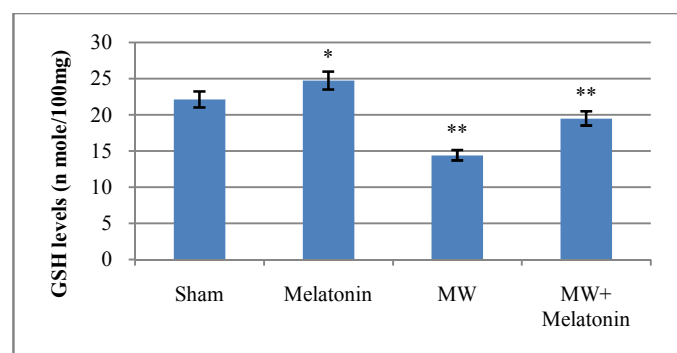


Fig 3 Variations in GSH levels (n mole/100mg) ± SD in whole brain of Swiss albino mice after 30 days exposure to 2.45 GHz microwaves in the presence and absence of melatonin supplementation

Statistical comparison:

Sham vs Melatonin, Sham vs Microwaves, Microwaves vs Microwaves+Melatonin

**-. p<0.001- highly significant, *- p<0.05- significant, n- non- significant

Superoxide Dismutase

A sharp fall in SOD activity was noticed after completion of microwave exposure. However the mice group supplemented with Melatonin (Group IV) showed an increased SOD activity. This implies that irradiation from microwaves resulted in the generation of superoxide radicals. Supplementation of Melatonin to microwaves exposed group (Group III) increased the levels of SOD in mice whole brain but it was still much lower as compared to sham. Only Melatonin treated groups (Group IV) exhibited an increased SOD activity to eliminate the radicals. These patterns of SOD were simultaneous with that of GSH (Fig.4).

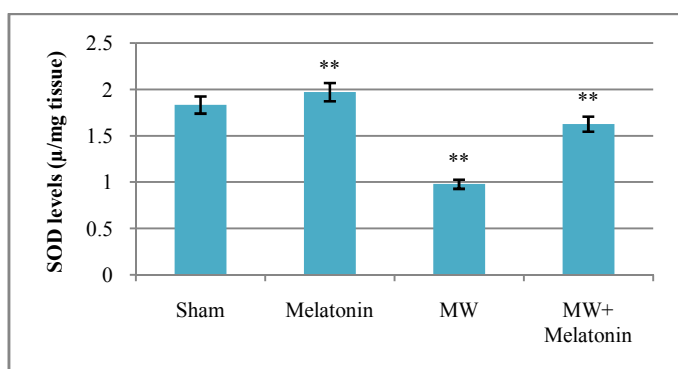


Fig 4 Variation in SOD levels ($\mu\text{/mg tissue}$) \pm SD in whole brain of Swiss albino mice after 30 days exposure to 2.45 GHz microwaves in the presence and absence of melatonin supplementation

Statistical comparison:

Sham vs Melatonin, Sham vs Microwaves,

Microwaves vs Microwaves+Melatonin

** - $p < 0.001$ - highly significant, * - $p < 0.05$ - significant, n - non- significant

Catalase

Microwave exposure (Group III) resulted in a highly significant increase in CAT activity compared to the sham exposed (Group I) mice. Supplementation of Melatonin prior to microwave exposure (Group IV) resulted in statistically highly significant decreased CAT activity in comparison to microwaves exposed group (Group III) (Fig.5)

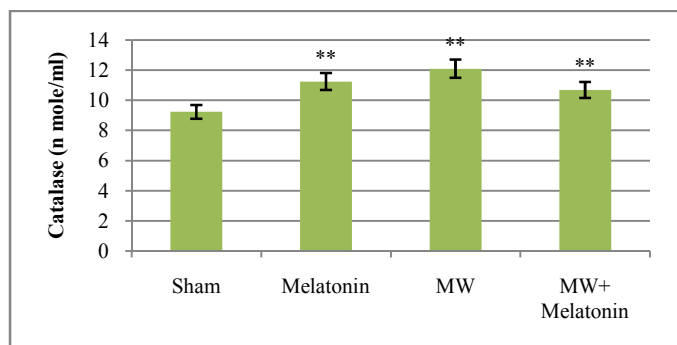


Fig 5 Variations in CAT (nmole/ml) \pm SD in whole brain of Swiss albino mice after 30 days exposure to 2.45 GHz microwaves in the presence and absence of melatonin supplementation

Statistical comparison:

Sham vs Melatonin, Sham vs Microwaves,

Microwaves vs Microwaves+Melatonin

** - $p < 0.001$ - highly significant, * - $p < 0.05$ - significant, n - non- significant

DISCUSSION

The potential of EMF adversely affecting the health of the human population is an issue which continues to receive a great deal of attention in both public and scientific forums. There is

growing evidence that the effects of microwave irradiation are mediated by the formation of ROS and free radicals, which are highly reactive, removing hydrogen atoms from fatty acids, causing lipid per oxidation and consequently cell death (Moustafa *et al.* 2001, Stopczyk *et al.* 2002). The LPO is a good biomarker of damage occurring due to radiation and inhibition of LPO is suggestive of radio protective action. Significant acceleration in the oxidation of lipids associated with depletion in antioxidant enzymes levels due to 2.45 GHz microwave exposure were noticed in our study. Lipid per oxidation not only damages cell membranes, but its products such as MDA also induce damage to other enzyme systems and DNA as well (Noda *et al.* 1993) Lipid per oxidation has been reported to be directly proportional to oxidative stress where the efficacy of various defense mechanisms is weakened. The defense mechanism may be strengthened by the addition of exogenous substance. Melatonin treatment significantly lowered the radiation-induced LPO in terms of MDA. Kumar *et al.* (2012) also reported increase in MDA in sperms of microwaves exposed male wistar rats. The inhibition of LPO in bio membranes can be caused by antioxidants. Earlier studies also showed that exposure to 10 GHz MW significantly increased LPO contents of mice spleen (Rifat *et al.* 2013, Sharma *et al.* 2014, Walani *et al.* 2014) and in blood serum which can be modulated by supplementation of exogenous substances (Singh *et al.* 2007, Sisodia *et al.* 2008, Sisodia *et al.* 2009, Sisodia *et al.* 2011, Sisodia *et al.* 2013). Increase in LPO after MW exposure in the present study gets support from the findings of Deshmukh *et al.* (2013) and Rifat *et al.* (2013). Mechanisms of antioxidative action of vitamin C are direct scavenging and blocking of ROS, as well as regeneration of other antioxidative systems (Griffith and Lunec 2001). Ascorbic acid, melatonin and ocimum flavonoids have also been reported to reduce lipid peroxidation in mice (Jagetia *et al.* 2003, Taysi *et al.* 2003). The presence of antioxidants in the Melatonin suppresses the formation of free lipid radicals and thus prevents the formation of endoperoxides. The antioxidative activity of Melatonin may be due to the reduction of hydroperoxides, inactivation of free radicals, chelation of metal ions or combinations thereof.

Glutathione is an important non-enzymatic antioxidant which plays a critical role in cellular defense system against toxic chemicals of exogenous and endogenous origin. Depletion of cellular GSH increases cell vulnerability to oxidative stress (Sharma *et al.* 2012). In the present study the decrease in the activities of antioxidant enzyme GSH in brain tissue noticed may be due to the damaging effect of free radicals produced following radiation exposure or alternatively could be a direct effect of formaldehyde formed from oxidation of free radicals, on these enzymes. The central nervous system (CNS) is inherently susceptible to oxidative injury, and because there are relatively low levels of endogenous antioxidants in the CNS, that sensitivity has been implicated as playing a causative or contributory role in a number of pathologic conditions. Microwaves produce thermal effects on biological systems at high power levels. The energy absorption at high power levels probably lead to nonspecific stimulation of hypothalamic-hypophyseal axis with liberation of corticosterone which causes sequestration of cells, an effect induced by any known stressor (Sri Nageswari 2003). Decreased activity of GSH in exposed group (Group III) may be due to decrease in its

formation, which requires NADPH and GR (Irmak *et al.* 2003, Zwirska-Korczała *et al.* 2005). Normally GSH enzyme works in parallel with SOD. Free radicals are produced continuously and detoxified by SOD, glutathione (GSH), and catalase (CAT). With excessive free radical production and the resulting consumption of antioxidants, endogenous defence mechanisms become insufficient. The decreased activities of both SOD and GSH in the brain exposed to electromagnetic radiation indicate the highly reduced capacity to scavenge hydrogen peroxide produced in brain in response to acute stress. Our results are in line with the studies reported of Kesari *et al.* (2011) which indicated a significant ($p < 0.005$) decrease in the level of glutathione peroxidase, superoxide dismutase and an increase in catalase activity of rat brain exposed to 900 MHz.

Normally, SOD enzyme works in parallel with GSH, playing an important role in the reduction of hydrogen in the presence of GSH forming GSSH (glutathione disulfide) and protects cell proteins and cell membranes against oxidative damage (Kumar *et al.* 2010). Lower levels of SOD should lead to higher levels of oxidative stress, which should in turn result in an increase in reactive species that are derived from superoxide, e.g., H_2O_2 , and existing data shows that the presence of such compounds can reduce the effects of a subsequent insult. Other studies also suggest that a decrease in the level of SOD activity may indicate an increase in the generation of reactive superoxide ions in the biological samples (Alvarez *et al.* 1989).

Treatment of rats before and after EMR exposure with melatonin (Lai and Singh 1997) and vitamin E (Koana *et al.* 1997) was found to block the adverse effect of EMR, possibly by affecting the lifetime of the radicals. Melatonin in the similar way may block the formation or scavenge the free radicals/ hydrogen peroxide resulting in maintenance of the levels of SOD in microwaves exposed mice.

The increased activity of catalase activity noted may be to compensate the overproduction of reactive oxygen species which are overproduced in brain by electromagnetic field (Kumar *et al.* 2010). The charge of detoxification is taken by CAT enzyme, which leads to increase in its activity. Zwirska-Korczała *et al.* (2005) also reported a decrease in activity of SOD, GSH and increase activity of CAT. Indeed CAT activity is enhanced when H_2O_2 levels are particularly high (Jones *et al.* 1981). Pierce *et al.* (1991) showed that H_2O_2 is able to induce apoptosis, which can be prevented by CAT. To overcome the effect of ROS on cells, more catalase is produced which in turn further induce apoptosis. When there is imbalance between production of ROS and its neutralization, it leads to oxidative stress. Such conditions can lead to necrosis or apoptosis and tumor promotion (Kesari *et al.* 2011).

Melatonin scavenges a diversity of reactive oxygen and nitrogen species including hydroxyl radical, singlet oxygen, hydrogen peroxide, nitric oxide and peroxyxynitrite anion. Based on the analyses of structure-activity associations, the indole moiety of the melatonin molecule is the reactive center of interaction with oxidants due to its high resonance constancy and very low activation energy barrier towards the free radical reactions. However, the methoxy and amide side chains also contribute significantly to melatonin's antioxidant capacity. The N-C=O structure in the C3 amide side chain is the functional group. The carbonyl group in the structure of N-C=O is key for

melatonin to scavenge the second reactive species and the nitrogen in the N-C=O structure is necessary for melatonin to form the new five membered ring after melatonin's interaction with a reactive species. The methoxy group in C5 appears to keep melatonin from exhibiting prooxidative activity. If the methoxy group is replaced by a hydroxyl group, under some in vitro conditions, the antioxidant capacity of this molecule may be enhanced (Tan DX *et al.* 2002).

The present investigation demonstrates that Melatonin supplementation protects the mice brain against microwave-induced damage. The protective activity of Melatonin against microwaves may be mediated through several mechanisms. The presence of Melatonin elevates the cellular antioxidants and enables it to scavenge free radicals in the irradiated system, which could be a leading mechanism for radioprotection. The reduced enzymatic antioxidant activity as a result of oxidative stress in the brain was also restored with Melatonin. Our results may indicate a probable role of ROS in the adverse effects of 2.45 GHz microwaves and also, indicated that Melatonin prevented these effects. However, there is a need for further studies with different frequencies and exposure periods in order to prove the protective effect of Melatonin on EMR-induced oxidative stress in hippocampal neural tissue.

CONCLUSION

In conclusion, this study demonstrates that oral pre-supplementation with Melatonin (2 mg/kg b. wt) reduces the effect of biochemical alterations induced by 2.45 GHz MW exposure at a statistical significant level. This study suggests that the melatonin offer protection against MW induced Brain damages in albino mice.

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