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Research Article

VARIATIONS IN HEMATOLOGICAL PARAMETERS IN BLOOD OF SWISS ALBINO MICE AFTER WHOLE BODY EXPOSURE AND ITS MODULATION BY MELATONIN

***Najendra Singh¹, Rajendra Jat¹, Sanjay Singh¹, Rashmi Sisodia¹ and Saxena V. K²**

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 objective of this investigation was to study the variations in haematological parameters of Swiss albino mice and the role of Melatonin drug against whole body irradiation with microwave radiations. For this objective mice (6-8 weeks old) from the inbred colony were selected and divided into four groups: Group I: Sham exposed, Group II: Melatonin treated, Group III: Microwave exposed, Group IV: Mel + Microwave exposed. Microwave irradiated mice were exposed with 2.45 GHz microwaves for 2 h /day for 30 consequent days. The power density during exposure was measured 0.174mW/cm² with an average whole body specific absorption rate (SAR) 0.373 W/kg. After the above experiment blood samples of mice were collected from all mentioned groups. Microwave exposure resulted in significant decrease ($P \leq 0.001$) in haemoglobin, neutrophil, packed cell volume and red blood cells. Whereas, lymphocytes, eosinophil, monocyte, platelets and white blood cells increased significantly ($P \leq 0.001$) after microwave exposure in comparison to the sham exposed. LPO and Alkaline phosphatase (ALP) also increased significantly ($P \leq 0.001$) after microwave exposure compared to sham exposed mice. Reduction was observed in total protein, blood sugar acid phosphatase and glutathione (GSH) level after microwave exposure compared to sham exposed mice. Under the experimental conditions tested, the data indicate that melatonin has the ability to influence the haematological parameters of Swiss albino mice.

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INTRODUCTION

We are surrounded by modern technology which is invented for human welfare. But these appliances produces electromagnetic radiation in enormous amount, due to which humans, animals, plants and even microorganism are exposed to these radiations. Microwaves are non-ionizing electromagnetic radiation wavelength ranging from 1 mm to 1 m and frequency between 0.3 and 300 GHz (Kumar, *et al.*, 2014; Timothy, 2004). In the present investigation 2-4 GHz frequency is used which lies in S-band. This frequency waves are being generated by weather radar, surface ship radar, and some communications satellites, especially those used by NASA to communicate with the International Space Station and the Space Shuttle video senders, cordless phones, and wireless headphones, among other consumer electronics uses, including microwave ovens, Bluetooth, amateur radio and amateur satellite operators.

Radiations emitted from these modern devices are reported to induce various types of biological effects which are of great concern to human health. A German study has indicated an increase in cancer around base stations. Mobile phones use electromagnetic radiation in microwave range (2G-900/1,800 MHz, 3G-2,100 MHz frequency band) which some believe may be harmful to human health. People living close to 2G and mostly 3G mobile phone masts or base stations frequently report symptoms of electromagnetic hypersensitivity such as dizziness, headaches, skin conditions, allergies, and many other problems. Different cells of hematopoietic tissues show differential sensitivity. It has been known for a decade that RF/microwaves from cell phones and towers transmitters cause damage in human blood cells that result in nuclei splintering off into micronuclei fragments. EMF has been reported to affect a wide range of other basic cellular functions. These effects include cell proliferation (Zhang *et al.* 2013), protein synthesis (Gerner *et al.* 2010), gene transcription and expression (Zhao *et al.* 2007), neurite outgrowth (McFarlane *et al.*

*Corresponding author: **Najendra Singh**

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

al. 2000), precancerous conditions (Kumar et al. 2012) and tissue damage in different organs of the experimental animals (Zare et al. 2007, Khayyat and Abou-zaid, 2009). Gagnon et al. (2000) observed gender specific immune response, and hematological changes, and gender differences in experimental mice. Experiments have pointed enhancement of the presence of free radicals after electromagnetic field exposure (Yoshikawa et al. 2000, Kumar et al. 2010). The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats (Şekeroglu et al. 2013).

Anti-oxidant treatments in animals and humans could be beneficial in preventing or reducing some complications of microwave radiation (Ilhan et al. 2004; Balci et al. 2007, Oral et al. 2008). Melatonin (N-acetyl-5-methoxy-tryptamine) is a neurohormone synthesized in and released from the pineal gland during the dark period. In addition to its neuroendocrine functions, melatonin seems to exert psychotropic effects in rodents, such as sedative, analgesic, anticonvulsant, hypnotic, and anxiolytic effects (Sugden, 1983). Its effects are mediated through the activation of two melatonin G-protein-coupled receptors, MT1 and MT2 (Reppert et al., 1994). Melatonin (a) protects nuclear and mitochondrial DNA, membrane lipids, and cytosolic proteins from oxidative damage; (b) it blocks oxidative mediators that initiate the neuroinflammatory response after traumatic brain injury, e.g. by reducing NF-kB activation; (c) interact with central γ -aminobutyric acid (GABA) neurotransmission; (d) it detoxifies ROS and nitrogen species (RNS); (e) it stimulates antioxidative enzymes; (f) it improves oxidative phosphorylation and stabilizes neuronal membranes (Reiter et al., 2002 and Maldonado et al., 2007). Melatonin protective actions against these adverse changes are believed to stem from its direct free radical scavenging and indirect antioxidant activities, possibly from its ability to limit free radical generation at the mitochondrial level and because of yet-undefined functions.

Therefore, the present investigation has been undertaken to evaluate the impact of 2.45 GHz on the hematopoietic system of Swiss albino mice and to evaluate the potential protective role of melatonin in reducing oxidative stress.

MATERIALS AND METHODS

Experimental Animals

Adult male Swiss albino mice, 6-8 weeks old and weighing 25±2 grams were used for the present study. Initially the mice were procured from Central Drug Research Institute (CDRI), Lucknow, India and maintained in the animal house as an inbred colony as per the norms established by Institutional Animal Ethical Committee (IAEC). The animals were housed in clean polypropylene cages and maintained under controlled conditions of temperature (25 ±1.5°C) and light (12 hours light: 12 hours dark). They were maintained on standard normal diet obtained from Ashirwad Industries Chandigarh, India and water ad libitum.

Drug Preparation

Melatonin was procured from Himedia Laboratories Pvt. Ltd., India. Distilled water was used as the solvent. The concentrations of melatonin in distilled water were made according to the body weight (2 mg per kg body weight) of mice according to earlier studies (Sokolovic et al. 2008).

2.45 GHz exposure and measurement of Specific Absorption Rate (SAR)

Mice were divided into four groups consisting of 6 mice in each group. Microwave radiation experimental bench (fig. 1) was used for exposure of mice. The bench consists of signal generator N5181A MXG RF Analog Signal Generator, 100 kHz to 6 GHz Agilent Company, 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. 6 Mice were kept in a rectangular partitioned cage made of plexiglas which was well ventilated with holes of 1 centimetre (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. Everyday, the cage with mice was placed in the same position facing the horn antenna. 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 Department of Physics, University of Rajasthan, Jaipur.

The emitted power of microwaves was measured by a power meter which is 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 in front of horn antenna. A similar experiment was performed with sham exposed animals without energizing the system. The power density at the cage location was 0.174 mW/cm² and the SAR was calculated as 0.373 W/Kg (watt per kilogram) following the work of Durney et al. (1984) using the formula-

$$SAR = \frac{(A_1 f^2 / f_0^2)}{(f^2 / f_0^2) + A_2 \left[\frac{f^2}{f_0^2} - 1 \right]^2} W / Kg$$

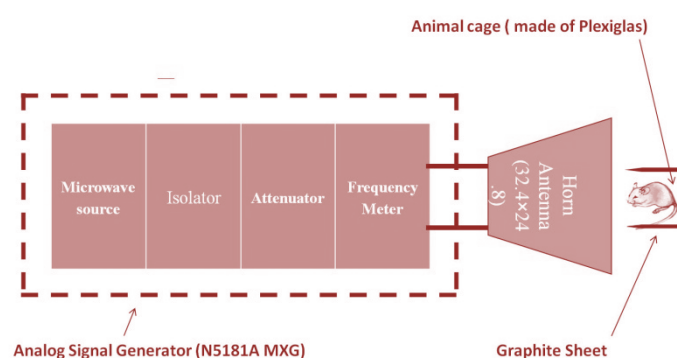


Figure 1 Diagrammatic view of 2.45 GHz microwave exposure setup (RF Analog Signal Generator N5181A MXG, 100 kHz to 6 GHz Agilent Company, USA).

Experimental design

24 mice were divided into four groups, each group consisted of six animals (n=6).

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.

Group IV: (Mel + MW exposed)

Mice were supplemented with Melatonin 2mg/kg once daily 1 hr before exposure to 2.45 GHz for 2hrs/day for 30 consecutive days.

Collection, preparation of smear and quantification of blood samples

At the end of experiment blood samples were collected from the venous sinus of medial canthus of eye. The mice was restrained, the neck gently scruffed and the eye made to bulge.

A capillary tube was inserted aseptically in to sinus and blood was allowed to flow by capillary action into the capillary tube.

For the hematological analysis, blood sample was received at a tube containing dipotassium ethylene diaminetetra acetate (EDTA) to stop it from clotting as recommended by Dacie and Lewis, 2006. Hemoglobin (Hb) content (gm %) of each animal was estimated by Sahli’s hemoglobinometer. Hematological parameters like total red blood cells (RBC), total white blood cells (WBC), differential white blood cells, platelet count, were estimated by Hematological Analyzer (Mindry Cellenium-19). PCV % was measured by the use of micro haematocrit method. In addition, for biochemical analysis blood sample was collected into a centrifuge tube without any anticoagulant and centrifuged at 25000 rpm for 20 minutes. Clear serum samples were separated in glass tubes and then they were subjected to different biochemical assays. All the biochemical assays were carried out on fresh serum samples. Blood sugar level was determined by braham and Tinder method (1972) measurement of total protein in serum was done by Bradford method (1976) determination of alkaline phosphatase and acid phosphatase activity was carried out by kinetic method using p-nitrophenyl phosphate (p-NPP). Estimation of LPO was done using method by Buege and Aust (1978) and GSH was done using method by Moron *et al.* (1979).

Statistical analysis

The values were expressed as mean ± SEM. Statistical analysis was performed using Student’s ‘t’ test and ANOVA.

Table 1 Variations in the different hematological parameters in the blood of Swiss albino mice in the presence /absence of Melatonin.

| Parameters | Sham Exposed (Group-I) | Melatonin (Group-II) | MW Exposed (Group-III) | MEL+MW Exposed (Group-IV) |
|-----------------------------------|------------------------|-------------------------|------------------------|---------------------------|
| HB% | 11.31±0.07 | 11.80±0.13* | 10.68±0.21* | 11.76±0.16* |
| TLC (Th/mm ³) | 8.41±0.09 | 8.25±0.07 ⁿ | 9.35±0.05** | 8.38±0.11** |
| Lymphocyte % | 55.16±0.08 | 54.83±0.01* | 60.5±.76** | 60.16±0.94 ⁿ |
| Eosinophil % | 1.35±0.07 | 1.23±0.13 ⁿ | 1.63 ±.06* | 1.28±0.07* |
| Monocyte % | 2.56±0.06 | 2.25 ±0.09* | 3.6±.06** | 2.43±0.13** |
| Neutrophil % | 38.20±0.28 | 38.25±0.41 ⁿ | 28.23±0.04* | 32.68±0.36** |
| Platelets (lakh/mm ³) | 1.63±0.08 | 1.71±.06 ⁿ | 0.78±0.04** | 1.25±0.09** |
| PCV% | 43.88±0.38 | 44.03±0.41 ⁿ | 38.25±0.37** | 39.45±0.37* |
| TRBC(millions/mm ³) | 6.15±0.02 | 6.25±0.01* | 3.88±0.04* | 5.95±0.12* |

Each value represents Mean ± SEM.
 Statistical comparison: Sham Vs MW, MW Vs Mel +MW, Sham Vs Mel
 **- p<0.001- highly significant, *- p<0.05- significant, n- non-significant

Table 2 Variations in the different serum biochemical parameters in the blood of Swiss albino mice in the presence /absence of Melatonin.

| Parameters | Sham Exposed (Group-I) | Melatonin (Group-II) | MW Exposed (Group-III) | MEL+MW Exposed (Group-IV) |
|----------------------|------------------------|---------------------------------------|---------------------------|------------------------------------|
| Blood sugar | 126.5±0.23 (100%) | 126.83±0.09 ⁿ (100.26%) | 112.13±0.79* (88.64%) | 121.33±0.49** (95.91%) |
| Total protein | 7.23±0.04 (100%) | 7.73±0.06* (106.91%) | 5.11±0.09** (70.67%) | 7.15±0.11* (98.89%) |
| Alkaline phosphatase | 83.66±0.24 (100%) | 83.16±0.70 ⁿ (99.40%) | 88.66±0.45** (105.97%) | 85.83±0.08* (102.59%) |
| Acid phosphatase | 6.01±0.33 (100%) | 6.95±0.15* (115.64%) | 3.10±0.43* (51.58%) | 3.08±0.42 ⁿ (51.24%) |
| LPO | 113.49±0.16 (100%) | 112.25±0.24* (98.90%) | 132.85±0.27* (117.05%) | 123.30±0.80* (108.64%) |
| GSH | 7.80±0.11 (100%) | 8.10±0.07* (103.84%) | 5.45±0.09** (69.87%) | 7.16±0.04** (91.79%) |

Each value represents Mean ± SEM.
 Statistical comparison: Sham Vs MW, MW Vs Mel +MW, Sham Vs Mel
 **- p<0.001- highly significant, *- p<0.05- significant, n- non-significant

RESULTS

Hematological analysis

MW exposure (Group III) resulted in significant decrease in different parameters of blood viz Hb, PCV, TRBC, and neutrophil compared to sham exposed mice. There was no significant change recorded in lymphocytes in Mel+MW exposed group (group IV). However, Melatonin supplementation prior to microwave exposure resulted in statistically significant increase ($p \leq 0.001$) in HB, PCV, platelets, neutrophil and RBC. WBC, lymphocytes, monocyte and eosinophils were found to be increased significantly ($P \leq 0.001$) after microwave exposure (group III) compared to sham (group I) but supplementation of melatonin prior to Microwave exposure (group IV) improved these parameters except lymphocyte count i.e. the presence of melatonin was able to restore the damage caused by microwave exposure.

Biochemical Analysis

MW exposure resulted in significant decrease ($p < 0.001$) in blood sugar, total protein, acid phosphatase and GSH levels compared to sham exposed mice. Melatonin supplementation prior MW exposure resulted in significant elevations ($p < 0.001$) in the blood sugar levels and total protein compared to exposed mice. Microwave exposure resulted in significant changes in levels of acid phosphatase which could not be modulated by melatonin supplementation. Significant increase in levels of ALP and LPO were noticed in MW exposed mice compared to sham exposed mice. Supplementation of melatonin prior MW exposure (group III) decreased the elevated levels of ALP and LPO.

DISCUSSION

Measurements of blood parameters are most important means to determine the health status of experimental animals. Blood and blood parameters are believed to be one of the primary particles that come in contact with RF EMF. Blood being ions are likely to react with induced EMF generated by EMF charges. The present study reported that MW exposure results in a significant decrease ($p \leq 0.001$) in the hematological constituents of blood viz. Hb, neutrophil, PCV, TRBCs, compared to sham exposed group.

In the present investigation the increase in lymphocytes may be due to the harmful action of MW exposure that stimulates the hematopoietic system to release more lymphocytes causing an increase in their number in the blood stream. Reddy (2017) reported increased the levels of White Blood Cells (WBC) ($P < 0.0001$) as a result of MWR from mobile phones. It indicates that the MWR triggers inflammation in exposed animals. Red blood cells declined significantly in experimental animals when compared with control groups. The depletion in red blood cells may be due to inflammation, similar conditions may be responsible for anaemia. The haemoglobin percentages of experimental and control groups were not affected significantly. whereas as the activity levels of Haematocrit, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin, Mean Corpuscular Haemoglobin Concentration, Platelet count were depleted significant ($P < 0.0001$) when compared with their respective control group.

Peighambarzadeh and Tavana, (2017) reported increased Blood Urea Nitrogen (BUN), Alanine Amino Transferase (ALT), and Aspartate Amino Transferase (AST) and the decreased in average amount of body weight, creatinine, fasting blood sugar, protein, albumin, cholesterol and thyroid Stimulating hormone (TSH) in the test group. Electromagnetic radiations have influence on the biochemical parameters in mice. Aziz *et al.*, (2010) also reported an increase in lymphocytes in cases of anemia, specifically macrocytic anemia, which arise under the influence of exposure to radiation, increased temperature, and increased resistance to the body's immune system. Our findings agree with previous studies done by Al-Uboody, (2015) who reported that several hematological variables are sensitive to mobile phone electromagnetic waves exposure. According to these authors, changes in the above parameters repeatedly occurred after mobile phone exposures. Aweda *et al.* (2011) after exposure of rat to 2.450 GHz microwave (MW) radiation reported effects on hematological parameters. They reported that 2.45 GHz MW, exposure resulted in decline in Hb, RBC, WBC, PCV, Platelets, neutrophil and lymphocyte counts were not significantly affected.

Supplementation of melatonin before exposure could modulate these parameters. Some of the parameters investigated by Aziz *et al.*, (2010) showed a significant increase in some blood parameters of WBC, MCV, blood platelets and a significant decrease in RBC, HB and MCH in EMF exposed mice compared to control mice They also reported that supplementation of vitamin C and vitamin E also modulated the effects of electromagnetic radiations in blood of Swiss albino mice. Our results are in agreement with the findings of Al-ubody, 2015 who reported that MW exposure to mice caused a significant decrease in blood glucose, PCV, neutrophil, Hb concentration as well as significant increase in lymphocyte, monocyte, and total WBC count. Similar studies, Singh *et al.* (2013) reported effects of electromagnetic radiations emitted from VDU (video display unit) on red blood cells of Swiss albino mice, 20 cm away, at power density of $0.295 \mu\text{w}/\text{cm}^2$. They found altered red blood cell count, Usman *et al.* (2012) studied the effects due to long term exposure of Swiss albino mice to RF EMF. They reported increase in PCV, RBC and Hb values with prolonged exposure in all the exposed groups. However, MCHC and WBC declined with prolonged exposure in all the exposed groups.

Alghamdi and Ghazaly, (2012) studied effects of electromagnetic fields on some hematological parameters of male white mice. They reported decline in hemoglobin, hematocrit, red blood cells count, in addition to the platelets count after short and long exposure to both types of mobile phone (Alcatel, Nokia). It was observed that the average number of white cells and lymphocytes increased significantly, indicating the increase to the body's immune response to radiation. Hassan (2011) found that exposure of female rats to electromagnetic field caused a significant decrease in total serum protein, total RBC count, PCV% and Hb concentration as well as significant increase in total cholesterol and total WBC count. Although these results were contrary to those found by (Amara *et al.* 2006) where they reported significant increase in the above parameters; they hypothesized that action of SMF on the geometrical conformation of hemoglobin was

reinforced by the fact that static magnetic field (SMF) induced a prominent effects on hemoglobin structure. Serum biochemical parameters viz ALP and LPO were found to be significantly increased ($p \leq 0.001$) in MW exposed mice compared to sham exposed. MDA a product of lipid peroxidation was found to increase during 2.45 GHz induced oxidative stress in the present investigation. High levels of serum MDA in 2.45 GHz exposed mice indicate that MW enhances LPO and produces oxidative stress by producing free radicals as reported earlier (Sisodia *et al.*, 2013; Faiza *et al.*, 2014).

In the present study, it was observed that melatonin treatment significantly lowered the microwave radiation-induced elevated levels of LPO in terms of malonaldehyde. The inhibition of LPO in biomembranes can be caused by antioxidants.

Decrease in serum transaminases alkaline phosphatase (ALP) in microwave treated mice compared to the sham exposed group is in agreement with the results of Moussa, 2009. Fathy-Assasa (2010) reported significant increase in alkaline phosphatase (ALP) in electromagnetic field treated rats compared to the control group. Ibrahim *et al.*, (2008) exposed Sparague-Dawely male rats to magnetic field and reported significant increase ALP compared to control group.

Rifat, *et al.*, (2010) exposed Swiss albino mice to microwaves and reported significant increase in levels of ALP. Serum alkaline phosphatase (ALP) exhibited significant increase ($P < 0.001$) in microwave exposed mice compared to the sham exposed group in our study is in agreement with the study done by Moussa, 2009. The activity of ALP was significantly lower in the groups treated with Melatonin before exposure indicating the modulatory role of Melatonin against radiation exposure. Sisodia *et al.* (2013) showed significant decreased ($P \leq 0.01$) in the level of total protein after microwave exposure compared with levels in the sham-exposed (control) mice.

Decrease in total protein concentration in the present investigation get support from the findings of Faiza *et al.* (2014) who also reported a significant decrease in total serum protein level. This decrease in MW exposed group, may be probably due to lysis or inhibition of protein synthesis, or may be by the depression of enzymes involved in the activation; this could be due to excessive damage to the genetic machinery. Increased protein concentration in the present study after melatonin supplementation may be due to improved ribosomal activities, which enhance protein synthesis. Hassan *et al.* (2011) showed that SMF exposure significantly decreased the total plasmatic protein level suggesting the change in protein metabolism of exposed rats.

The present study shows a sharp decrease in amount of blood sugar following MW exposure. Higher blood sugar level in melatonin-treated group compared to sham exposed group shows that it protects the enzymatic mechanism in the liver. Melatonin has been reported as scavengers and inhibitors of lipid per oxidation.

CONCLUSION

From the present study, it can be concluded microwave induced damage in blood can be reduced by supplementation of melatonin prior to MW exposure. Anti-oxidative activity of

melatonin may be responsible for the protective activity noticed in the present study.

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