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10 Ghz Microwaves Induced Biochemical, Learning and Memory Alterations in Swiss Albino Mice Brain

KEYWORDS: Microwaves, Brain, Radial arm maze, Biochemical assays

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ABSTRACT *The present study is an attempt to study the effects of 10 GHz (Giga Hertz) microwaves irradiation in Swiss albino mice. The animals of 6-8 weeks of age were divided into 2 groups - (i) sham exposed (ii) microwaves. The mice were exposed to 10 GHz microwaves daily for 2 hours per day for 30 days. The power density was 0.25 mW/cm2 (milliwatt per centimeter square). Average whole body specific absorption rate (SAR) was calculated 0.1790 W/kg. After the exposure period the animals were sacrificed at different autopsy intervals viz., 1-30 days and the brain was excised to study various biochemical parameters. The animals were also tested in a radial arm maze to assess the learning and memory performance. Chronic exposure of these electromagnetic field radiations resulted in significant decrease in antioxidant enzyme activity of SOD (superoxide dismutase) and GSH (glutathione) with increased catalase activity (CAT) at all post treatment follow up intervals. Lipid peroxidation significantly increased at all the autopsy intervals. The protein content of the brain depleted upto 7th day after microwave exposure Radial arm maze test revealed that 10 GHz leads to significant depletion in spatial learning and memory performance in Swiss albino mice. Free radicals causing oxidative stress may be responsible for altered biochemical levels of the brain and impairment of learning and working memory of Swiss albino mice after microwave exposure.*

1. INTRODUCTION

The X- band is widely used in communication systems for civil and military application devices such as aircrafts, weather forecast systems and various types of radars. The 10 GHz (Giga Hertz) band is one of the easiest microwave bands to get on, primarily as a result of its proximity to frequencies heavily used by different radars and the resulting equipment availability. This band has an advantage of giving a reasonable energy density in a waveguide of convenient size from a moderately powered microwave generator. Another advantage of using 10 GHz lies in the fact that it is present in the central region of the frequency range which is commonly considered hazardous. This particular frequency also penetrates tissues to a depth of about 0.5 mm in biological body and an appreciable proportion of energy is absorbed (Carney et al. 1968). Increased usage of microwaves in every field has caused potential threat to human health, resulting in growing public and scientific concern resulting in large number of publications. There is evidence that microwaves may produce adverse biological effects in the nervous system (Kesari and Behari 2009, Sharma et al. 2014), even at low levels of radiation power (Jauchem 2008, Pooley 2010). The hippocampus is suggested to be especially vulnerable to microwave radiation, which may result in memory and learning deficits. Nevertheless, the long-term effects of microwaves on cognitive function remain controversial (Nittby et al. 2008).

The effect of microwave (MW) radiations on biological systems is primarily identified as due to an increase in temperature i.e. thermal (Stuchley 1988) though non thermal effects have also been identified (Paulraj and Behari 2004). Researches have pointed enhancement of the presence of free radicals after electromagnetic field (EMF) exposure (Kumar et al. 2010, Yoshikawa et al. 2000) which leads to a wide variety of clinical disorders and environmental stress (Galli et al. 2005, Houten et al. 2006). Microwave radiation may alter the level of antioxidants due to free radicals formation (Kesari et al. 2010).

The balance between production and neutralization of ROS levels can increase dramatically, which may cause damage to the cell structures leading to behavioral, histopathological, biochemical alterations and impairment of learning as a result of oxidation of poly-unsaturated fatty acids in lipids, amino acids in proteins and damage to DNA. Cells have a variety of defence mechanisms against the harmful effects of ROS like Superoxide dismutase (SOD), vitamin E and C. Cells have their own set of antioxidant defence mechanism to fight with free radical formation and to overcome the limit of damaging effects. SOD, catalase (CAT) and glutathione (GSH) are the enzymatic defence systems of cells against oxygen radicals. The brain is considered abnormally sensitive to oxidative damage (Robbins and Zhao 2004) because it has an abundant lipid contents, a high oxygen consumption rate and relatively scarce antioxidant enzymes compared with peripheral tissue (Kikuchi et al. 2003). It contains relatively low levels of SOD, CAT and glutathione peroxidase (GSH-Px) (Singh et al. 2008, Sisodia et al. 2011). Therefore, we studied effects of 10 GHz microwaves induced biochemical, learning and memory alterations if any on whole brain of Swiss albino mice.

2. Materials and Methods

2.1. Experimental Model (Swiss albino mice)

Adult male Swiss albino mice of 6-8 weeks old, 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 the controlled conditions of temperature (25 ±1.5°C) and light (12 Light: 12 Dark). They were fed on standard normal diet obtained from Hindustan Lever, Delhi, India and water was provided ad libitum.

2.2. Animal Exposure

Mice were divided into two groups-

Group I (Sham exposed): Mice were kept vertically below the horn antenna but without energizing the system.

Group II (Microwaves exposed): Mice of this group were exposed to the 10 GHz radiation source through the antenna for 2 h/ day (hours/day) for 30 days at pulsed density of 0.25 mW/cm^2 (Fig. 1).

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Two mice were housed at a time in a rectangular partitioned cage made of plexiglass which was well ventilated with holes of 1 cm diameter. The dimensions of the cage (4.5×9×9cm) were such that the animals were comfortably placed, though they could not move. The head part faced the horn antenna. 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 dimensions of the cage were of the order of wavelength. At near field distance from the horn antenna, it was found that the power density measured was 0.25 mW/cm^2 which was maximum. Everyday, cage with the mice was placed in the same position facing the horn antenna. The whole microwave exposure system was procured from Wavetech, Faridabad, Haryana, India.

2.3. Specific Absorption Rate (SAR)
The emitted power of microwaves was measured 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, Kansas, USA). The power density at the cage location was 0.25 mW/cm^2 and the SAR was calculated as 0.1790 W/Kg (Watt/ Kilogram) (estimation done according Durney et al. (1984).

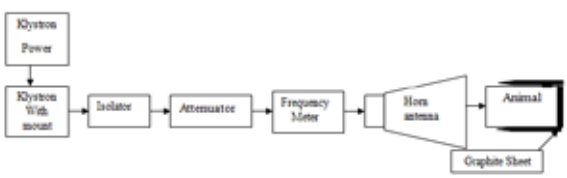


Figure 1: Schematic representation of 10-GHz microwave source

2.4. Removal of Brain Tissue
Six mice were sacrificed by cervical dislocation from each group for biochemical assays at various follow up intervals ranging between 1- 30 days after completion of 30 days exposure. 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 quantitative estimation of LPO (Lipid Peroxidation), GSH, protein, SOD and CAT.

2.5. Biochemical Assay
2.5.1. Lipid Peroxidation (LPO) Assay- The amount of tissue TBARS was measured by the thiobarbituric acid assay (TBA) as previously described by Buege and Aust (1978).

2.5.2. Glutathione (GSH) Assay- The reduced GSH content of tissue samples was determined in brain by the method of Moron et al. (1979).

2.5.3. Protein Assay- Estimation of protein was based on the method proposed by Bradford (1976).

2.5.4. Superoxide Dismutase (SOD) Assay - SOD was measured by the method of Marklund & Marklund (1974).

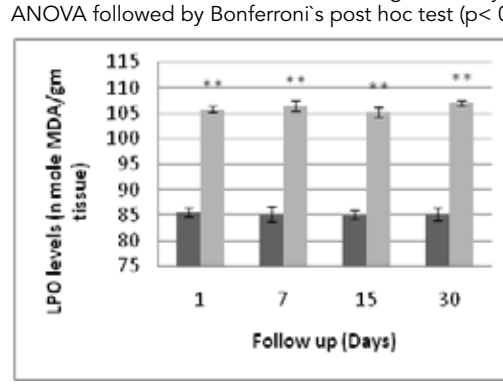
2.5.5. Catalase (CAT) Assay - Estimation of catalase enzyme was based on the method proposed by Aebi et al. (1984).

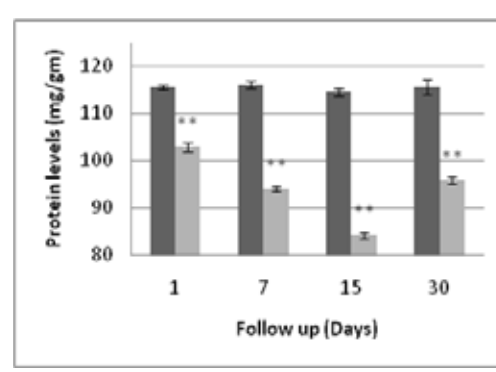
2.6. Radial Arm Maze (RAM) test- Six mice from each group were subjected to radial arm maze test (Rao et al. 2005) to assess the learning and memory. The radial arm maze has a centrally placed octagonal platform (20 cm long side) to which are attached 8 radial arms, 60 cm long and 8 cm wide, numbered from A to H clockwise. Each arm has a sunken feeder of 2 cm diameter and 1.5 cm deep at a distance of 2 cm from the far end. Mice were put on a deprived schedule for 15 days prior to the experiment; food was available for only 1 h daily. The food-deprived animal was placed on the central platform and allowed to explore for 15 min and given its daily ration of food afterwards. On the next day, 35 mg food pellets were placed into the feeders and exploration was repeated. The actual experiment started as soon as the animal went straight to the feeders and ate the pellets. The sequence of channels entered (all four feet) and time spent there were recorded. The animals were allowed to make 8 choices and then removed from the apparatus. This trial was repeated 3 more times. Additional food was given to the animals 30 min after the last trial.

2.7. Statistical Analysis
The results obtained in the present study were expressed as mean ± SD. The statistical difference between various groups was analysed by using two way ANOVA followed by Bonferroni`s post-hoc test. The significance was observed at the $p < 0.001$ (Highly significant) and $p < 0.05$ (significant) level.

3. Results
3.1. Biochemical Estimations-
Lipid peroxidation product as reflected by thiobarbituric acid substance (TBARS) equivalent content was augmented after 10 GHz microwave exposure at all the follow up intervals viz., 1-30 days compared to sham irradiated mice ($p<0.001$) (Fig. 2). Whereas GSH content decreased significantly ($p< 0.001$) at all follow up autopsy intervals in whole brain of mice compared to sham. Protein content also decreased significantly ($p< 0.001$) at all autopsy intervals in mice whole brain of mice compared to sham. Maximum decline was recorded at day 7 post irradiation. Thereafter, recovery was evident though the protein level was much below the sham level.

A sharp fall in SOD activity was noticed 24 hrs after completion of microwave exposure. Thereafter increase ($p<0.001$) in SOD level was noted on day 3, which remained almost stable upto the last interval studied. This implies that irradiation from microwaves resulted in the generation of superoxide radicals ($p<0.001$). Microwave exposure resulted in a significant increase in CAT activity compared to the sham exposed mice. All the results were found to be significant by two way ANOVA followed by Bonferroni`s post hoc test ($p< 0.001$).





Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

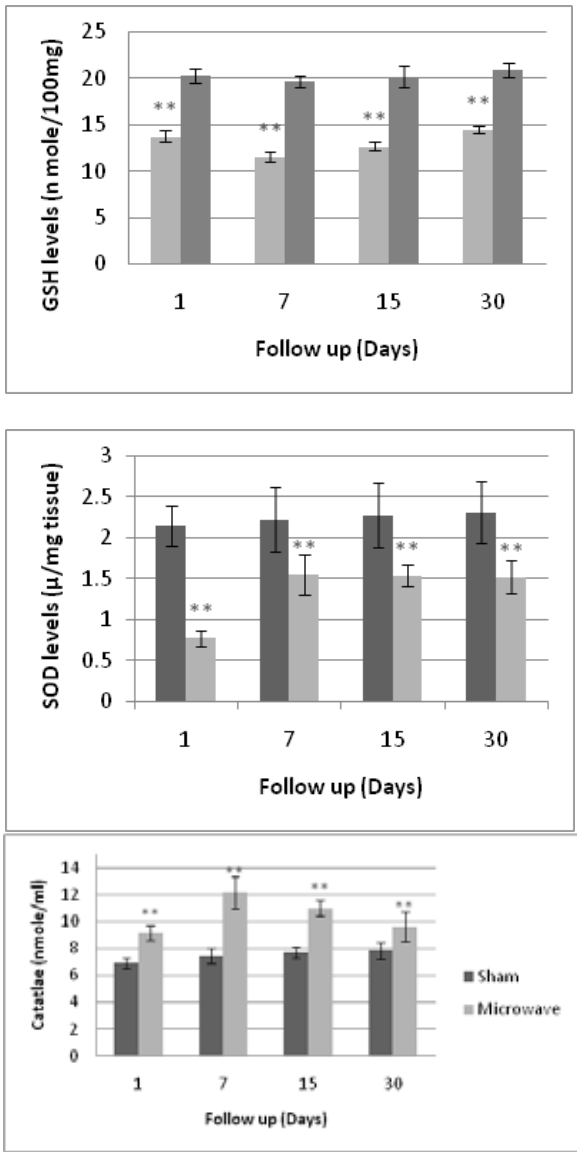


Fig. 2: Variations in (a) LPO levels (n mole MDA/gm tissue) (b) GSH levels (n mole/100mg) (c) Protein levels (mg/gm) (d) SOD levels (µ/mg tissue) (e) CAT (nmole/ml) in microwave (10 GHz) exposed mice. Values are mean ±SD. N =6. **- p< 0.001.

3.2. Radial Arm maze performance

Radial arm maze performance of mice from the two groups was also observed. Microwave exposure (group II) resulted in significant decrease (p<0.001) in number of correct entries as well as significant increase (p< 0.001) in time taken to complete the test compared to sham (Table 1).

Table 1: Performance in the radial arm maze of mice exposed to 10 GHz microwaves (Mean± SD).

Groups	No. of correct entries	Total time taken(s)
Sham	7±0.26	152.3±1.86
Microwaves	4.5± 0.34**	247.3±2.74**

****= p<0.001, n= non significant. Data are compared as Sham vs Microwaves**

4. Discussion

There has been a long standing debate about the effect of electromagnetic field on biological systems and its mechanism of interaction. There are several reports which indicate that free radicals are involved in EMR induced tissue injury and effects parameters such as genotoxicity and oxidative stress (Ilhan et al. 2004, Mailankot et al. 2009).

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). 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 (Kesari and Behari, 2009).

Malondialdehyde (MDA) is the breakdown product of the major chain reactions leading to oxidation of polyunsaturated fatty acids and thus serves as a reliable marker of oxidative stress mediated lipid peroxidation (LPO) in neural tissue and other organs (Irmak et al. 2003). The peroxides via lipid peroxidation damage cell membrane and other components of the cell. In the present study, there was considerable increase in TBARS content after EMF exposure.

Reports suggest that electromagnetic field increase the concentration of free radicals which may enhance the probability of damage to the biological system. Damage to body occurs when intracellular antioxidant mechanism are overwhelmed by ROS. GSH, CAT and SOD protect cells against ROS. In the present study, decreased activities of the key antioxidants, GSH and SOD were found in the whole brain of mice exposed to 10 GHz. SOD plays a key role in defence mechanism against free radical activity. 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). 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. Zwirska-Korczala et al. (2005) also reported a decrease in activity of SOD, GSH and increase in activity of CAT.

Moustafa et al. (2001) reported a decrease in plasma SOD activity in humans. This decrease in activity of SOD was related to accumulation of superoxide amino radicals in cells. The detoxification is mainly done by SOD. Hydrogen superoxide, a product of SOD activity, is also a strong inhibitor of SOD enzyme (Kula et al. 2002). The charge of detoxification is taken by CAT enzyme, which leads to increase in its activity.

Decreased activity of GSH in exposed group may be due to decrease in its formation, which requires NADPH and GR (Irmak et al. 2003, Zwirska- Korczala 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. Kerman and Selon (2012) reported that in the EMR exposed group (900 MHz) neural tissue MDA levels increased while SOD, CAT, and GSH-Px activities were reduced which could be reversed by oral administration of melatonin.

Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

Decrease in protein content after exposure to EMF's might be due to either decline in the rate of protein synthesis or increase in the comsumption of protein. It may also be the result of the depression of enzyme involved in the activation of amino acid and transferring to t-RNA or by the inhibition of release of synthesized polypeptides from polysomes. Some studies have indicated that oxidative stress diminishes and the levels of some proteins vary during the progression of alzheimer's disease (Wu et al. 2004). Studies suggest that local protein synthesis has crucial roles in synaptic plasticity, the change in neuronal communication efficiency that is probably a cellular basis of learning and memory. Induced by neuronal activity, local protein synthesis provides key factors for the modification of activated synapses. A review by Klann and Sweatt (2008) summarizes a contemporary model proposing a role for altered protein synthesis in memory formation and its subsequent stabilization. It has long been shown that protein synthesis can occur in neuronal dendrites, but its significance remained unclear until recently. Fragopoulou et al. (2012) reported that long-term irradiation from both EMF sources altered significantly ($p<0.05$) the expression of 143 proteins in total (as low as 0.003 fold down regulation up to 114 fold overexpression). Several neural functions related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha-synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. These results suggest that EMF induces an oxidative stress within the brain tissue of mice. The change in antioxidants (SOD, CAT and GSH), lipid peroxidation and total protein levels may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the microwaves exposure.

Changes in behavior and cognition are important outcomes used to assess the effects of exposure of microwaves on the brain (Keetley et al. 2006, Papageorgiou et al. 2006). Radial Arm Maze (Olton and Samuelson, 1976) and Morris Water Maze (Morris, 1984), have been developed to assess rodent spatial memory and learning. It has been comprehensively reported that rats show distinct age-related deficits in such spatial learning tasks (Barnes 1990) and there is direct evidence that spatial memory tasks are sensitive to hippocampal injury (Grant et al. 1992). The exposure to EMF may have a facilitatory effect on brain functioning, especially in tasks requiring attention and manipulation of information in working memory (Koivisto et al. 2000). Studies also indicate that microwave induced hyperthermia can impair learning and memory (Moghimi et al. 2009). The hippocampus encodes the spatial relationships between components of scenes or contexts and in the absence of this representation animals with hippocampal lesions will not be able to form the object-place configurations that are important in episodic memory (Lee and Solivan, 2008, Narayanan et al. 2009). Spatial memory is a kind of short-term memory which is responsible for recording surroundings and spatial orientation. Eight-arm radial maze is recognized as an ideal model to study animal spatial memory, which can effectively exclude interference on learning and memory caused by animal's movement dysfunction (Goonawardena et al. 2010, Saito et al. 2010).

Zhao et al. (2012) have also reported disrupted learning and memory ability after microwave exposure. They also concluded that the hippocampus can be influenced by long term microwave exposure, which might result in impairment of cognitive function due to neurotransmitter disruption. Some studies have demonstrated that spatial learning and memory that depend on the hippocampus are impaired by chronic exposure to MW irradiation in rodents (Li et al. 2008, Nittby et al. 2008). Paulraj and Behari (2004) have also reported significant decrease in the protein kinase C (PKC) level in the whole brain and hippocampus of the Wistar rats exposed to microwaves. This is suggestive that this type of radiation could affect membrane bound enzymes associated with cell signaling, proliferation and differentiation. This may also suggest an effect on the behavior of chronically exposed rats. Liu et al. (2003) suggested that decline in learning and memory is associated with a very significant increase in two parameters of oxidative stress in the brain i.e. levels of lipid peroxidation and protein oxidation. The results of our experiments showing simultaneous decrease in protein levels of brain and learning memory after irradiation may be correlated to the altered protein synthesis or less protein synthesis during this stage of translation which stabilizes long term memory (Sharma et al. 2014).

5. Conclusions

It may be concluded that ROS overproduction by microwaves may cause depletion in the learning and memory, activity of SOD, GSH enzymes and increase in CAT activity and lipid peroxidation levels.

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Volume : 4 | Issue : 5 | May 2014 | ISSN - 2249-555X

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