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Effect of low level microwave radiation exposure on cognitive function and oxidative stress in rats

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Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health. The present study aimed to evaluate the effects of 900 MHz MW radiation exposure on cognitive function and oxidative stress in blood of Fischer rats. Animals were divided into two groups (6 animals/group): Group I (MW-exposed) and Group II (Sham-exposed). Animals were subjected to MW exposure (Frequency 900 MHz; specific absorption rate 8.4738×10^{-5} W/kg) in Gigahertz transverse electromagnetic cell (GTEM) for 30 days (2 h/day, 5 days/week). Subsequently, cognitive function and oxidative stress parameters were examined for each group. Results showed significant impairment in cognitive function and increase in oxidative stress, as evidenced by the increase in levels of MDA (a marker of lipid peroxidation) and protein carbonyl (a marker of protein oxidation) and unaltered GSH content in blood. Thus, the study demonstrated that low level MW radiation had significant effect on cognitive function and was also capable of leading to oxidative stress.

Keywords: Cognitive function, Microwave exposure, Oxidative stress, 900 MHz

Increased usage of mobile communication has raised serious concerns in our society about the possible adverse effects of electromagnetic radiation on human health. Microwave (MW) radiations are a type of non-ionizing electromagnetic radiation ranging from 300 MHz to 300 GHz. Mobile phones are low power radio devices that transmit and receive radio frequency radiation at frequencies in the range of microwave radiation 900-1800 MHz through an antenna used close to the user's head. As these devices are held close to the brain during communication, there may be a possibility of adverse effects of these radiations on brain.

MW radiations might induce or promote cancer and the symptoms associated with their use include sleep disturbances, memory problems, headache, nausea and dizziness[1]. In addition, MW radiation may affect biological system by changing the permeability of blood brain barrier, electroencephalographic activity, blood pressure and increase free radicals, leading to oxidative damage[2-4]. Several studies have reported an association between MW exposure and human health with emphasis on neurodegenerative diseases[5,6].

Exposure to 2450 MHz MWs at specific absorption rate (SAR) of 0.6 W/kg causes reduction in performance of rats in the radial-arm maze[7]. MW exposure may be involved in the formation of reactive oxygen species (ROS) and increased oxidative stress in tissues[8]. It has been known that production of ROS may enhance lipid peroxidation and

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Abbreviations: EMF, electromagnetic field; EPM, elevated plus maze; GSH, reduced glutathione; GTEM, Gigahertz transverse electromagnetic cell; IAL, initial acquisition latency; ITL, initial transfer latency, MDA, malondialdehyde; MW, microwave; MWM, Morris water maze; ROS, reactive oxygen species; SAR, specific absorption rate; TBA, thiobarbituric acid; TCA, trichloroacetic acid.

protein oxidation and may also change level of antioxidant like GSH. Therefore, the present study has been designed to investigate the effect of 900 MHz microwave radiation exposure on the cognitive function and oxidative stress in blood of Fischer-344 rats.

Materials and Methods

Chemicals

Reduced glutathione (GSH), 2,4-dinitrophenyl-hydrazine (DNPH), 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) and 2-thiobarbituric acid (TBA) were procured from Sigma-Aldrich Co. (St. Louis, Mo, USA). All other chemicals used were of analytical grade and obtained either from Sisco Research Laboratories or Qualigens Fine Chemicals, Mumbai, India.

Microwave exposure setup and dosimetry

The Gigahertz Transverse Electromagnetic (GTEM) cell, GTE 10 was used to evaluate the biological effects of microwave radiation on experimental animals (Fig. 1A & B). It was designed with the help of Center for Applied Research in Electronics (Microwave Laboratory), Indian Institute of Technology, New Delhi and Amitech Electronics Ltd. Sahibabad (UP, India). GTEM cell had a pyramidal tapered, dual terminated section with its outer cell dimension (l x b x h) as 220 cm, 120 cm, 80 cm, respectively. MWs were generated from microwave generator SMC 100 (Rohde & Schwarz GmbH & Co., Germany). The microwave source consisted of a signal generator operating at frequency range from 9 KHz to 3.2 GHz, an amplifier, a DC regulator and a power meter. During exposure, rats were restrained in a closed box (L:30 cm, B:15 cm, H: 20 cm) divided into 4 compartments with holes of 1 cm diameter to facilitate easy movement and breathing, kept at a distance of 100 cm from source. One box could hold 4 rats and two such boxes could be placed within the GTEM cell for exposure. The microwave chamber was lined with absorbers to minimize the possibility of any reflections. Electric field was experimentally checked using an E-field probe inserted into the TEM cell through a slit wall. The GTEM cell was placed in a temperature controlled room under constant lighting conditions.

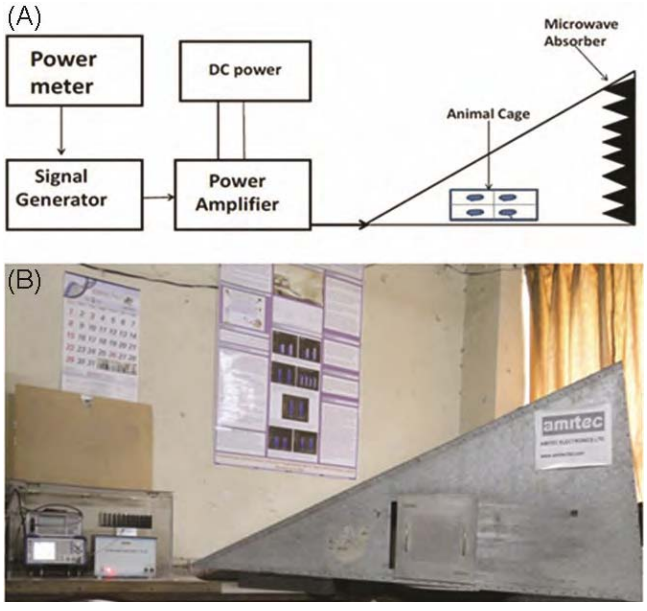

Fig. 1—(A) Schematic diagram of MW exposure set-up with position of rats in cages shown during exposure; (B) Photograph of MW exposure set-up (GTEM cell)

Animals and treatment

Male Fischer-344 rats, weighing 150-200 g were obtained from Central Animal House Facility of the Institute and placed in individual raised, galvanized wired cages, kept under standard conditions (temperature 22 ± 2°C) under alternating 12 h light and dark cycle. They were provided with nutritionally adequate standard diet obtained from Nutrilab (Bangalore, India) and water ad libitum. Animals were divided randomly into two groups: sham-exposed and MW-exposed group with 6 animals in each group. The MW-exposed group was exposed to 900 MHz at a power level of -10.00 dbm (0.1 mW) in a GTEM cell for 2 h daily, 5 days per week, during light period and every day at the same time for 30 days. During the exposure, rats were placed in closed boxes (L: 30 cm, B: 15 cm, H: 20 cm) with holes of 1 cm diameter to facilitate breathing. The sham-exposed group was subjected to similar conditions, except the MW exposure. Appropriate permission was obtained from Institutional Animal Ethics Committee (IAEC), University College of Medical Sciences, Delhi and appropriate care of the animals was undertaken as per guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India for laboratory animal facilities.

Specific absorption rate (SAR) distribution was calculated by power balance method[9] using the equation: P_{abs} per mouse = 1/n (P_{in} − P_{out} − P_{refl}), where, P_{abs} = RF power in watt absorbed per animal,

n = number of animals within the cell, P_{in} = input power (Watt), P_{out} = output power (Watt) and P_{refl} = reflected power (Watt).

Assessment of cognitive function

Elevated plus maze (EPM) paradigm

The EPM has been described as a simple method for assessing behavioral response in rodents. It has two opposite open arms (50 cm × 10 cm), crossed with two closed arms of same dimensions with 40 cm high wall, the arms are connected with central square (10 cm × 10 cm). The rats were trained on EPM one day prior to microwave exposure and acquisition was measured in terms of seconds. They were placed individually at one end of an open arm facing away from the central square and allowed to enter either of the closed arms and explored for 20 s. The time taken to enter one of the closed arms was recorded as initial transfer latency (ITL). The animal which could not enter the closed arm within 90 s were gently pushed to in one of the closed arms and the ITL was assigned as 90 s. Retention of memory after 24 h was assessed in the same manner[10].

Morris water maze

The acquisition and retention of a spatial navigation task was examined using a Morris water maze[8]. Animals received a training session consisting of four trials in a day for four days prior to exposure in Morris water maze (180 cm diameter × 60 cm) filled with water. An escape platform was hidden 2 cm below the surface of water in a fixed location in one of four quadrants half way between the wall and middle of the pool. The water was made opaque during the task with a non-toxic dye. Each trial consisted of releasing a rat into the water facing the wall of the pool at one of four starting compass positions (N, S, E, W), so that each position could be explored well. The time to reach the escape platform (latency in seconds) was recorded up to a maximum of 3 min. The animal which could not find the platform up to 3 min were deliberately placed on the platform and allowed to sit for 30 s. The time taken by a rat to reach the platform on fourth day was recorded as initial acquisition latency (IAL). Following 24 h after initial acquisition latency, a probe test was done, where there was no platform and each rat was randomly released from any one of the positions and tested for the retention of acquired memory. During retention, the time taken by each rat to locate the target quadrant (quadrant in which platform was placed during training) and time spent in target quadrant for four 15 s interval over 60 s was recorded.

Assessment of oxidative stress parameters

The lipid peroxidation in serum was measured as thiobarbituric acid reactive substance (TBARS). Briefly, 0.5 ml serum was precipitated with 20% trichloroacetic acid (TCA) and the precipitate was suspended in 0.05 H_2SO_4 and TBA (0.07% in 1 M sodium sulfate) and incubated in boiling water bath for 30 min. The malondialdehyde (MDA)-TBA adduct, thus formed was extracted with butanol and measured at 532 nm. The results were expressed as nmoles/ml[11].

Protein carbonyl concentration, a marker of oxidative modification of proteins was determined spectrophotometrically using 2,4 dinitrophenylhydrazine (DNPH), a traditional carbonyl reagent[12]. Reactive carbonyl derivatives were calculated using the DNPH molar extinction coefficient at 370 nm and expressed in nmol/mg of protein.

The reduced glutathione (GSH) content in blood was estimated using 5,5'-dithiobis-2 nitrobenzoic acid (DTNB) as described previously[13]. In this method, GSH was oxidized by DTNB and then reduced by GSH reductase with NADPH as hydrogen donor. The oxidation of GSH by DTNB was detected photometrically by a change of absorption at 412 nm and content was expressed as mg/dl.

Statistical analysis

The values were expressed as the mean ± SD. Statistical analysis was performed using SPSS 17. Student's t-test and Mann-Whitney U-Test were used to determine significant difference between groups. Statistically significance was accepted at $p<0.05$.

Results

Effect on cognitive function

The result of the elevated plus maze (EPM) test showed a significant alteration in transfer latency (TL) on 30th and 31st day in MW-exposed group when compared to sham-exposed group (Fig. 2 A & B). Rats exposed to MW radiation at 900 MHz and average whole body SAR as 8.4738×10^{-5} W/kg for 30 days

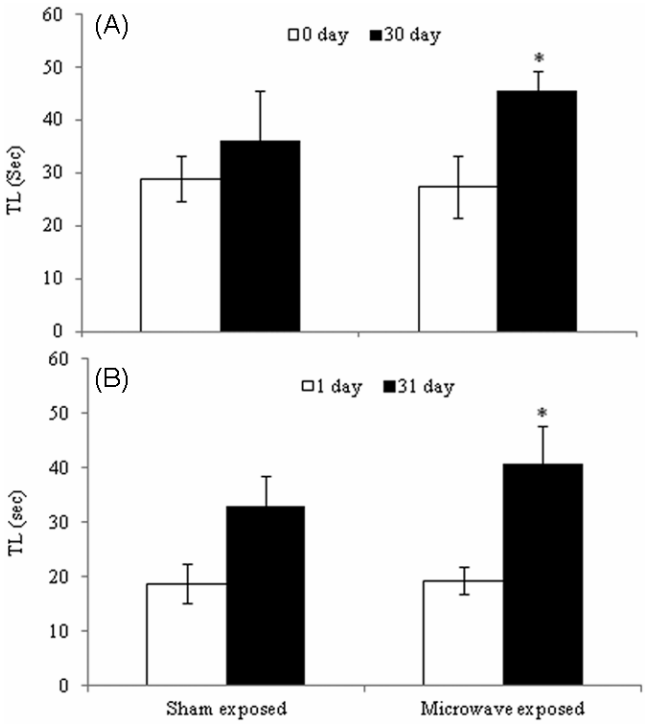

Fig. 2—Effect of MW exposure at 900 MHz (A) acquisition and (B) retention transfer latency [Values expressed as mean ± SD. Statistical significance was considered at *p<0.05]

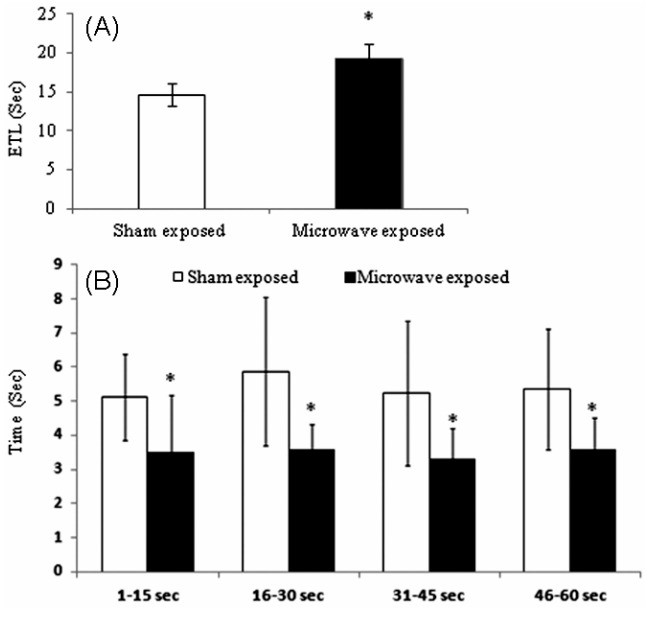

Fig. 3—Escape latency time (ELT) of rats during water maze test to locate the hidden platform and time spent in Q-4 (target quadrant) during last day when platform was removed, i.e., probe test [Statistical significance was considered at *p<0.05]

showed significant alteration in time taken to enter one of the closed arms of EPM on 30th as well as 31st day of MW exposure when compared to sham-exposed group.

MWM test was performed to evaluate spatial memory performance in rats. MW exposure caused significant alteration on escape transfer latency (ETL) in comparison to sham-exposed group. It was observed that MW-exposed rats took longer time to locate the place where platform was placed. The latency to reach the target quadrant was significantly longer and time spent in the target quadrant was significantly shorter in MW-exposed group when compared to sham exposed group (Fig. 3 A & B).

Effect on oxidative stress

MW exposure at 900 MHz led to significant increase in the level of MDA in blood, a marker of lipid peroxidation when compared with sham-exposed group (Fig. 4 A). MW exposure also caused significant

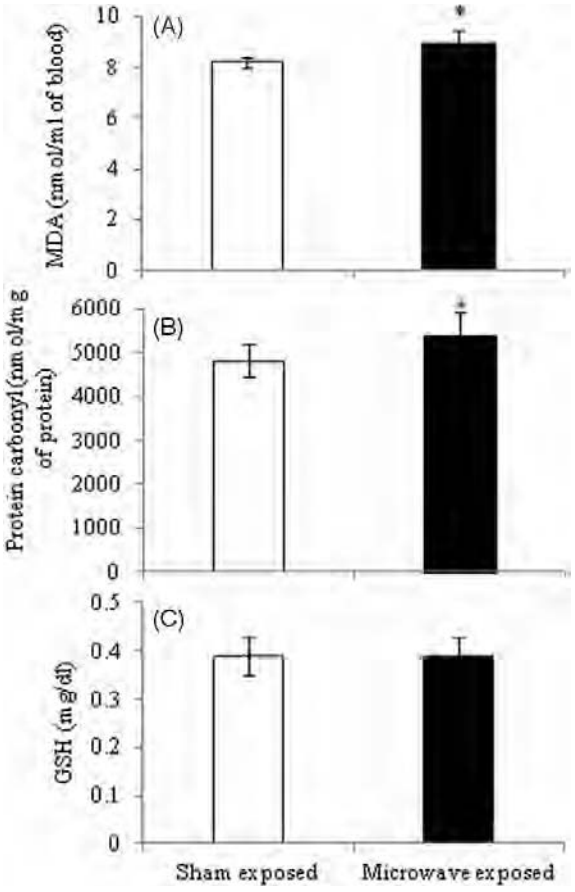

Fig. 4—Effect of microwave exposure at 900 MHz on (A) lipid peroxidation (MDA level nmol/ml), (B) protein oxidation (nmol/mg protein) and (C) GSH (mg/dl) in rat blood [Values are expressed as mean ± SD. Statistical significance was considered at *p<0.05]

increase in the carbonyl content, a marker of protein oxidation in blood of MW-exposed rats in comparison to non-exposure group. (Fig. 4 B). However, there was no change in the blood GSH (most prevalent and important intracellular anti-oxidant) content, when compared with sham-exposed group (Fig. 4 C).

Discussion

Rapidly growing concerns about possible adverse effects of MW radiation emitted from mobile phones and other wireless communication devices on human health have been discussed in many countries. The present study was carried out to evaluate the biological effects of 900 MHz MW radiation in experimental animals using specially designed microwave exposure system, the GTEM cell. The GTEM cell allows the generation of MW radiation in range of mobile phone frequencies. The SAR value in the present study was far below than the limit of 2W/kg for possible exposure to head in humans as per the International Commission on Non-Ionizing Radiation Protection (ICNIRP)[14]. We observed significant alterations in cognitive function and oxidative stress parameters.

Several effects of these radiations on central nervous system such as sleep disturbances, electrical activity, neurotransmitters imbalance and blood brain barrier permeability have been reported[15]. An earlier study has reported that relatively low intensity electromagnetic field (EMF) is capable to interact with molecular and cellular processes associated with carcinogenesis[16]. Because of insufficient evidences, the existing knowledge about detrimental effects of MW radiation still remains obscure and contradictory.

The present study provided two important findings. Firstly, our results indicated that 900 MHz MW exposure for 30 days caused cognitive decline in the rats. During the cognitive function assessment, we observed significant changes in transfer latency between sham-exposed and MW-exposed rats after 30 days of exposure. In spatial memory test, their latency time to reach the target quadrant was found longer and the time spent in the target quadrant was shorter, suggesting that MW radiation exposure might lead to impairment in cognitive function.

Earlier, it has been reported that mobile phone exposure of GSM (900/1800 MHz) with 50 missed calls/day for 4 weeks affects acquisition of learning response in Morris water maze test[8]. Also, the long-term GSM 900 MHz MWs at whole body SAR value of 0.6 and 60 mW/kg significantly alters the performance of rats during the episodic-like memory test, which indicates impairment in memory function after long-term exposure[17]. The underlying mechanisms for the changes of memory functions are still not clear. One of the possible mechanisms for deranged cognitive function might be increased oxidative stress in rat brain due to MW exposure.

It has been shown earlier that mobile phones exposure at frequency 900 MHz and SAR 0.043-0.135 W/kg causes oxidative damage by increasing levels of MDA, carbonyl groups and xanthine oxidase activity and decreasing catalase activity[18]. Several studies have also reported that reduced memory functions observed are correlated to hippocampal alterations induced by mobile phone exposure[19,20] at frequency of 1800 MHz and SAR 2.4 W/kg. All the above-mentioned studies had limitations of exposure system i.e. directly mobile phones were used for giving MW radiation exposure, but in the present study, we used a well-controlled system for MW exposure i.e. GTEM cell.

Oxidative stress resulting from excessive generation of ROS or deterioration of antioxidant defense capacity has been closely linked to the pathogenesis of neuronal dysfunction or death[21]. The generation of free radicals *in vivo* exceeds the rate at which endogenous antioxidants scavenge them, thus macromolecules like proteins, lipids etc. become targets for oxidative modification, which leads to deterioration of cellular architecture and signaling and ultimately death. The MDA is often considered as an index of free radical generation which increases in condition of oxidative stress[22].

The present study also showed that 900 MHz MW radiation caused oxidative stress in experimental animals. Significant increase in MDA level, a marker of lipid peroxidation and protein carbonyl content, a marker of protein oxidation clearly indicated that 900 MHz MW radiation exposure for 30 days was capable of causing oxidative damage. Interestingly, no significant alteration was observed in GSH level, indicating that MW exposure led to increase in oxidative stress by lipid and protein oxidation, but might not able to alter one of the important molecules of redox cycle for antioxidant defense.

Ilhan *et al*[23] have reported significant increase in MDA level in brain of Wistar rats after exposure to MWs at 900 MHz through mobile phones for

7 days. A significant increase in protein carbonyl content in brain tissue of Wistar rats after exposure to microwave radiation at 900 MHz and SAR 0.043-0.135 W/kg for 20, 40 and 60 days has also been reported[18]. In both the above-mentioned studies, no controlled system of MW exposure, such as TEM cell was used. However, these reports might support indirectly to our findings that MW exposure induced oxidative damage in experimental animal model. Presumably, MW exposure might produce oxidative stress in brain, which might be associated with impairment in cognitive function.

In conclusion, the present study demonstrated that the MW exposure at the 900 MHz and SAR of 8.4738×10^{-5} W/kg caused impairment of cognitive function and oxidative stress level in blood of experimental animals. Findings of the present study might shed further light upon the mechanisms underlying the cognitive and oxidative alteration of macromolecules without affecting GSH level. However, the limitation of our study was that we did not analyze oxidative stress in brain tissue and assessed other parameters of glutathione redox system, such as glutathione peroxidase, glutathione reductase etc and other antioxidant enzymes, such as superoxide dismutase, catalase etc.

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