Original Article

Effect of Low Level Subchronic Microwave Radiation on Rat Brain

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Abstract

Objective The present study was designed to investigate the effects of subchronic low level microwave radiation (MWR) on cognitive function, heat shock protein 70 (HSP70) level and DNA damage in brain of Fischer rats.

Methods Experiments were performed on male Fischer rats exposed to microwave radiation for 90 days at three different frequencies: 900, 1800, and 2450 MHz. Animals were divided into 4 groups: Group I: Sham exposed, Group II: animals exposed to microwave radiation at 900 MHz and specific absorption rate (SAR) 5.953×10^{-4} W/kg, Group III: animals exposed to 1800 MHz at SAR 5.835×10^{-4} W/kg and Group IV: animals exposed to 2450 MHz at SAR 6.672×10^{-4} W/kg. All the animals were tested for cognitive function using elevated plus maze and Morris water maze at the end of the exposure period and subsequently sacrificed to collect brain tissues. HSP70 levels were estimated by ELISA and DNA damage was assessed using alkaline comet assay.

Results Microwave exposure at 900-2450 MHz with SAR values as mentioned above lead to decline in cognitive function, increase in HSP70 level and DNA damage in brain.

Conclusion The results of the present study suggest that low level microwave exposure at frequencies 900, 1800, and 2450 MHz may lead to hazardous effects on brain.

Key words: Brain; Cognitive function; Comet assay; DNA damage; HSP70; Microwave radiation

Biomed Environ Sci, 2016; 29(12): 858-867 *doi: 10.3967/bes2016.115* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

INTRODUCTION

Current exposure to microwave radiation (MWR) is comparatively high because of the heavy use of Wireless Fidelity (Wi-Fi) communication devices and mobile phones. Advances in mobile phone technology have been accompanied by a progressive increase in the intensity and frequency of the emitted electromagnetic waves without consideration of health consequences. This is leading to increased concerns about potential harmful effects of MWR on impairment of cognitive function such as learning ability and concentration[1-3]. Exposure to 900 MHz

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Biographical note of the first author: Pravin Suryakantrao Deshmukh, male, born in 1984, PhD, majoring in microwave radiation and health hazard.

electromagnetic field radiation for 28 d has been reported to impair spatial memory in rats by activating the mkp-1/ERK pathway[4]. Chronic microwave exposure also induces cognitive deficit and 5-HT system in rats[5]. Whereas, microwave exposure at 900 MHz has been reported to cause no effects on spatial memory in male rats at sub-chronic and chronic level[6]. In other few studies also no differences were observed in cognitive performance in response to microwave exposure and no clear evidences have been established that mobile phone signals affect cognitive function[7-8]. Thus, the reports on effects of MWR on cognitive function are still inconsistent and remain controversial.

Heat shock responses are activated by stress and a variety of other stimuli that are potentially harmful to cells and microwave radiation is one of the recent additions to the list of physical stimuli[9-10]. Heat Shock protein 70 (HSP70) is one of the most studied heat shock proteins and it is the central component of the cellular network of molecular chaperones and folding catalysts. HSP70 protects cell against a variety of environmental stressors[11]. The function of HSP in general is to act as molecular chaperones that bind the partially damaged or denatured proteins and this is important why HSP70 is itself one of the best examples of altered protein conformation[12]. Therefore, it is thought that HSPs are important markers of stress.

The effect of microwave radiation depends on the energy absorbed by biological tissue which varies with how the energy is delivered in space and time. Moreover, the effects of MWR depend upon its electromagnetic characteristics such as frequency, intensity and exposure duration. DNA is continuously damaged by endogenous and exogenous factors and then repaired by DNA repair enzymes. DNA damage and/or its faulty repair can result in accumulation of DNA adducts that can eventually lead to changes in cellular functions, cell death or cancer[13-14]. The damage can be in the form of single and double strand breaks. The genotoxic effects of MWR exposure for 30 and 60 d have been studied in our laboratory using most commonly used method called comet assay, where we have reported that low level MWR can induce DNA damage in rat brain[15-16].

The hippocampus is an utmost important part of brain which controls behavioural and cognitive functions including spatial and working memory and has been reported to be vulnerable to microwave exposure. Thus, the present study is focused on hippocampal region of brain[17-20]. Till date no study has reported effects of microwave frequencies (900 MHz, 1800 MHz, and 2450 MHz) at low power level for long duration on cognitive function, HSP level and DNA damage. Therefore, the current *in vivo* study was undertaken to investigate the effects of low level MWR at three different frequencies (900 MHz, 1800 MHz, and 2450 MHz) on cognitive function, HSP70 and DNA damage.

MATERIALS AND METHODS

Microwave Exposure Set Up and Dosimetry

The Gigahertz Transverse Electromagnetic (GTEM) cell was designed in collaboration with Center for Applied Research in Electronics (Microwave Laboratory), Indian Institute of Technology, New Delhi and Amitech Electronics Ltd. Sahibabad, Ghaziabad (Uttar Pradesh, India) to estimate biological effects of MWR exposure in experimental animals (Figure 1A & B). GTEM cell is a pyramidal tapered, dual terminated section with its outer cell dimension Length (L): 220 cm × Breath (B): 120 cm × Height (H): 80 cm. Microwaves are generated through microwave generator SMC 100 (Rhode & Schwarz GmbH & Co, Germany). The microwave source consists of a signal generator operating at frequency range from 9 kHz to 3.2 GHz, amplifier, Direct Current (DC) regulator and a power meter. During the exposure rats were restrained in closed boxes (dimension as 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 and kept at a distance of 100 cm from the source. The microwave chamber is lined with absorbers which minimize the possibility of any reflections. The electric field was experimentally checked using an electric field (E-field) probe inserted into the Transverse Electromagnetic (TEM) cell through a slit wall. Pre-exposure validation was conducted using spectrum analyzer to ensure the uniformity of the field strength across the volume of GTEM cell. The E-field strength was observed homogeneous inside GTEM cell. The microwave radiation used in the study is continuous wave and linearly polarized. The GTEM cell was placed in a temperature controlled room (22±2 °C) under constant lighting conditions. Specific absorption rate (SAR) distribution was calculated by the power balance method using following equation[21].

$$P_{abs/rat} = 1/n\ (P_{in} - P_{out} - P_{refl}) \quad (1)$$

Where, P_{abs} = Radio frequency (RF) power (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).

Animal Exposure

Male Fischer-344 rats (60 d old, weighing 150-200 g) were obtained from central animal house facility of the institute and placed in individual raised, galvanized wired cages. They were acclimatized to laboratory conditions for 5 d and were kept under standard conditions (temperature 22±2 °C, constant humidity 40%-50%) with alternating 12 h light and dark cycle. Rats were provided with nutritionally adequate standard diet obtained from Nutrilab (Bangalore, India) and water *ad libitum*. Animals (24 rats) were divided into four groups (6 rats in each group): Group I (sham exposed): animals kept under the same conditions as that of other groups except microwave exposure, Group II: animals exposed to microwave radiation at 900 MHz, SAR 5.953×10^{-4} W/kg, Group III: animals exposed to 1800 MHz, SAR 5.835×10^{-4} W/kg and Group IV: animals exposed to 2450 MHz, SAR 6.672×10^{-4} W/kg. At a time 6 rats were given whole body microwave exposure at frequencies 900, 1800 and 2450 MHz (power level 0.00 dBm) for 90 d (2 h/d, 5 d/week) during light period at the same time every day. Rats had no access to food/water during the exposure and were returned to their home cages after exposure. The protocol and study method was approved by the Institutional Animal Ethics Committee (IAEC), University College of Medical Sciences, Delhi and care of the animals was undertaken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India. Body temperature of rats was noted by rectal measurements before and after the microwave exposure in all the groups. The microwave exposure resulted in no change of body temperature. All the animals were tested for cognitive function using elevated plus maze and Morris water maze after the termination of exposure period and subsequently sacrificed to collect brain tissues.

Assessment of Cognitive Function

Elevated Plus Maze (EPM) Paradigm Elevated plus maze (EPM) is a simple method for assessing behavioural response in rodents[22]. EPM has two opposite open arms (50 cm x 10 cm), crossed with two closed arms of the same dimensions with 40 cm high wall. The arms are connected to a central square (10 cm x 10 cm) (Figure 2). The rats were trained on EPM one day prior to microwave exposure and acquisition was measured in terms of seconds. Rats 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

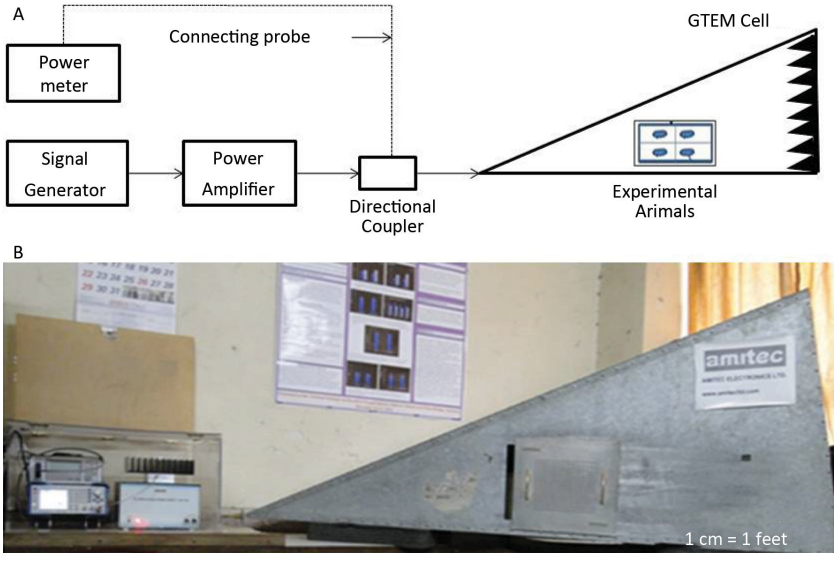

Figure 1. (A) Schematic diagram of microwave exposure setup. (B) Gigahertz Transverse Electromagnetic cell (GTEM cell).

of the closed arms was recorded as initial transfer latency (ITL). The animal which could not enter the closed arm within 90 s was gently pushed into one of the closed arms and ITL was assigned as 90 s. Retention of memory after 24 h was assessed in a similar manner.

Morris Water Maze (MWM) Test The acquisition and retention of a spatial navigation task were examined using Morris water maze method[23]. Animals received a training session consisting of four trials in a day 4 d prior to microwave exposure. The Morris water maze (180 cm diameter x 60 cm height) was filled with water. An escape platform was hidden 2 cm below the surface of water in a fixed location in one of the four quadrants halfway between the wall and the middle of the pool. The water was made opaque during the task with a nontoxic dye. Each trial consisted of releasing a rat into the water facing the wall of the pool, at one of four starting compass positions (North, South, East, West), 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 was 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, with 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 was recorded.

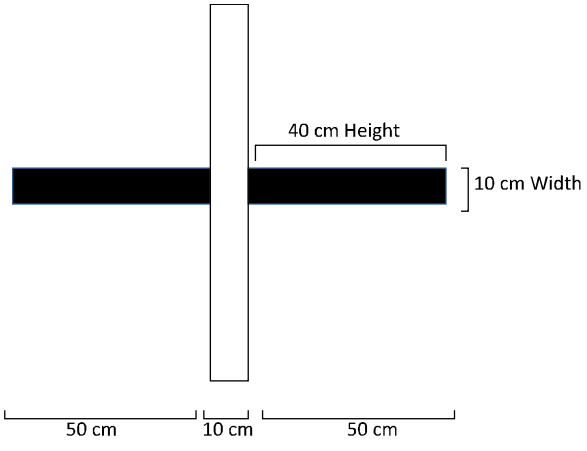

Figure 2. Schematic diagram of elevated plus maze. Black coloured; closed arms and white coloured; open arms.

Preparation of Brain Samples and Estimation of HSP70 Level

After the termination of exposure the rats from each group were anesthetized and decapitated to isolate brain tissues. Hippocampal portions were dissected out and washed with phosphate buffer solution (PBS) pH 7.4 and subsequently homogenized with appropriate amount of PBS at 4 °C with protease inhibitors and then centrifuged to collect supernatant which was stored at -80 °C until use. The level of HSP70 (pg/mL) was determined by a commercially available enzyme linked immunosorbent assay (ELISA) Kit (Assay design, NY USA) using an experimental protocol according to manufacturer's instructions.

Measure of DNA Damage Using Alkaline Comet Assay

DNA damage was evaluated using the alkaline comet assay with some minor modifications[13]. Slides were prepared in duplicates per sample. Briefly, the remaining half of the hippocampus minced in 1 mL chilled mincing solution (Hank's balanced salt solution, with 20 mmol/L ethylene diamine tetra acetic acid (EDTA) and 10% dimethyl sulfoxide (DMSO) in a petri dish and chopped into small pieces with a pair of scissors to get a uniform cell suspension. Slides were precoated with 600 µL of low melting agarose (LMA, 1.0%) prepared in PBS. 600 µL of diluted sample (50 µL cell suspension mixed with 600 µL of 0.75% low melting agarose, LMA) was added to form the second layer. The slides were kept on ice for 5 min to allow the gel to solidify. The slides were immersed in freshly prepared chilled lysing solution containing 2.5 mol/L NaCl, 100 mmol/L EDTA, 10 mmol/L Tris (pH 10) with 10% DMSO and 1% Triton X-100 (added just before use). The slides were left in the lysing solution for 1 h at 4 °C. The slides were placed in fresh and chilled electrophoresis buffer (1 mmol/L Na_2EDTA and 300 mmol/L NaOH, pH > 13) for 25 min to allow DNA unwinding and expression of alkali-labile sites as DNA strand breaks. Electrophoresis was conducted at 0.9 V/cm for 60 min at 4 °C. All these steps were performed under dim light and the electrophoresis tank was covered with black paper to avoid additional DNA damage due to stray light. Tris buffer (0.4 mol/L, pH 7.5) was added drop-wise and left for 5 min to neutralize excess alkali and was repeated

three times. Air dried slides were stained with 100 µL of ethidium bromide (20 mg/mL) for 5 min. Slides were randomized and coded to blind the scorer for analysis. All slides were scored by one person to avoid inter scorer variability. Slides were scored using an image-analysis system (Kinetic Imaging, Liverpool, UK) attached to a fluorescence microscope (BX51, Olympus Japan). Images from 100 cells (50 from each replicate slide) were analyzed. Undamaged cells had an intact nucleus without a tail and damaged cells had the appearance of a comet. To quantify DNA damage, the parameters such as percent of DNA content in the head and tail, Olive tail moment (OTM), tail extent moment and tail length (TL) were evaluated using Komet 6.0 software (Kinetic Imaging, Liverpool, UK) as described by Tice[24].

Statistical Analysis

Statistical analysis was performed with SPSS (version 16.0). All values were expressed as mean ± Standard Deviation (SD). Significance of difference among groups was determined by one way analysis of variance (ANOVA) using Tukey's test. Statistical significance was accepted at $P < 0.05$.

RESULTS

Effect on Cognitive Function

Elevated Plus Maze (EPM) The influence of MWR on cognitive function was evaluated using elevated plus maze. All the microwave exposed groups showed significantly higher transfer latency (TL) as compared to sham exposed group but when TL was compared between the microwave exposed groups (900 MHz, 1800 MHz, and 2450 MHz), no significant difference was observed. TL on the first day (on 90th day, end of exposure duration) shows the acquisition of learning behaviour of animals, whereas TL on next day (24 h after 90 d of microwave exposure) shows retention of information or memory. Significant differences were observed in TL between sham exposed and microwave exposed groups (Figure 3A and B). Animals exposed to MWR for 90 days took more time to enter one of the closed arms of elevated plus maze when compared to sham exposed animals. Thus, increase in TL indicates significant impairment in learning and memory.

Morris Water Maze (MWM) Spatial memory performance was evaluated using Morris water maze in all the experimental groups (Figure 3C and D). Significant difference with respect to escape time was observed between microwave exposed and sham exposed groups. During the probe trial (with the removed platform) microwave exposed rats took longer time to locate the place where the platform was placed. Time to reach the target quadrant was significantly longer in microwave exposed groups and the time spent in the target quadrant was significantly shorter in microwave exposed groups when compared to the sham exposed group. Whereas no significant differences were obtained when

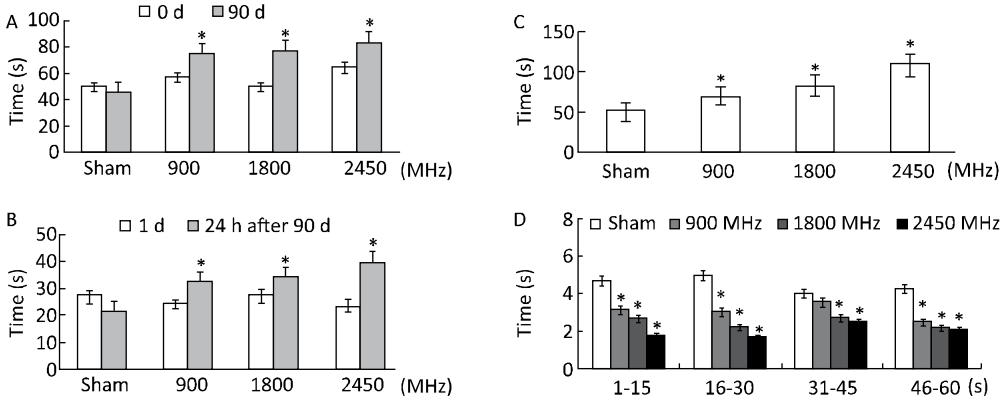

Figure 3. Effect of microwave radiation exposure on cognitive function. (A) Time taken by rats to enter one of the closed arms during elevated plus maze (Acquisition). (B) Time taken by rats to enter one of the closed arms during elevated plus maze (Retention). (C) Escape latency time (ELT) of rats during Water maze test to locate hidden platform. (D) Time spent in target quadrant (time was divided into 4 intervals of 15 s). * shows significant difference from sham exposed group ($P < 0.05$). Values are expressed as mean ± SD (6 animals per group).

compared between microwave exposed groups (900, 1800, and 2450 MHz).

Effect on Heat Shock Protein (HSP70)

Exposure to 90 d MWR resulted in significant increase in HSP70 level in all the groups (900, 1800, and 2450 MHz), when compared with sham exposed group ($P < 0.001$, Figure 4). Interestingly, significant increase in the level of HSP70 was observed in 1800 MHz ($P < 0.05$) and 2450 MHz ($P < 0.001$) exposed groups in comparison with 900 MHz exposed group.

Effect on DNA Strands

Comet assay performed in hippocampal tissues following MWR exposure showed significant increase in the percent of DNA in tail, tail extent moment, Olive tail moment, and tail length in all microwave exposed groups when compared to sham exposed animals (Figure 5). The percent of DNA migrating into the tail region was significantly enhanced in all the three groups, i.e., 900 MHz ($P < 0.05$), 1800 MHz, and 2450 MHz ($P < 0.001$) when compared to sham exposed group. Correspondingly, the percentage of DNA in the head was significantly decreased in all the microwave exposed groups, i.e. 900 MHz ($P < 0.05$), 1800 MHz, and 2450 MHz ($P < 0.001$) (Figure 6A). The head and tail DNA content in 2450 MHz exposed group was significant ($P < 0.001$) when compared with 900 MHz exposed group. The Olive tail moment was also increased significantly ($P < 0.05$) in animals exposed to microwave radiation at all the three frequencies ($P < 0.001$, Figure 6B) when compared to sham exposed group. The Olive tail moment in 2450 MHz exposed group was significantly ($P < 0.001$) increased as compared to 900 MHz exposed group.

Similarly, the tail length of comet was increased significantly in animals exposed to 900 ($P < 0.05$), 1800, and 2450 MHz ($P < 0.001$) exposed groups when compared to sham exposed group. Significant ($P < 0.05$) difference in tail length was observed in 2450 MHz exposed group in comparison with 900 MHz exposed group (Figure 6C). Significant ($P < 0.001$) increase in tail extent moment was noted in all microwave exposed groups when compared to sham exposed group. Similarly, the tail extent moment in 2450 MHz exposed group showed significant difference ($P < 0.001$) when compared with 900 MHz exposed group (Figure 6D).

DISCUSSION

The present study was carried out using a specially designed microwave exposure system, the GTEM cell for irradiation to experimental animals and provides evidence that low level MWR exposure for 90 d results in cognitive impairment, elevation in HSP70 protein level and DNA damage in brain of Fischer rats. Microwaves emitted from cell phones fall within the range between 300 MHz to several gigahertz. The GTEM cell allows the generation of microwave radiation in the range of mobile phone frequencies. At the cellular and sub-cellular level, microwaves may exert direct or indirect effects on cell membranes, cytoplasm, and nucleus[25].

Brain is the most sensitive target organ, the damaging effects of microwave radiation on the brain includes brain dysfunction and brain structural

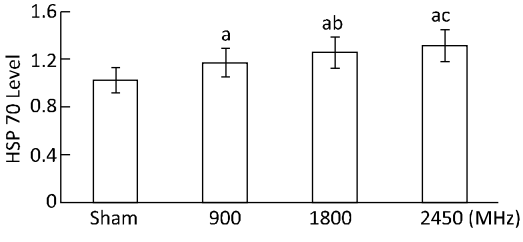

Figure 4. Effect of microwave radiation exposure on HSP70 level (pg/mL) in rat hippocampus. [a]$P < 0.001$, when compared with sham exposed group. [b]$P < 0.05$, [c]$P < 0.001$ when compared with 900 MHz exposed group. Values are expressed as mean ± SD (6 animals per group).

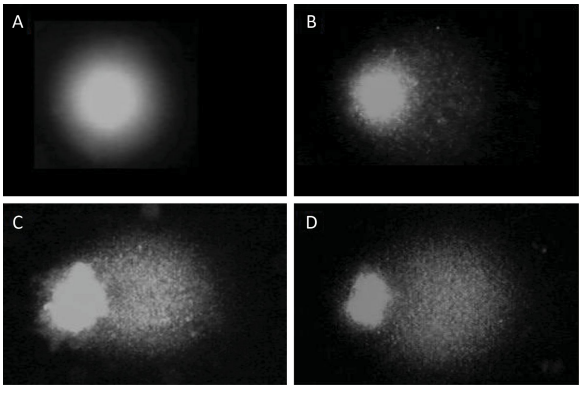

Figure 5. Representative picture of comet (DNA damage) at different frequencies in rat hippocampus observed in fluorescent microscope at 40 ×. (A) Sham exposed, (B) 900 MHz exposed, (C) 1800 MHz exposed, and (D) 2450 MHz exposed.

damage[26]. Hippocampus which is an utmost important part of brain, controls behavioural and cognitive functions including spatial and working memory has been observed to be vulnerable to microwave exposure[16-17,20,27]. In the present study, cognitive function was found to be declined in the rats exposed frequencies 900 MHz, 1800 MHz, and 2450 MHz for 90 d. Our earlier study also suggests that MWR exposure for 30 d at low level, i.e., 900 MHz at 5.953×10^{-4} W/kg, 1800 MHz at 5.835×10^{-4} W/kg, and 2450 MHz at 6.672×10^{-4} W/kg, causes impairment in learning and memory[28]. Microwave exposure for 30 d at 900 MHz even at very low level affects cognitive function[29]. The declined cognitive function could be due to direct or indirect interaction of microwave radiation with brain of experimental rats[30]. Li et al. has shown that long term microwave exposure at 2.856 GHz with the average power density of 5, 10, 20, or 30 mW/cm^2 respectively for 6 min (3 times a week) up to 6 weeks induces cognitive deficit in Wistar rats[31]. In another study by Narayanan et al. it has been reported that animals exposed to the GSM mobile phone exposure at (900/1800 MHz) with 50 missed calls/day for four weeks showed alterations in acquisition of learning response in the Morris water maze test[23]. Nittby et al. reported significant impairment in cognitive function after 55 weeks of exposure in rats exposed to MWR at GSM-900 with whole body SAR value of 0.6 and 60 mW/kg[32]. Dubreuil et al. used radial arm maze and dry land spatial navigation task to evaluate memory effect on head exposure in rat at 900 MHz for 45 min (1 and 3.5 W/Kg), and they reported no significant change[33]. In our recently reported study, it has been revealed that low intensity microwave radiation causes alternations in monoamine neurotransmitters associated with learning and memory[14]. Our observations were not found correlated with Dubreuil et al. (2002), might be due to the inadequate duration of exposure in their study[33]. Cognitive impairment could be due to the damage in the blood brain barrier and the cells in the brain which are concerned with learning, memory and movement[34-37].

In the present study, MWR triggered elevation in level of HSP70 in all the groups, i.e. 900 MHz, 1800 MHz and 2450 MHz (SAR 5.953×10^{-4} W/kg, 5.835×10^{-4} W/kg and 6.672×10^{-4} W/kg respectively). Some of the observations have reported that non-thermal radio frequency energy induces heat shock response in various cellular targets and observed different results in cell sensitivity to electromagnetic fields[38-40]. In our earlier study, we have reported that MWR exposure for 30 d at low level leads to elevation in level of HSP70[28]. A study by Yang et al. demonstrates that exposure to electrom- agnetic fields at 2450 MHz, SAR 6 W/kg, elicits a stress response as indicated by increased level of HSP70 in rat hippocampus[9]. Hippocampus controls the behavioural and cognitive functions including spatial and working memory. The elevation in level of HSP70, in line of the evidences of above reported

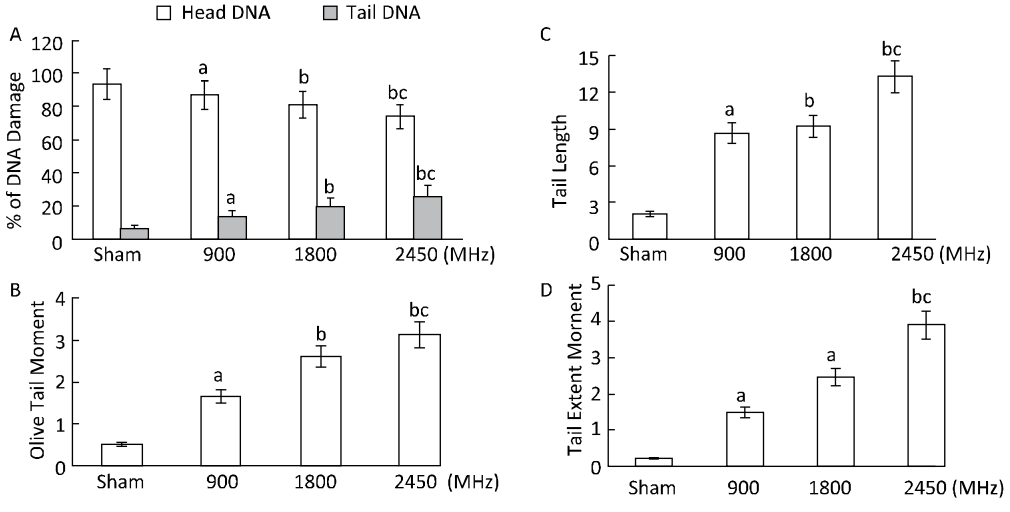

Figure 6. Effect of microwave exposure on DNA sensitivity in rat hippocampus. (A) Percent DNA in head and tail, (B) Olive tail moment (arbitrary unit), (C) Tail length (μm), and (D) Tail extent moment. [a]$P < 0.05$, [b]$P < 0.001$ when compared with sham exposed group. [c]$P < 0.001$ when compared with 900 MHz exposed group. Values are expressed as mean ± SD (6 animals per group).

studies suggest that increase in HSP70 might be one of the possible causes for cognitive decline in experimental animals. Narayanan et al. have showed that microwave exposure at 900-1800 MHz leads to shrunken darkly stained neurons in the CA[3] region of the hippocampus of rat brain[41]. The exposure to GSM 900 MHz, SAR 6W/kg showed the damaging effect on glial cells, which alters neuronal activity in the rat hippocampus[42]. Zhao et al. demonstrated that low level long term MWR exposure at average power densities of 2.5, 5, and 10 mW/cm^2 with average SAR of 1.05, 2.1, and 4.2 W/kg respectively leads to marked alterations in the structure and function of the hippocampus in rat brain[43]. It has been reported that weak MWR (15-20 µW/kg) can alter proteins, explaining the way of stress activation[44]. Jorge-mora et al. reported that electromagnetic fields affect the heat shock protein levels[45].

In the present study, DNA damage was observed at subchronic low level MWR exposure in brain of experimental rats. It is apparent from our study that, at such low level of MWR and the range of frequency from 900 to 2450 MHz may lead to genotoxicity in brain. In one of our earlier studies, we have reported that low intensity MWR exposure for 30 d is capable of interacting with DNA by unknown mechanism and causes single strand DNA breaks[13]. The biochemical compounds in living cells are composed of charges and dipole that can interact with electric and magnetic fields. Electrons have been shown to move in DNA and biochemical reactions may be modulated by electromagnetic field[3,46]. Indirect theory attributes DNA damage to oxidative stress through reactive oxygen species (ROS)[47-48]. ROS may play a role in mechanism of biological effects caused by MWR[13,49].

Kesari et al. have reported that high frequency electromagnetic field (2.45 GHz, 50 Hz modulated) exerts their genotoxic effects in male Wistar rats. They have observed significant increase in DNA strand breaks in brain cells of rats after 2 h exposure/day to MWR (whole-body SAR of about 0.11 W/kg) for 35 d [50]. Lagroye et al. reported no significant change in DNA strand breaks in brain cells of rats exposed to 2450 MHz field for 2 h at 1.2 W/kg[51]. Vershaeve et al. observed that long-term exposure (2 h/day, 5 d/week for 2 years) of rats to 900 MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells[52]. This negative finding may be the result of the variation in the experimental setup and exposure system. Thus the results on genotoxic effects of radiofrequency electromagnetic fields are still contradictory.

Campisi et al. have shown increase in oxygen radicals accompanied by increase in DNA strand breaks in primary rat glial cells after exposure to 900 MHz field[38]. To achieve this effect exposure time of 20 min in electric field strength of 10 V/m (safety limit: 41 V/m) was sufficient. Xu et al. have also demonstrated the genotoxic potential of mobile phone radiation[53]. They have reported that the DNA adduct rate caused by oxygen radicals in the mitochondria of primary cultured neurons is significantly increased after a 24 h GSM exposure. Lai et al. reported increased in single and double strand DNA breaks in brain cells of rats exposed to 2450 MHz for 2 h at whole body specific absorption rate 0.6 W/kg[54-55]. Guler et al. observed lipid and DNA damage in brain of pregnant and non pregnant rabbits, but not in their new borns after exposure (15 min a day for 7 d) to 1800 MHz signals (electric field strength: 14 V/m; safety limit: 58 V/m)[56]. The present study demonstrates that microwave exposure may cause genotoxic alterations in the brain of whole body exposed rats. Paulraj and Beharireported single strand DNA breaks in Wistar rat brain exposed to low intensity MWR exposure for 35 d (2.45 and 16.5 GHz, SAR 1.0 and 2.01 W/kg respectively)[57]. Aweda et al. reported that low SAR 2.45 GHz microwave radiation exposure can induce single strand breaks in brain cells of rats[58]. Thus, DNA damage has been reported to occur in brain cells of experimental rats exposed to radiofrequency electromagnetic field below the valid safety limits of 2 W/kg (ICNIRP guideline 1998)[59]. Thus, the question arises-whether or not the same deleterious alterations may also occur in the brain tissue of regular mobile phone users.

CONCLUSION

In conclusion, the present study suggests that low level subchronic microwave radiation leads to potentially significant effects on rat brain as evidenced by DNA damage and increased HSP70 level in hippocampus tissues which could induce cognitive impairment in rats. Further *in vivo* experimentation is warranted to better understand the molecular mechanism of action.

ACKNOWLEDGEMENTS

The authors are grateful to Indian Council of

Medical Research (ICMR), New Delhi for grant in the form of the extramural research project vide sanction letter No. 5/8/4-4(env) 07-NCD-I dated 3-08-09. One of the authors Pravin Suryakantrao Deshmukh is grateful to ICMR for Senior Research Fellowship (SRF) support. Mr. Digvijay Singh is duly acknowledged for his technical help during the animal experiments.

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interests.

Received: May 22, 2016;
Accepted: November 30, 2016

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