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2.45 GHz (CW) MICROWAVE IRRADIATION ALTERS CIRCADIAN ORGANIZATION, SPATIAL MEMORY, DNA STRUCTURE IN THE BRAIN CELLS AND BLOOD CELL COUNTS OF MALE MICE, MUS MUSCULUS

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Abstract—Present study examines biological effects of 2.45 GHz microwave radiation in Parkes strain mice. Forty-day-old mice were exposed to CW (continuous wave) microwave radiation (2 h/day for 30 days). Locomotor activity was recorded on running wheel for 12 days prior to microwave exposure (pre-exposure), 7 days during the first week of exposure (short-term exposure) and another 7-day spell during the last week of the 30-day exposure period (long-term exposure). Morris water maze test was performed from 17th to 22nd day of exposure. At the termination of the exposure, blood was processed for hematological parameters, brain for comet assay, epididymis for sperm count and motility and serum for SGOT (serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate

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pyruvate transaminase). The results show that long-term radiation-exposed group exhibited a positive Ψ (phase angle difference) for the onset of activity with reference to lights-off timing and most of the activity occurred within the light fraction of the LD (light: dark) cycle. Microwave radiation caused an increase in erythrocyte and leukocyte counts, a significant DNA strand break in brain cells and the loss of spatial memory in mice. This report for the first time provides experimental evidence that continuous exposure to low intensity microwave radiation may have an adverse effect on the brain function by altering circadian system and rate of DNA damage.

1. INTRODUCTION

Microwaves (MW) are the electromagnetic waves with the wave length ranging from 1 mm to 1 m (frequency between 0.3 GHz to 300 GHz). These non-ionizing electromagnetic radiations have a large frequency band pattern and induce a lot of biological effects which are of great concern to human health due to their increasing use in daily life. These non ionizing radiations, generally produce heating effects, alter chemical reactions and induce electrical currents in the tissues and cells of biological system [1]. Numbers of studies have focused on RF (Radio frequency) and MW radiation-induced potential health effects and epidemiological studies have revealed that there is an increased risk of brain tumors among analogue cellular phone users [2]. In contrary, relation between exposure to RF field and cancer has not been found [3]. On the other hand, RF radiation (at 2.45 GHz and 27 MHz) is reported to increase cell proliferation [4], blood brain barrier permeability [5], Ca^{2+} efflux [6] and ornithine decarboxylase activity characteristic of many cancer promoters [7]. Protein Kinase C (PKC), a key enzyme involved in the signal transduction from membrane receptors in response to action of cytokines, growth factors, hormones etc. has also been reported to decrease in brain cells of rat exposed to RF radiation [8, 9]. These radiations are also reported to affect/ depress different neurotransmitters [10, 11] as well as structural and genomic changes in the brain and testis of rat [12]. Further, while no DNA damage in brain cells of rat has been observed after acute exposure (2 or 4 h exposure to 2450 MHz-CW) [13, 14], chronic exposure to low intensity MW (2.45 GHz and 16.5 GHz, 2 h/day/35 days) caused an increase in DNA single strand break in developing rat brain [15, 16]. Although it is well known that DNA damage in brain cells could affect neurological functions and also possibly lead to neurodegenerative disorders [17–19], it is yet to be elucidated how RF radiation may induce DNA strand break. However,

causal relation of EMF (Electro magnetic field) and biological effects such as neurological and degenerative changes can not be ruled out especially in response to chronic exposure.

A number of studies in mammals also report the effects of 2.45 GHz EMF on hematopoiesis in pregnant mice [20], spermatogenesis in mouse [21], brain development in mice [22], radial maze in rats [23], micronucleated erythrocytes in rats [24], and cholinergic activity in the rat [11]. Since susceptibility or responsiveness of various tissues and organ systems towards any stimulation or stressor including EMF exposure of different intensity and duration differs, conflicting and sometimes contradictory results are not surprising [25]. In spite of large number of studies focusing on either one or two parameters at a time, there is no comprehensive study describing the effect of EMF on various parameters of body metabolism combining behavioral responses and effects at the molecular level (DNA breakage). Moreover, there is complete lack of information on the circadian system/activity pattern following the exposure to microwave radiation. Hence present experiment was designed to study the effect of 2.45 GHz microwave radiation on various parameters, such as circadian system (locomotor activity pattern), brain function (DNA damage, spatial memory), liver function, haematological and spermatogenic parameters (sperm count, motility) of Parkes strain mice.

2. MATERIALS AND METHODS

2.1. Animals and Microwave Irradiation (Exposure Setup)

Forty day old male mice (Parkes Strain) were obtained from the mice colony of our laboratory maintained under light:dark cycle (LD12:12). Animals were supplied with food (standard rodent food pellets supplied by Pashu Aahar Kendra, Varanasi, India) and tap water *ad libitum*. Randomly selected 10 mice were divided into two groups (control and experimental) of five mice each ($N = 5$). The mice of the experimental group were exposed to 2.45 GHz microwave radiation (2 h/per day, from 09:00 h to 11:00 h for 30 days) as described below. The control group was subjected to sham exposure. The whole experiment was conducted in accordance with institutional practice and within the framework of the revised animals (Scientific procedures) act of 2002 of the Government of India on Animal welfare. Body weight was recorded weekly. Water maze test with respect to the MW exposure is presented in Fig. 1.

The experimental setup for microwave exposure is shown in Fig. 2. The microwave source consisted of Analog Signal Generator covering a frequency range from 250 kHz–20 GHz. The source (model No. E

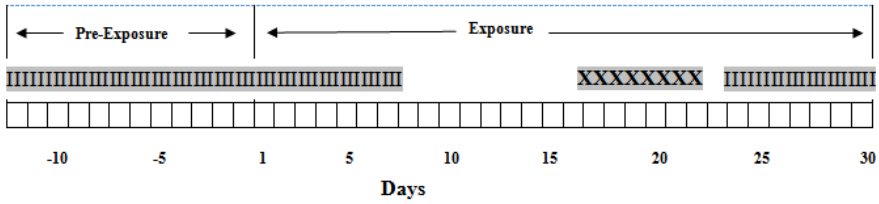


Figure 1. Diagrammatic representation of the period of monitoring of wheel running activity (IIIII) and Water maze test (XXXX) with reference to exposure to microwave radiation.

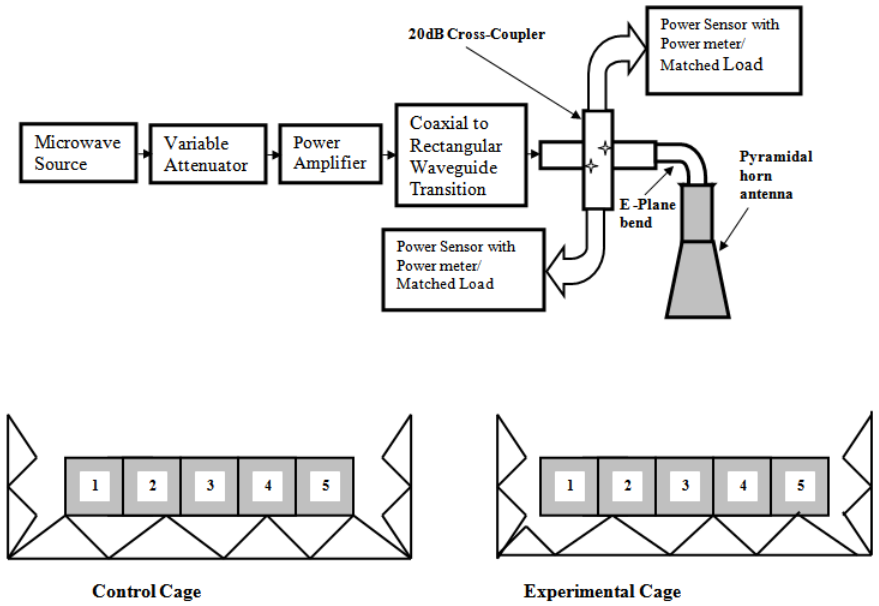


Figure 2. Diagrammatic representation of the setup for microwave radiation and the position of the animal cages during exposure.

8257D PSG) was manufactured by Agilent Technologies, USA. The required frequency was adjusted to 2.45 GHz continuous wave (CW). The microwave power was delivered to horn antenna through a coaxial attenuator, microwave amplifier (model No. 8349B, Hewlett Packard Co., USA), coaxial to waveguide transition, 20 dB cross coupler and E-plane bend. The maximum output from the amplifier was 19.8 dBm measured by the power sensor and meter having range from 30 µW to 3 W (model Nos. 8481H and 836A, Hewlett Packard Co., USA). Silver

polished brass material was used for pyramidal horn antenna. The throat dimensions of the antenna are 7.2 cm × 3.2 cm and those of horn antenna mouth are 9.0 cm × 5.0 cm. The axial length of the antenna from the throat is 10.0 cm. The distance between the pyramidal horn and the mid plane of the body of animal (assuming the body length to remain horizontal during exposure) was estimated through far field criterion ($R \geq 2D^2/\lambda$) to be 25 cm. The cross-sectional dimensions of the animal cage in E- and H-planes (electric and magnetic field planes respectively) were designed on the basis of 1.24 dB and 1.69 dB beam-widths in corresponding planes of the horn respectively. For this the horn radiation pattern were also measured at 2.45 GHz in E- and H-planes. The dimension of the animal cage was 25 cm × 16 cm × 6.5 cm accommodating five mice at a time. The cage and its partitions were made up of transparent low dielectric constant material polyethylene material (dielectric constant = 2.25). Small circular holes were drilled in the cage to keep the animals aerated during exposure and also to reduce the dielectric constant of the top surface for reducing the reflections and increasing the transmission of microwave into the cage. Each animal was kept in a pre-specified compartment of the cage throughout the exposure period. The partition was made in such a way that mice remain restrained in the cage during the period of microwave irradiation and they were exposed parallel to E field. The animal cage was placed on the carbon impregnated styrofoam microwave absorber to minimize reflection and was also tied to the absorber unit to avoid any change in the position of the cage with reference to the horn antenna. Everyday both control and experimental mice were placed in their designated cage and the compartment for two hours, i.e., from 09:00 to 11:00.

The 20 dB cross coupler, power sensor and meter having the range 30 µW to 3 W (model Nos. 8481H and 836A, Hewlett Packard Co., USA) were used to measure the power input to antenna and reflections from it. Power transmitted from the antenna was estimated by subtracting the power reflected from the antenna when it looks towards free space from the measured input power. This way power transmitted by the antenna was estimated which is equal to 54 mW. The effective relative permittivity ε_{reff} of the top surface of the perforated plastic animal cage was calculated using the following formula:

$$\varepsilon_{reff} = \frac{\varepsilon_{r1}V_1 + \varepsilon_{r2}V_2}{V_1 + V_2}$$

where ε_{r1} and ε_{r2} are the relative permittivity of the air and plastic respectively and V_1 and V_2 are their respective volumes [26]. The term 'effective' is used because the effective value of the parameters was estimated for a composite dielectric consisting air and plastic.

The values of ε_{r1} and ε_{r2} equals to 1.0 and 2.25 respectively. The expression has been used successfully in finding the effective relative permittivity of the discrete rods supporting the helix of a travelling-wave tube. For this purpose, the structure with discrete dielectric media is smoothed out as an equivalent continuous dielectric medium of an effective relative permittivity [27, 28]. The effective relative permittivity is computed to be 2.2. This was used to compute the power transmitted into the animal cage from its top surface, which is 49.54 mW. The power density was computed using the following formula:

$$Power\ Density = \frac{P_t G_t}{4\pi R^2}$$

where P_t is the power transmitted in to the cage, G_t is the gain of the horn and R is the distance between the horn aperture and mid plane of the body of exposed mice of the exposure cage and it equals to 0.02564 mW/cm^2. The SAR (specific absorption rate) was estimated for body length parallel to the electric field, as per the actual placement of mice [29]. Computed value of whole-body averaged SAR was found to be 0.03561 W/Kg.

Animals were kept in far field and exposed to very low intensity. These animals were normal through out the period of study and did not show any sign of heat stress or otherwise any discomfort. However, we have measured air temperature in the exposed and sham exposed animal cages but we did not find any difference in the temperature over the whole duration of exposure and also between the two groups.

2.2. Circadian Locomotor Activity

Both control and experimental mice were placed in individual running wheels (Clock Lab, USA) housed in a photoperiodic schedule of LD 12:12 (Light Onset at 06:00) maintained by an electronic timer (L&T; Larsen and Toubro Ltd, India). Food and water were supplied *ad libitum*. Each revolution of the wheel was taken as a count *via* a magnetic read-relay switch. Data were collected via the ACT-557 breakout box, the CL-200 data collection interface and analyzed as per the Clocklab analysis package (Coulbourn, USA) manual to generate actograms. The actograms were appropriately highlighted using Microsoft Paint software. The handling times were appropriately marked on the actograms. Individual actograms were analyzed to elucidate the phase angle difference (Ψ) with reference to the time of onset of darkness following the methods described earlier [30, 31]. The period of 3 hours consisting of exposure and handling duration was excluded to compensate for the effects of handling stress. The average phase angle difference in hours for each group was also plotted.

2.3. Morris Water Maze

Morris water maze experiment is used to test the spatial and working memory of each individual mouse. For this test each animal was placed into a small pool of water, which contains an escape platform hidden a few millimeters below the water surface. The mice were released in the four different quadrants sequentially for checking the escape latency time (ELT). Three different types of tests include: visible platform training (1 day), hidden platform test (4 days) and the probe test (without platform, 1 day). The timings (ELT) obtained for both groups were compared. This whole experiment was continued for 6 days, including last day for the probe test [32].

All the mice were taken to the experimental Morris water maze pool. The mice were allowed for 20 s to locate the platform in a target quadrant (Q4), and then were allowed to stay on the platform for 20 s. If the mice were unable to locate the platform within the time then they were guided by hand to the platform and were allowed to remain there for 120 s. Every time, the reaching time to the platform (ELT) was noted. After four-day of the hidden platform test, on the last day the platform was removed and the time spent by each animal in the target quadrant was noted. Starting position of mice on each day to conduct four acquisition trials were changed regularly.

2.4. Hematology

After the microwave exposure of 30 days, blood was collected from the tail vein to determine the number of erythrocytes & leucocytes by hemocytometric method and hemoglobin by hemometer (Sahli's method).

2.5. Spermatogenic Count

The mice were sacrificed by decapitation. Body was dissected; the epididymis was excised and placed in a pre-warmed Petri dish containing 0.5 ml of 0.9% NaCl at 37°C. The tissue was minced with scalpel for approx. 1 min and then placed in a 37°C incubator for 10 min prior to determining sperm motility. The suspension was stirred, one drop was placed on a warmed microscope slide, and a 22×60 mm cover slip was added on the top. For the determination of sperm motility, at least 10 microscopic fields were observed at $40\times$ magnification using a standard optical microscope, and the percentage of motile sperm was determined [33]. Thereafter, the cover slip was removed and the spermatozoa suspension was allowed to dry in air. The dried sample was stained with 1% eosinY/5% nigrosin and

examined at 40× for assessing sperm counts according to the WHO laboratory manual [34, 35].

2.6. Comet Assay

For the Comet assay experiment brain was taken out immediately from the mice sacrificed as described above and processed for the preparation of a single-cell suspension according to the method of Hartmann et al. [36] as described by Patel et al. [37]. Briefly, brain was placed in 1 ml chilled mincing solution (Hank's balanced salt solution, with 20 mM EDTA and 10% DMSO) in a Petri dish and chopped into small pieces with a pair of scissors. The pieces were allowed to settle and the supernatant containing the single cells was taken. Normal melting agarose (NMA, 1.0%) was prepared in Milli-Q water, microwaved and kept at 60°C. Conventional glass slides were then dipped into molten NMA up to two-thirds of their frosted end, the lower surface was wiped clean and the slides were left to dry. On this first layer, 80 µl of diluted sample (100 µl cell suspension mixed with 100 µl of 1% low melting point agarose, LMA) was added to form the second layer. A cover slip (size 24 mm × 60 mm) was placed gently to evenly spread the cells in the agarose. The slides were kept on ice for 5 min to allow the gel to solidify. The cover slips were removed and a third layer of 0.5% LMA (90 µl) was added onto the slide and placed over ice for 10 min. Finally, the cover slips were removed and the slides immersed in freshly prepared chilled lysing solution containing 2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10) with 10% DMSO and 1% Triton X-100 being added just before use. The slides remained in the lysing solution over night at 4°C, followed by electrophoresis according to the method of Singh et al. [38]. The slides were placed in a horizontal gel electrophoresis tank side-by-side and avoiding spaces, with agarose ends nearest to the anode. Fresh and chilled electrophoresis buffer (1 mM Na_2EDTA and 300 mM NaOH, pH > 13) was poured into the tank to a level of approximately 2.5 mm above the slides. The slides were left in this solution for 25 min to allow DNA unwinding and expression of alkali-labile sites as DNA strand breaks. Electrophoresis was conducted at 24 V (0.7 V/cm) and a current of 330 mA using a power supply (Electra Comet III from Techno Source India Pvt. Ltd., Mumbai, India) for 30 min at 4°C. All these steps were performed under dimmed light and the electrophoresis tank was covered with black paper to avoid additional DNA damage due to stray light. After electrophoresis, the slides were drained and placed horizontally in a tray. Tris buffer (0.4 M; pH 7.5) was added drop-wise and left for 5 min to neutralize excess alkali. Neutralizing of slides was repeated thrice. Each slide was stained with 75 µl EtBr (20 µg/ml) for 5 min and dipped in chilled distilled water to wash off

excess EtBr, and cover slip placed over it. Slides were placed in a dark humidified chamber to prevent drying of the gel. The slides were scored within 24 h.

Slides were scored using an image-analysis system (Kinetic Imaging, Liverpool, UK) attached to a fluorescence microscope (Leica, Germany) equipped with appropriate filters (N2.1, excitation wavelength of 515–560 nm and emission wavelength of 590 nm). The microscope was connected to a computer through a charge-coupled device (CCD) camera to transport images to software (Komet 5.0) for analysis. The final magnification was $\times 400$. The Comet parameters recorded were Olive tail moment (OTM, arbitrary units), tail DNA (%) and tail length (migration of the DNA from the nucleus, μm). Images from 100 cells (50 from each replicate slide) were analyzed as per the *in vivo* guidelines [36].

2.7. SGOT and SGPT Test

Trunk blood of the decapitated mice was collected in a tube and centrifuged at 4000 rpm for 15 min. Serum was separated and stored in -20°C until assayed. The level of SGOT (serum glutamate oxaloacetate transaminase) and SGPT (serum glutamate pyruvate transaminase) in the serum was measured with the help of SGOT-SGPT kits as per manufacturer's protocol (Span Diagnostics Ltd., India).

2.8. Statistical Analysis

The means of the control and experimental groups were compared using Student's 't' test. The null hypotheses were rejected at $p < 0.05$.

3. RESULTS

There was no significant difference in the body weight of the MW-exposed mice as compared to control or its initial weight.

3.1. Circadian Locomotor (Wheel Running) Activity

All mice exhibited positive phase angle differences (Ψ) between lights-off and the onset of activity. A statistically significant difference was witnessed when average Ψ of the long term (30 days) microwave-exposed group was compared with that of its time qualified control group and all other groups. The average Ψ of the short term (7 days) microwave-exposed group was not significantly different from that of its time-qualified control and pre-exposed control groups (Fig. 3).

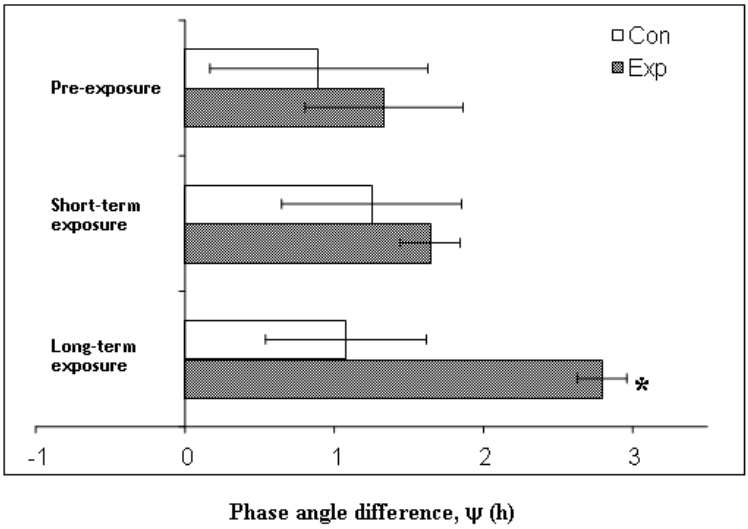


Figure 3. Phase angle difference (Ψ) in hours (h) between lights-off and the onset of activity of mice. $^{*}p < 0.05$, significance of difference from control group.

There was a marked shift in the activity bouts after long-term exposure to radiation, reflecting most of the activity occurring within the light fraction of the LD cycle. In contrast, in pre-exposure groups and to some extent in short-term exposed groups, most of the activity was restricted to the dark phase (Fig. 4).

Note marked shift in the activity bouts after long-term exposure to radiation (mostly occurring within the light phase of the LD cycle) in comparison to its pre-exposure and short term exposure period. Most of the activity is restricted to the dark phase in the sham exposed control group.

3.2. Morris Water Maze

In the Morris water maze experiment there was no significant difference among the ELT of control and exposed mice during hidden platform test. However, the mice exposed to MW radiation spent significantly less time in the Q-4 during probe test (6th day) as compared to control (Fig. 5).

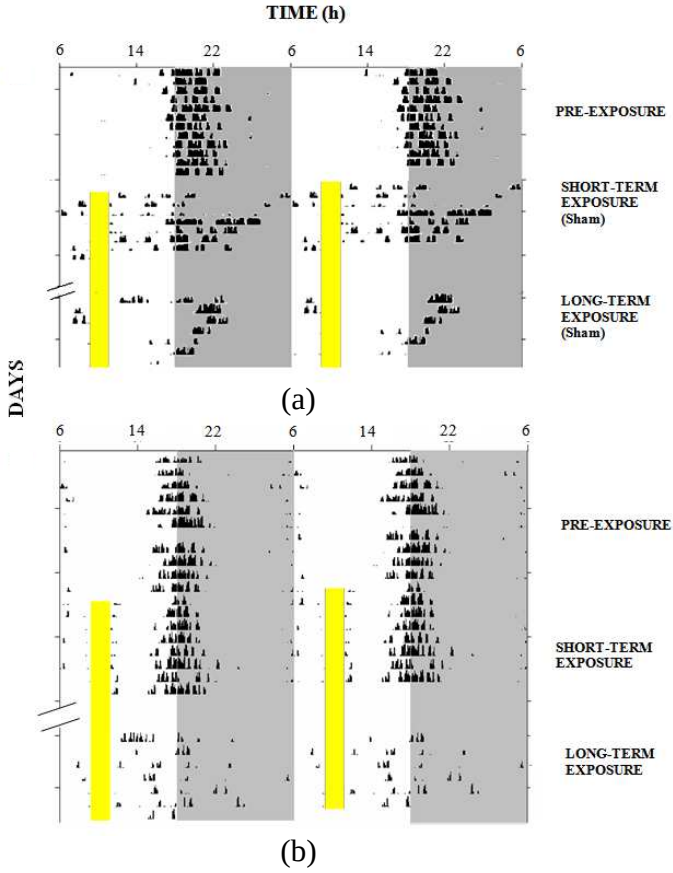
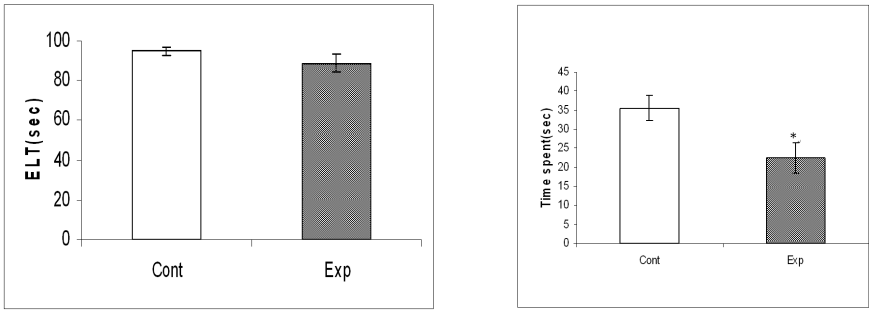


Figure 4. Representative actograms showing wheel running activity of control (upper panel; (a)) and microwave radiation exposed (lower panel; (b)) mice maintained under LD 12:12. Vertical bars represent period of microwave exposure (09:00–11:00).

3.3. Hematology

After the long-term MW radiation exposure, although there was no significant change in the hemoglobin content, the total number of erythrocytes and leucocytes increased significantly in the experimental mice as compared to the control (Fig. 6).



(a) (b)

Figure 5. Escape latency time (ELT) of mice during water maze test to locate the hidden platform; (a) and the time spent in Q-4 (target quadrant) during last day when platform was removed, i.e., probe test; (b) $^{*}p < 0.05$, significance of difference from control.

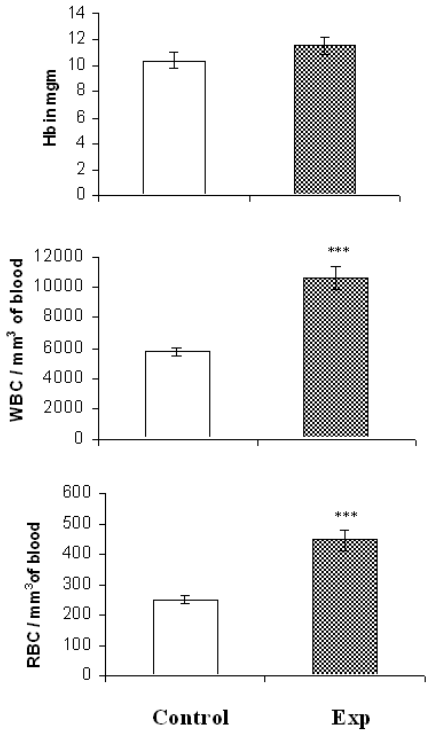
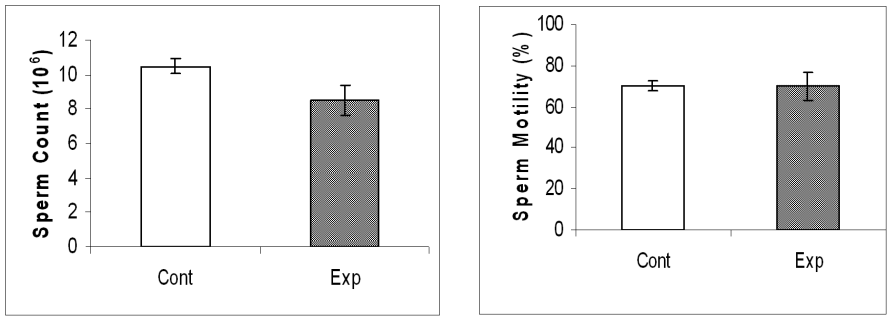


Figure 6. Effect of 2.45 GHz microwave radiation on the hemoglobin (Hb), white blood cell (WBC) and red blood cell (RBC) counts of mice. $^{***}p < 0.001$ significance of difference from control.

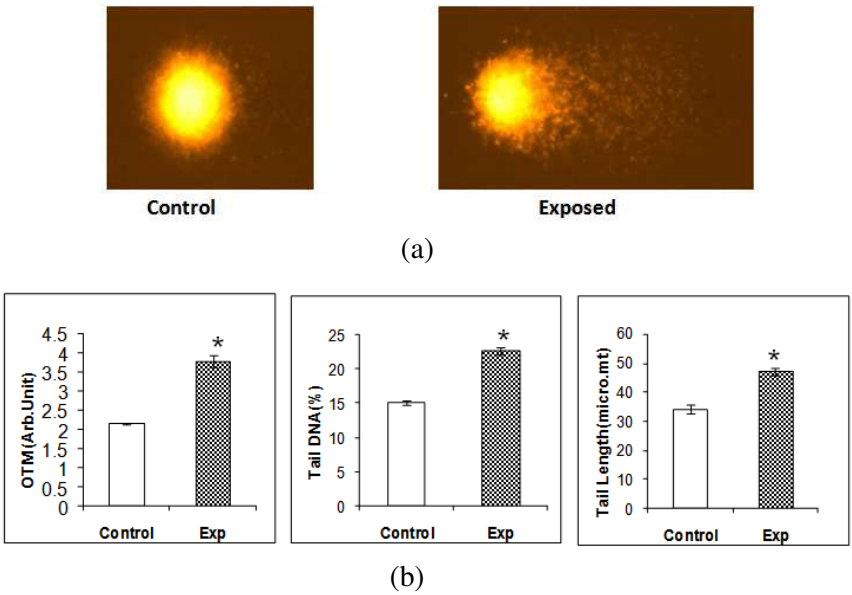
3.4. Sperm Counts

The sperm counts and the % of sperm motility were not altered in the treated mice as compared to the control (Fig. 7).



(a) (b)

Figure 7. Effect of 2.45 GHz microwave radiation on the sperm count (a) and sperm motility (b) of mice.



(a)

(b)

Figure 8. Effect of 2.45 GHz radiation on the DNA damage of brain cells in mice monitored by Comet assay.

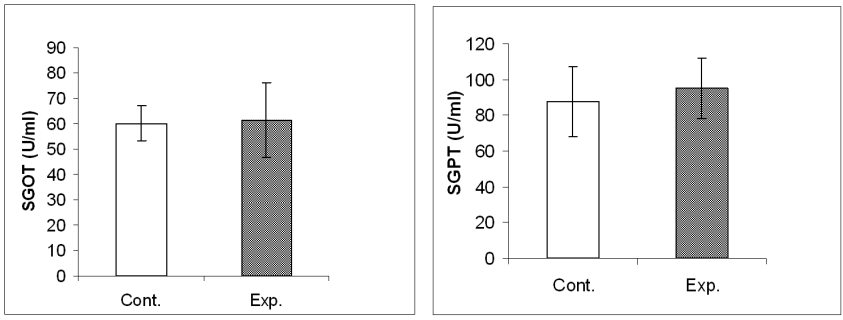
Upper panel of the Fig. 8(a) shows the photographs of intact (control) and damaged DNA (exposed) following long term exposure. In the comet assay image the magnification is 400×. Note significant increase in the tail length following DNA breakage in the exposed group. Lower panel (b) shows quantitative measurement of olive tail moment, % tail DNA and tail length. $^*p < 0.05$, significance of difference from control.

3.5. Comet Assay

A significant ($p < 0.05$) increase in the Olive tail moment (OTM), tail length and % tail DNA were observed in brain cells of microwave-exposed mice when compared to control brain cells (Fig. 8).

3.6. SGOT and SGPT Test

The levels of SGOT and SGPT are used as blood based markers of liver damage. There were no significant differences in the levels of SGOT and SGPT in the exposed mice as compared to the control (Fig. 9).



(a) (b)

Figure 9. Effect of 2.45 GHz microwave radiation on the serum glutamate pyruvate transaminase (SGOT; (a)) and serum glutamate pyruvate transaminase (SGPT; (b)) of mice.

4. DISCUSSION

In general, although the chronic exposure (2 h/day for 30 days) to 2.45 GHz microwave (CW) had no effect on the general body metabolism, it affected the circadian organization and spatial memory of mice. The long-term exposure to this non-ionizing radiation also led to strand DNA breakage in the brain cells as judged by the comet assay.

Monitoring the wheel running activity during pre-exposure, shot-term exposure and long-term exposure period clearly indicates that chronic exposure to 2.45 GHz MW radiation alters the characteristics of the circadian rhythms in locomotor activity of mice. From the actograms, it is apparent that under the long-term, but not short-term exposure regime, the activity tends to be suppressed in intensity. The large positive Ψ (advance of onset) exhibited by the animals exposed to radiation for longer durations could be due to the exposure of MW radiation early in the subjective day. Moreover, the activity seems to fragment into several components and shows a tendency towards splitting, even in the LD condition. It also appears that, long-term exposure to MW radiation caused a suppression of activity and reduced entrainment response to the imposed LD schedule. It may be surmised that the radiation is a weaker Zeitgeber than light, albeit it could be an effective one.

Further, these animals exhibit a bout of post-replacement activity, after wheel deprivation during the handling period, as reported earlier [39]. However, all the experimental animals tend to continue running after the post-handling bout, whereas control animals show a clear separation between the post-handling bout and the onset of activity. Further studies, especially in DD (constant darkness) or LL (constant light) are required to investigate the possible circadian-rhythm modifying effects of this radiation and to elucidate a phase-response curve. It may be tentatively stated that, the rest-activity ratios may be altered by this radiation. Moreover, the duration of long-term exposure could be increased further, in expectation of a concomitant increase in the phenomena discussed above. Present rhythm study indicates that microwave radiation has chiefly deleterious effects on the circadian physiology of mammals, evinced by suppression and disorganization of locomotor activity rhythm, shifting of the onset of locomotor activity, and failure to entrain to an LD cycle, not with short-term exposure, but when the exposure is relatively chronic.

The water maze test developed by Morris [32] is a behavioral procedure designed to test spatial memory in rodents. The spatial memory is part of memory responsible for recording information about one's environment. It helps in gathering and processing sensory information about its surroundings. In mammals the spatial memory is hippocampus dependent. The Morris water maze has proven to be a robust and reliable test that is strongly correlated with hippocampal synaptic plasticity and NMDA (N-methyl D-aspartate) receptor function. Present study shows that the learning increases continuously with the trials in both control and the exposed mice since they show improvement in their performance in locating the

hidden platform and ELT start decreasing suggesting that non-ionising MW radiation has no obvious adverse effect on the learning process. However, significant difference was seen in the probe test. The radiation exposed mice spent significantly less time in the target quadrant as compared to control. This preliminary study although suggests that spatial memory may be affected by such exposure, further studies especially the effect of short- and long-term exposures on memory, are required to strengthen this suggestion.

Microwave radiation increased the number of erythrocytes and leucocytes in irradiated mice, a condition similar to that observed in polycythemia (increased number of RBC) and leukemia (increased number of WBC) but there was no significant change in hemoglobin content. In rats an increase in leucocytes number but no difference in differential leucocytes count after four weeks of exposure at 100 Hz magnetic field has been reported [40]. Leucocytes are much more sensitive than erythrocytes. Black and Heynick [41] reported that exposure to radiofrequency electromagnetic fields can stimulate lymphocytes to become lymphoblasts (active cells undergoing mitosis). Although present findings indicate a significant increase in blood cell counts following microwave radiations, mechanism of such increase and its pathology needs to be confirmed before drawing meaningful conclusion.

Our data obtained from alkaline comet assay illustrated that prolonged exposure to microwave radiation causes DNA strand breaks in brain cells of mice. The data is consistent with a study by Paulraj and Behari [16], which demonstrated that thirty five days prolonged exposure to low intensity microwave can cause DNA strand break in the brain cells of rat. Another study reported that even acute low intensity microwave exposure (2 h) can produce a significant increase in the DNA strand breaks in rat brain [17]. In spite of number of reports indicating radiation induced DNA damage, the precise mechanism of DNA strand breaks due to microwave radiation is still unknown. However, there may be two possible pathways of DNA strand break: (i) an induction in DNA damage could be due to a reduction in the DNA damage repair processes in the cells or due to DNA–DNA and DNA–protein cross links and DNA adduct formation etc. which are generated as an intermediate step, during DNA repair; (ii) microwave radiation creates own magnetic field which can initiate Fenton reaction that generate free radical in brain cells leading to DNA strand breaks [9, 15, 17]. DNA strand break even after 2 hours of exposure indicates extreme sensitivity of brain cells to EMF, suggesting its adverse effect on brain function.

5. CONCLUSION

Based on the present findings, it is concluded that chronic exposure to microwave radiation alters blood picture and has degenerative effects on brain performance gauged from changes in spatial memory, circadian organization and extent of DNA damage. Although MW radiation exposure has no adverse effect on the reproductive and metabolic performance, prolonged exposure may lead to neurodegenerative disorder. Since brain performance is directly correlated to human health risk assessment; similar to ionizing radiation, safe limits to the frequency and duration of microwave radiation must be also prescribed in view of its increased use in the daily life of the modern society.

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