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Fifty-gigahertz Microwave Exposure Effect of Radiations on Rat Brain

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Abstract The object of this study is to investigate the effects of 50-GHz microwave radiation on the brain of Wistar rats. Male rats of the Wistar strain were used in the study. Animals of 60-day age were divided into two groups—group 1, sham-exposed, and group 2, experimental (microwave-exposed). The rats were housed in a temperature-controlled room (25 °C) with constant humidity (40–50%) and received food and water ad libitum. During exposure, rats were placed in Plexiglas cages with drilled ventilation holes and kept in an anechoic chamber. The animals were exposed for 2 h a day for 45 days continuously at a power level of 0.86 μW/cm^2 with nominal specific absorption rate 8.0×10^{-4} w/kg. After the exposure period, the rats were killed and homogenized, and protein kinase C (PKC), DNA double-strand break, and antioxidant enzyme activity [superoxides dismutase (SOD), catalase, and glutathione peroxidase (GPx)] were estimated in the whole brain. Result shows that the chronic exposure to these radiations causes DNA double-strand break (head and tail length, intensity and tail migration) and a significant decrease in GPx and SOD activity (p=<0.05) in brain cells, whereas catalase activity shows significant increase in the exposed group of brain samples as compared with control (p=<0.001). In addition to these, PKC decreased significantly in whole brain and hippocampus (p<0.05). All data are expressed as mean ± standard deviation. We conclude that these radiations can have a significant effect on the whole brain.

Keywords Glutathione peroxidase · Superoxidase · Catalase · Microwave radiation · Protein kinase C

Introduction

Microwaves may affect biological systems by increasing free radicals, which may enhance lipid peroxidation, and by changing the antioxidative activities of the brain cells, thus

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leading to oxidative damage. The effect of microwave radiations on biological systems is primarily identified as due to an increase in temperature, i.e., thermal [1], though nonthermal effects have also been identified [2, 3]. The present study was designed to determine the effects of 50 GHz nonthermal microwave radiation on rat brain. This frequency was chosen because 50 GHz has a very small penetration depth, which may cause an increase in the random molecular motion due to free-radical processes within the cell. So far, most of the investigations are confined to a frequency up to 10 GHz. Above-range frequencies will have limited penetration into the body, and their mode of interaction with the system, including those at cellular and molecular levels, will be interesting to examine. It will be of further interest to establish their commonality with after-exposure parameters at comparatively lower frequencies. The study also aims to spread over the electromagnetic field (EMF) effect over a wider band of nonionizing electromagnetic spectrum.

Protein kinase C (PKC) and antioxidant enzymes play an important role in functioning of the central nervous system. PKC plays a key role in a variety of pathologic states, including oncogenesis [4, 5]. This may affect the cellular responses to extracellular stimuli participated in cell differentiation and apoptosis [6]. PKC modulates ion conductance by phosphorylating membrane proteins such as channels, pumps, and ion exchange proteins mobilization into the cytosol. This has been implicated in phosphorylation of several neuronal proteins, which are thought to regulate neurotransmitter release and long-term potentiation in memory formation [7]. The activation of this enzyme is thought to be biochemically dependent on Ca^{2+}. Tumor-promoting phorbol esters, such as 12,O-tetradecanoyl phorbol-13-acetate (TPA), have a structure very similar to diacylglycerol and activate PKC directly, both in vitro and in vivo [8]. TPA has a specific membrane receptor in the cell membrane [9, 10]. In order to stimulate cell proliferation in cells, growth factor and PKC are needed to induce the signal pathways. Furthermore, tumor promoters TPA have a membrane receptor in the membrane of all cells. This receptor is considered to be calcium phospholipid-dependent protein kinase (PKC). It is involved in the regulation of a variety of cellular events, including modulation of receptor functions for major hormones and certain enzymes such as adenylate cyclase and ornithine decarboxylase.

In the present investigation, PKC has been estimated in hippocampus, whole brain, and the remaining portion. The hippocampus is understood to be responsible for learning and memory. In addition to this, antioxidant enzyme estimation has also been performed. It is well known that these enzymes play a major role in protecting the cells by removal of free radicals, which are generated by microwave radiations. It has been established that the overproduction of reactive oxygen species (ROS) comes through free radicals formation and may change the levels of superoxides dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activity in the whole brain. These parameters were undertaken to determine the possible site of the EMF biointeraction with ROS. Very recently, Mahfouz et al. [11] reported that microwave/radiofrequency radiation may lead to oxidative stress due to overproduction of ROS. Others workers, several from our laboratory, have shown that these radiations affect cholenergic systems, brain Na+–K+ ATPase activity [12, 13], growth-related enzymes [14], PKC activity [3], single-strand DNA breaks in brain cells [15, 16], and reduced infertility in male rats [17].

In continuation, DNA double-strand breaks were observed and comet scoring was carried out with tail and head-length migration and intensity. The first study of DNA strand break was observed by Lai and Singh [18] in rat brain cells at continuous and pulsed RF radiation. These field exposures induced the formation of DNA–protein and DNA–DNA cross-links in brain cells of rats [19], which could be the result of free-radical damage

involving iron cations [20, 21]. The results of Ivancsits et al. [22–25] indicate that the interaction of these fields with DNA is quite complicated and apparently depends on many factors, such as the mode of exposure, the type of cells, and the intensity and duration of exposure. Some researchers found conflicting results; some observed that there is no effect of radio frequency radiation exposure in DNA strand break of mammalian somatic cells [26, 27]. To support these data and to confirm their pathological implications, we have measured the activities of antioxidant enzymes, namely, SOD, CAT, and GPx and tumor promoter PKC in rat brain cells.

Material and Methods

Material

The GPx (catalog No. 703102), CAT (catalog No. 707002), and SOD (CAT No. 706002) antioxidant enzyme kit was purchased from the Cayman Chemical Company, Ann Arbor, MI, USA. P^{32} radioactive labeled ATP was purchased from BRIT, Hyderabad, India. The rest of the chemicals were purchased from Thomas Baker Chemicals Limited, Marine Drive, Mumbai, India.

Animals Exposure

Male Wistar rats (60 days old and 190±20 g body weight) were obtained from the animal facility of Jawaharlal Nehru University, New Delhi. They were divided into two groups, a sham-exposed group (n=6) and an experimental group (n=6). In the animal exposure and subsequent experimentation, a blind study was conducted, where the experimental and control code was not disclosed before the data analysis. All animals were housed in an air-conditioned room, where the temperature was maintained at 25 °C with constant humidity (40–50%). The air circulation was constantly maintained to keep it in equilibrium with the room temperature. The animals were provided with standard food pallets (prepared by Brook Bond India, Mumbai, India) and water ad libitum.

The protocol and study method was approved by the Institutional Animal Ethical Committee and the Committee for Purpose of Control and Supervision of Experimental on Animal.

Exposure Chamber

Two rats at a time were placed in a Plexiglas cage, which was quite ventilated with holes of 1-cm diameter. The dimension of the exposure cage was made in such a way that animals moved freely. The chamber is lined with radar-absorbing material (attenuation, 40 db) to minimize the possibility of any reflections. At far field distance from the horn antenna, it was found that the field is homogeneous in the vertical plane midline of the beam. Rats were exposed with 50 GHz continuous source through the antenna, 2 h a day for 45 days (Fig. 1). The power density at receiving end was measured (0.86 $\mu W/cm^2$) and the nominal specific absorption rate (SAR) value was calculated (8.0×10^{-4} w/kg). The animals faced E filed orientation parallel to the exposure chamber. Since the free space wavelength at this frequency is 0.6 cm, it is assumed that the exposure is limited to the subcutaneous surface. Every day, the cage was placed in the same position facing the horn antenna and the same number of rat positions was filled.

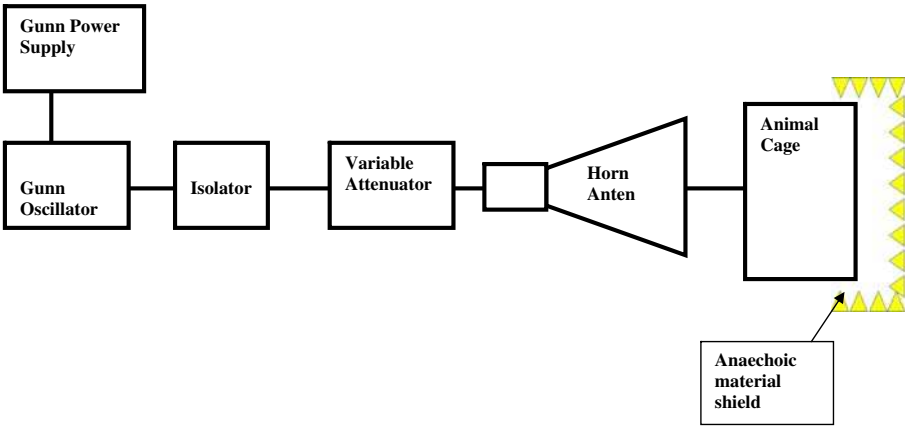

Fig. 1 Schematic diagram of 50-GHz radiation source

Sample Preparation and Tissue Homogenate

In the present investigations, enzyme assay was used to determine the enzyme activity (SOD, GPx, and CAT) in exposed Wistar rats. Immediately after exposure, animals were killed by overdose of anesthesia and the brain was collected in ice-cold buffer. The brain was homogenized in cold buffer (50 mM Tris–HCl, pH 7.5, 5 mM EDTA, and 1 mM DTT per gram tissue) for GPx, 5–10 ml HEPES buffer for SOD, and (50 mM potassium phosphate, pH 7.0, containing 1 mM EDTA per gram tissue) for CAT. The sample was centrifuged at 10,000×*g* for 15 min at 4 °C. Supernatant was collected and enzyme assay was performed. Different sets of animals with the same age group were taken for given parameters (DNA strand break, PKC, and antioxidant enzyme).

Estimation of GPx Activity

One hundred twenty microliters of assay buffer and 50 μl cosubstrate mixture were added in nonenzymatic wells. One hundred microliters of assay buffer, 50 μl of cosubstrate mixture, and 20 μl of diluted GPx were added in other wells as control samples, whereas the same amount of assay buffer and cosubstrate including 20 μl of brain sample in place of GPx were added in all the wells. Immediately, reaction was initiated by adding 20 μl of cumene hydroperoxide to all the wells being used. Finally, the well plate was placed in a microplate reader spectrophotometer, and the absorbances of the samples were taken at 340 nm

Estimation of Superoxidase Activity

Twenty microliters of SOD standard was diluted with 1.98 ml of sample buffer. SOD standard wells were prepared by using 200 μl of the diluted radical detector and 10 μl of diluted standard. Sample wells were also prepared by adding 200 μl of the diluted radical detector and 10 μl of sample to the wells. The reaction was initiated by adding 20 μl of diluted xanthine oxidase to all the wells. The sample plate was kept in microplate reader temperature, and absorbance was taken at 450 nm.

Estimation of CAT Activity

One hundred microliters of assay buffer, 30 μl of methanol, and 20 μl of standard were added to wells, which contained 10 μl of formaldehyde and 9.99 ml of sample buffer and formaldehyde wells were prepared. Control wells were prepared by adding 100 μl of diluted assay buffer, 30 μl of methanol, and 20 μl of diluted CAT. Thirdly, the sample wells were prepared by adding 100 μl of diluted assay buffer, 30 μl of methanol, and 20 μl of tissue samples. The reaction was initiated by adding 20 μl of diluted hydrogen peroxide to all the wells. Sample plate was incubated for 20 min at room temperature and 30 μl of potassium hydroxide was added to terminate the reaction. Thirty microliters of purpald (chromogen) was added to each well and thereafter incubated for 10 min at room temperature on a shaker. Ten microliters of potassium periodate was added to each well, incubated for 5 min at room temperature on shaker, and the absorbance of samples was taken at 540 nm.

Calcium-dependent Protein Kinase (PKC) Assay

After 45 days of exposure, the animals were killed with rat cocktail anesthesia (Ketamine, Xylazine; IP) and then decapitated. Brain tissue was taken out from the cranial cavity immediately and put into a deep freezer for a short while to become tissue hard. The hippocampus was taken out and assays were performed in three sets as follows: (1) hippocampus, (2) whole brain minus hippocampus (remaining brain), (3) whole brain.

Each brain tissue was homogenized separately in 40 vol of ice-cold, 1-mM sodium bicarbonate (pH 7.5). The homogenate was centrifuged at 600 g for 10 min at 4 °C. The supernatants were centrifuged at 20,000×*g* for 30 min at 4 °C. The pellet was pipetted with ice-cold, 1-mM sodium bicarbonate and centrifuged at 20,000×*g* for 30 min at 4 °C. The pellet was resuspended in incubation buffer (100 mM HEPES, 120 mM NaCl, 1.2 mM MgSO4, 2.5 mM KCl, 15 mM NaHCO3, 10 mM glucose, 1 mM EDTA, pH 7.4) and protein concentration was measured by Lowry's method [28]. Protein kinase activity was assayed in a total volume of 0.5 ml incubation medium [50 mM HEPES, 10 mM MgCl2, 0.5 mM CaCl2, and 0.2 mM EGTA (free calcium level of 0.1 mM), pH 7] with a total protein concentration of 100 μg. P^{32}-labeled ATP (specific activity 3,000 Ci/mmol ATP) was added to initiate the reaction and then incubated at 25 °C. Fifty-microliter samples were taken out and pipetted upon 3-mm filter discs (pretreated with 10% trichloroacetic acid, 20 mM sodium pyrophosphate, and 10 mM EDTA). These filter discs were dropped into 500 ml of the TCA mixture (10% trichloroacetic acid, 20 mM sodium pyrophosphate, and 10 mM EDTA) and left overnight at 4 °C. Filters were washed once in 5% TCA, heated to 90 °C for 15 min in 10% TCA. Furthermore, 5% TCA wash was extracted in hot ethanol/ether (3:1 v/v) before drying. Radioactivity was measured in a Hewlett Packard scintillation counter

DNA Double-strand Breaks Estimation

In the present investigations, comet assay (also referred to as single-cell gel electrophoresis) is used to determine DNA damage (DNA strand break). Assay was performed according to the technique of Singh [29]. Immediately after the exposure period, one rat at a time was anesthetized by placing it in a glass jar containing cotton dipped in anesthetized ether. Animals were killed and brain was homogenized in phosphate buffer and a single-cell suspension was made by using pipette. From the suspension, 10 μl of its suspension was

mixed with 0.2 ml, 0.7% agarose. Agarose was suspended in phosphate buffered saline with 3:1 agarose higher resolution and kept at 37 °C to maintain physiological conditions [18]. The mixture was pipetted out and poured onto a fully frosted slide, immediately covered with coverglass (24×60 mm). These slides were kept in an ice-cold steel tray on ice for 1 min to allow the agarose to gel. Again, a layer was made over the gel with 100 μl of agarose as before, after removing the coverglass [29, 30]. These slides were immersed in ice-cold lysing solution with the addition of DNAase free proteinase K (0.5 mg/ml) and kept overnight at 4 °C. After lysing overnight, the slides were removed and placed in a horizontal slab of an electrophoresis assembly. One liter of electrophoresis buffer was gently poured into the assembly. After 20 min to allow for unwinding, electrophoresis was started at 250 mA (12 V) for 30 min. The slides were removed from the electrophoresis apparatus and placed in coplin jar containing neutralizing buffer. After 30 min, the slides were transferred to another jar of neutralizing solution. After one more change of 30 min, the slides were left vertical at room temperature to dry and stained with ethidium bromide (EtBr of 0.05 mg/ml) covered with a 24×60-mm coverglass. Microscopic slides were prepared with each individual animal separately.

Images were taken at 100× magnification using a charge-coupled device camera GW525x (Genwac, Orangeburg, NY, USA) attached to Leica DMLB fluorescence microscope (Leica, Wetzlar, Germany) with an excitation filter of 490 nm, a 500-nm dichroic filter, and an emission filter of 515 nm. The images of double strand DNA break in brain cells were recorded with fluorescence microscope.

Comet Scoring

Slides were assayed for double-strand DNA breaks. Twenty cells were selected from each slide. Therefore, from each animal, 40 cells (two slides) were scored. Head and tail length (μm), intensity (%), and tail migration (μm) from the beginning of the nuclear area to the last five pixels of DNA perpendicular to the direction of migration at the leading edge were measured. Tail and head length, migration, and intensity of individual cells were measured. The scoring of comet assay was done by using Comet assay IV 4.2 version software (Perceptive Instrument, Haverhill, UK). Data are presented as mean ± standard deviation. The difference between exposed and control groups was tested for significance by using one-way analysis of variance (ANOVA). A difference at $p<0.05$ was considered statistically significant.

Results

Antioxidant Enzymatic Activity in Brain Cells

Compared with the control group (16.82±2.96), those exposed to the 50-GHz showed a significant decrease (12.66±0.87 nmol min^{-1} ml^{-1}; $p<0.001$) in GPx activity (Fig. 2a). The exposed group also showed a significant decrease of SOD activity (169.09± 15.34 U/ml; $p<0.006$) as compared to the control group (189.50±13.13) (Fig. 2b). However, the exposed group of animals showed a significant increase in CAT activity (9.81±1.60 nmol min^{-1} ml^{-1}; $p<0.001$) as compared to the control group (6.86±0.76) (Fig. 2c). The results occulting significant changes occurring in the exposed group of brain cells in all antioxidants enzymatic activities of GPx SOD and CAT are shown in Fig. 2a–c.

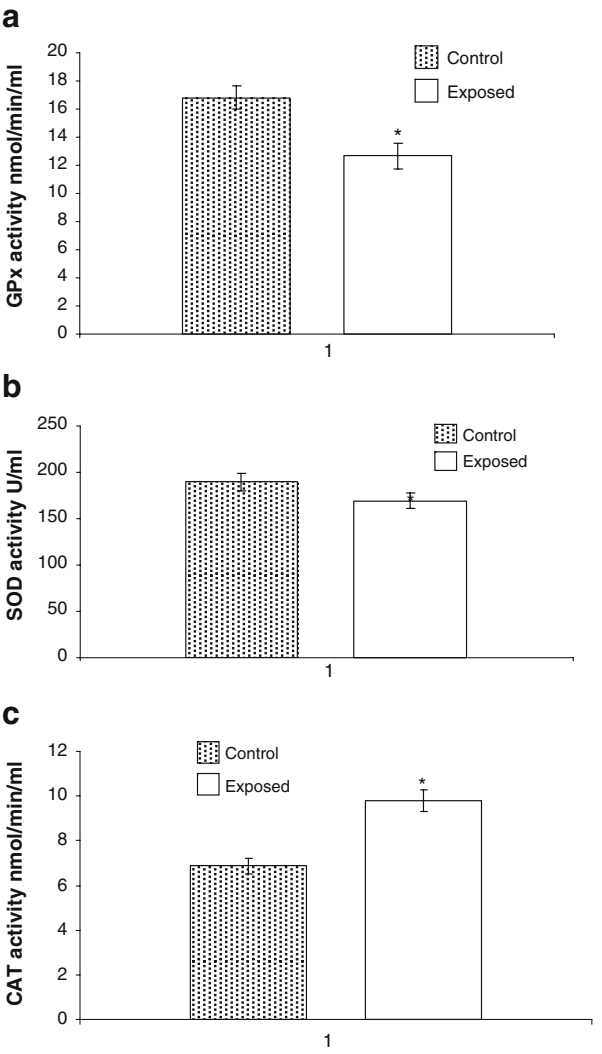

Fig. 2 Antioxidant enzyme activities in rat brain cells in 50 GHz exposed Wistar rats. Result shows significant decrease in exposed group of brain GPx (**a**) and SOD (**b**). However, the exposed group shows significantly increased brain CAT enzyme activity compared to control (**c**). Data are expressed as mean ± standard deviation (SD). *$p<0.05$ vs control group

PKC Activity

The important role of PKC is the transduction for the activation of many cellular functions and control of cell proliferation. Cells, which are subjected to prolonged exposure to tumor promoter phorbol esters, showed depletion in PKC level. PKC activity in whole brain is reduced significantly ($p<0.005$) in the chronically exposed group (3,198.0±1,259.28/mg protein), as compared to their sham exposed counterpart (6,321.33±2,141.55/mg protein) (Fig. 3a). In the hippocampus group, the experimental results also show a significant decline. For the sham-exposed group, it was 3,990.0±1,494.27/mg protein, and for the exposed group, it was 2,703.0±1,213.46/mg protein ($p<0.03$) (Fig. 3b).

However, in the remaining brain, the experimental data do not show a significant difference when compared to the sham-exposed group ($p>0.05$). For the sham-exposed group, we measured 6,455.33±4,061.20/mg protein, whereas for the exposed group, it

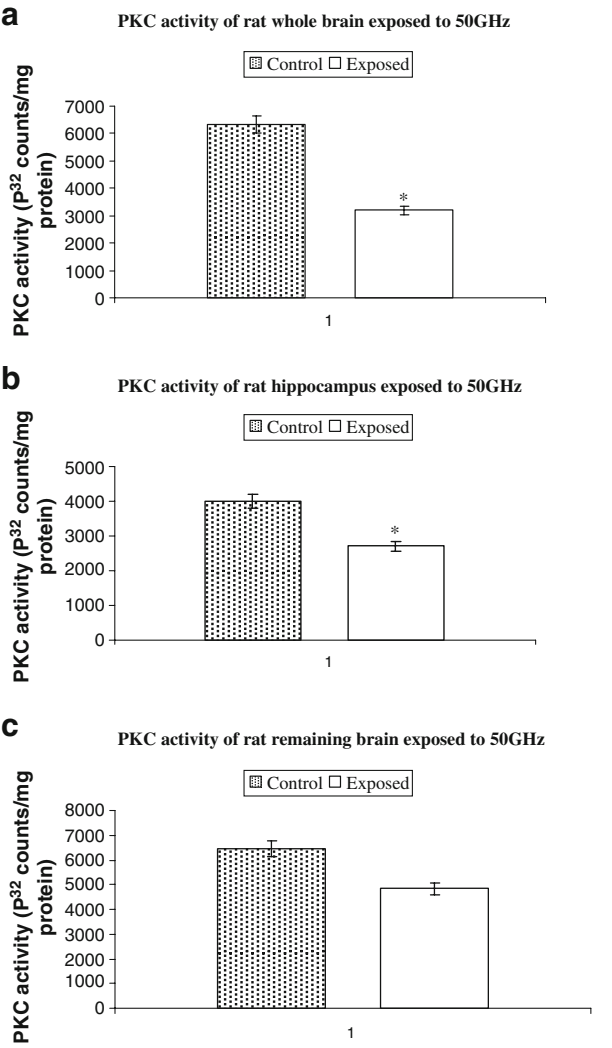

Fig. 3 Effect of chronic exposure to 50 GHz microwave radiation on PKC activity in whole brain (**a**), hippocampus (**b**) was statistically significant ($p<0.05$) as compared to sham-exposed group. However, whole brain minus hippocampus (remaining brain) (**c**) shows no significant result. *Asterisks* show significant results

was 4,850±4,119.14/mg protein (Fig. 3c). All statistical work was done by one-way ANOVA.

DNA Double-strand Break

In the qualitative picture of DNA double-strand break, it is shown that more tail migration has taken place in the exposed brain (Fig. 4) as compared to control (Fig. 5). Our results show that the prolonged chronic exposure to 50 GHz causes reproducible increase in double-strand DNA breaks in brain cells of rats in all the exposed-group animals. The average values of head and tail length, intensity, and tail migration of rat brain cells exposed to 50-GHz continuous wave are given in Table 1. It shows that there is a significant increase in the head and tail length of DNA, and tail migration was also recorded as compared to the control group. A comparison for the control group, the average values of

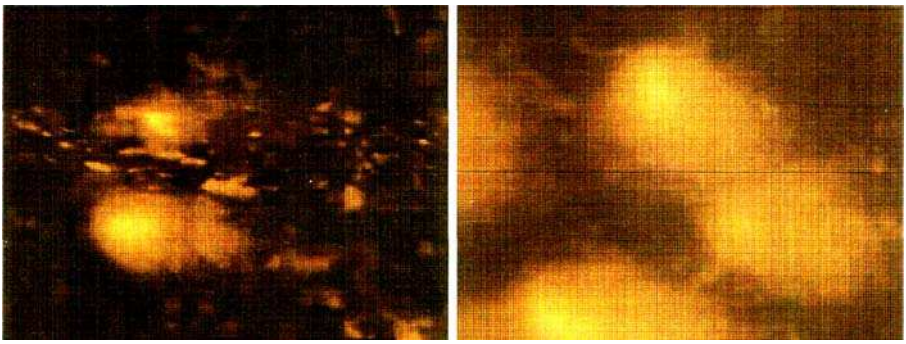

Fig. 4 DNA double-strand break of exposed rat brain cells observed in fluorescent microscope at 40×

head length (93.26±0.41 μm), tail length (66.26±0.69 μm), and tail migration (19.63± 0.70 μm), was recorded, whereas for the exposed group, it was head length (109.17±1.13 μm; $p<0.01$), tail length (176.56±2.35 μm; $p=0.0023$), and tail migration (122.23±2.19 μm; $p<0.005$). At the same time, the total average value of head and tail intensity was also recorded. A significant decrease was observed in head intensity, whereas significant increase was observed in tail intensity of the exposed group. For the control group, the average value of head intensity (87.05±1.2%) and tail intensity (12.94±1.20%) was scored. Alternatively, in the exposed group, the average values in head intensity (49.76± 1.04%; $p<0.03$) and tail intensity (48.24±2.00%; $p<0.02$) were recorded. The present study was aimed to find out the effect of chronic exposure of radiation. Data obtained show that prolonged (45 days) exposure to microwave radiation (50 GHz) causes double-strand DNA break in brain cells.

Discussions

SOD and GPx play a key role in the biological system protecting the body from destructive free-radical activity. An absence or decrease in activity of these enzymes may have noxious metabolic outcomes. The mechanism of detoxication is not confined to the SOD alone, but a product of SOD activity is also a strong inhibitor of this enzyme [31]. It is suggested that an effective detoxication of active oxygen takes place with concordant SOD and CAT action. CAT is involved in the detoxification of hydrogen peroxide (H_2O_2), a ROS.

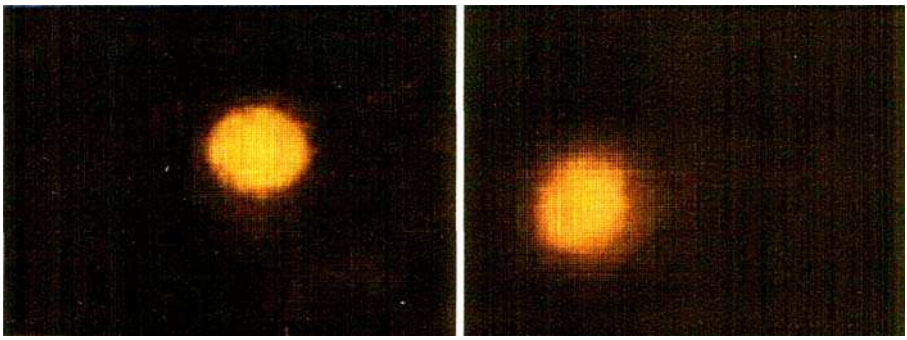

Fig. 5 No DNA double-strand break of exposed rat brain cells observed in fluorescent microscope at 40×

Table 1 Average value of six animals were scored by using comet assay IV 4.2 version (Perceptive Instrument) has presented in mean ± SD (standard deviation).

	Head length	Tail length	Tail migration	Head intensity	Tail intensity
Control	93.26±0.41	66.26±0.69	19.63±0.70	87.05±1.2	12.94±1.20
Exposed	109.17±1.13	176.56±2.35	122.23±2.19	49.76±1.04	48.24±2.00
p Value	$p<0.01$	0.0023	0.005	$p<0.03$	$p<0.02$

In our study, SOD and GPx activities were significantly decreased ($p<0.05$) due to the effect of EMF on the brain, whereas CAT activity was significantly increased in the exposed group ($p<0.05$). Other studies also suggest that a decrease in the level of SOD activity may indicate an increase in the generation of reactive superoxide ions in the biological samples [32]. The decrease in GPx activity might have been due to the excessive production of free radicals. Although GPx is a relatively stable enzyme, it can be inactivated under conditions of severe oxidative stress [33, 34]. In addition to these, the hydrogen superoxide formed during the detoxification process is then illuminated by CAT. Indeed CAT activity is enhanced when H_2O_2 levels are particularly high [35]. Earlier, Rotilio [31] demonstrated that the product of SOD activity (hydrogen superoxide) inhibits the enzyme activity of SOD itself. The possible mechanism defines that EMF exposure leads to generation of ROS [36], which are able to damage many biomolecules, including DNA, enzymes, lipids, and proteins [37]. Under subtoxic conditions, free radicals are also known to play an important role in cellular signal transduction processes [38]. We can hypothesize that a reduction or an increase in antioxidative enzyme activities observed in our study may be related to overproduction of ROS under microwave field exposure.

There is another enzyme, PKC, which may also play a pivotal role in mediating cellular stimuli involved in proliferation, differentiation, apoptosis, and exocytotic release in a number of neuronal and nonneuronal systems. This indicates that any alteration may finally lead to affect the normal growth of the cells. However, in the brain, the hippocampus is probably a preferential site for EMF biointeraction [3]. This is in line with many other reports that a chronic exposure of electromagnetic radiation affects learning and memory functions [39] by affecting hippocampus. Butler et al. [40] reported that cells might be functionally depleted of protein kinase by prolonged exposure to biologically active phorbol esters. They reported that the activity was reduced to 92% as compared to control [40]. An earlier study from our laboratory also suggests that there was a decrease in the activity of PKC in rats exposed to 147-MHz amplitude-modulated at 16 Hz as compared to the control group [22]. It is suggested that protein kinase in the membrane may be a target for microwave radiations, which leads to a variety of altered intracellular events in the cells [41].

Our study on DNA double-strand breaks is in agreement with other scientific reports that have occulted the real picture of mutagenic effects due to EMF radiations. Lai and Singh [18, 42–44] first reported an increases in single- and double-strand DNA breaks in brain cells of rats exposed for 2 h to 2,450-MHz field at 0.6–1.2 W/kg. More recently, this has been confirmed by Paulraj and Behari [16], who reported an increase in single-strand breaks in developing brain cells of rats after 35 days of exposure to 2.45- and 16.5-GHz fields at 1 and 2.01 W/kg. Nikolova et al. [45] reported a low and transient increase in DNA double-strand break in mouse embryonic stem cells after acute exposure to 1.7-GHz field. Aitken et al. [46] reported significant damage to both the mitochondrial genome ($p<0.05$) and the nuclear -globin locus ($p<0.01$) in exposed mice to 900-MHz RFR at a SAR of 0.09 W/kg for 7 days at 12 h per day. A study by Diem et al. [47] reported an effect on

exposed human fibroblasts and rat granulosa cells to mobile phone signal (1,800 MHz; SAR 1.2 or 2 W/kg; during 4, 16, and 24 h) and suggested that the exposure may induce DNA single- and double-strand breaks as measured by the comet assay.

Several reports suggested that DNA damage in cells could have an important implication on human health because they are cumulative. Normally, DNA is capable of repairing itself efficiently through a homeostatic mechanism, whereby cells maintain a delicate balance between spontaneous and induced DNA damage. DNA damage accumulates if such a balance is altered. Most cells have considerable ability to repair DNA single-strand breaks; for example, some cells can repair as many as 200,000 breaks in 1 h. However, DNA double-strand breaks, if not properly repaired, are known to lead to cell death or apoptosis. Indeed, we have observed an increase in apoptosis and decrease in sperm count [26, 48, 49] and DNA double-strand break in sperm and brain cells exposed to microwave RFR [17, 27, 42, 44].

The correlation between oxidative damage to cells (DNA strand break, antioxidant enzyme, and PKC level changes) and ROS has been established in the present paper. ROS are free radicals, which may play a role in mechanisms of the biological effects induced by electromagnetic radiation. It is suggested that the outcome of oxidative damage induced by EMFs will therefore depend on various factors, including the oxidative status of the cell, capability of endogenous antioxidation enzymes, and processes to counteract free radical buildup, availability of exogenous antioxidants, iron homeostasis (a balance of iron influx, storage, and use), the parameters of exposure (e.g., intensity and duration of exposure and, possibly, the wave shape), and whether the oxidative damage is cumulative. In aerobic cells, ROS are generated as a by-product of normal mitochondrial activity. If not properly controlled, ROS can cause severe damage to cellular macromolecules, especially DNA and antioxidative enzyme. DNA damage and alteration in enzyme activities are clear indications of tumor promotion.

Conclusion

We conclude that prolonged exposure to 50 GHz field radiation may decrease the level of PKC, cause the DNA double-strand break, and also make the changes in antioxidant enzymes in neurological system of male rats due to free radicals formation. It also confirms that the possible site of action of such radiation is the hippocampus, the region responsible for control of learning and memory in the brain.

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