Whole body 900 MHz radiation exposure effect on enzyme activity in male wistar rats

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Abstract

Microwave (MW) from cellular phones affects biological system by increasing free radicals, which may enhance lipid peroxidation (LP) and by changing the antioxidative activities which leads to oxidative damage.
The investigation concerned with the effect of low intensity microwave on whole body exposure of male wistar rats. Twenty rats were divided into two groups i.e. control (n=10) and exposed (n=10). Animals were exposed with 900 MHz frequency continuously for 2 hours a day for 35 days. The whole body specific absorption rate (SAR) was 0.9 W/Kg. After the exposure period, rats were sacrificed to analyze the enzyme activity (superoxides dismutase, catalase and glutathione peroxidase) in brain, liver and sperm.

Our findings shows a significant decrease in GPx and SOD of exposed rat brain, liver and sperm ($p< 0.001$), where as Catalase activity shows significant increase in brain ($p <0.001$), liver ($p <0.05$) and sperm ($p <0.001$) samples as compared to control. All data's are expressed as mean ± standard deviation (SD) and were analyzed by analysis of variance (One way- ANOVA). Our results coveys that the regular use of mobile phone at domestic level can have negative impact on human enzymatic activity. This may to make the criteria for safe exposure.

1. Introduction

Microwaves (MW) from cellular phones may affect biological systems by increasing free radicals, which may enhance lipid peroxidation (LP), and by changing the antioxidative activities of the liver, brain and sperm cells. The effect of microwave radiation on biological system is primarily due to an increase in temperature i.e. thermal [1], though non-thermal effects have also been studied [2]. Dasdag et al. [3] used commercially available 890-915 MHz GSM (global signal module) with 0.141 W/kg SAR and reported the decrease in seminiferous tubule diameter in male rat testes after exposure. Recently, Aitken et al. [4]) found significant damage to mitochondrial and nuclear genome in epididymal spermatozoa of mice, when exposed to RF 900 MHz radiations. Fejes et al, [5] and Ji-Geng et al, [6] reported that carrying mobile phones near reproductive organs for longer time may have negative effects on the sperm motility and male fertility. Some authors investigated that the electromagnetic fields (EMF) penetrate the living organism and alter the cell membrane potential [7]. This alteration may affect free-radical processes within the cell and alters the activities of antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in different organs. The exact mechanism of MW-interactions is not well understood, although a few studies have suggested the involvement of lipid peroxidation and free radical formation [8] and also the biochemically induced oxidative stress. Since less information about the mechanism of microwave interaction with organ system, this study has been carried out at 900 MHz mobile phone exposure to clarify the activities of free radical scavenger enzymes in brain, liver and testicular tissue. Previous reports from our laboratory have also showed that these radiations affect cholenergic systems and brain Na+- K+ ATPase activity [9], growth related enzymes [10], protein kinase C activity [11] and reduced fertility [12] of rats.

2. Material & Methods

2.1 Material

The Glutathione Peroxidase (GPx, catalog No. 703102), Catalase (catalog No. 707002) and Superoxide Dismutase (Catalog No. 706002) antioxidant enzyme kit was purchased by the Cayman Chemical Company, Ann Arbor, MI, United Sate of Amrica. Rest of the chemicals were purchased from Thomas Baker Chemicals Limited, Marine Drive, Mumbai.

2.2 Animals Exposure

Male Wistar rats (70 days old and 200 ± 20 gram body weight) were obtained from animal facility of Jawaharlal Nehru University, New Delhi. They were divided in two groups, control (n=10) and exposure group

(n=10) for the frequency. All animals were housed in an air conditioned room, where the temperature was maintained at 25^0C with constant humidity (40-50%) and kept on 12/12 hour light/dark cycle throughout the experiment. They were provided with standard food pallets (prepared by Brook Bond India Limited) and water *ad libitum*.

The protocols for animal experimentation described in this study were approved previously by the Institutional Animal Ethical Committee (IAEC) and Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). All subsequent animal experiments adhered to the 'Guidelines for Animal Experimentation' of the University.

2.3 Exposure System

Male Wistar rats were exposed continuously to 900 MHz Frequency at a specific absorption rate of approximately 0.9 W/Kg for 35 days at 2 hours per day. Rats were placed in Plexiglas cages with drilled ventilation holes 1cm diameter, which have been attached with mobile phone hand set. Rats were prepared for the mobile phone exposure (n=10). Similar experiment was performed with control animals without mobile ring and vibrator (sham exposed).

2.4 Sample preparation and Tissue Homogenization

Exposed animals were sacrificed by over dose of anesthesia and brain, liver, sperm were collected in ice cold buffer. Perfused brain and liver were homogenized with 5-10 ml of cold buffer for GPx, 5-10 ml HEPES buffer for SOD and 5-10 ml of cold buffer for catalase. Sperm were collected from caput and cauda region of epididymis and diluted with cold buffer. Sample was centrifuged at 10,000xg for 15 min at 4^0C. Supernatant was collected and enzyme assay was performed.

2.5 Estimation of Glutathione peroxidase activity

120 µl of assay buffer and 50 µl co-substrate mixture was added in non-enzymatic wells. 100 µl of assay buffer, 50 µl of co-substrate mixture and 20 µl of diluted GPx was added in other wells as control sample where as same amount of assay buffer and co-substrate including 20 µl of brain, liver and sperm samples 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 wells plate was placed in micro-plate reader spectrophotometer and absorbance of the samples were taken at 340 nm

2.6 Estimation of Superoxide dismutase activity

20 µl of SOD standard was diluted with 1.98 ml of sample buffer. SOD standard wells prepared by using 200 µl of the diluted radical detector and 10 µl of diluted standard. Whereas, 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 micro-plate reader temperature and absorbance was taken at 450nm.

2.7 Estimation of catalase activity

100 µl of assay buffer, 30 µl of methanol and 20 µl of standard was 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. 30 µl of potassium hydroxide was added to terminate the reaction. 30 µl of purpald (chromogen) was added to each wells and there after incubated for 10 min at room temperature on a shaker. 10 µl of potassium periodate was added to each wells, incubated for 5 min at room temperature on shaker and the absorbance of samples were taken at 540 nm.

3. Results

3.1 Glutathione peroxidase and Superoxide dismutase activity

Our results suggests that the GPx activity of exposed group of brain (11.66 ± 0.77), liver (14.13 ± 1.57), and sperm (2.38 ± 0.09) significantly decreased ($p<0.001$) as compared to control brain (15.82 ± 2.86), liver (21.96 ± 3.16), and sperm (4.13 ± 0.19) as shown in figure 1. Whereas, the SOD activity in brain (231. 48 ± 25.72; $p< 0.05$), liver (28.51 ± 4.97; $p< 0.01$) and sperm (150.19 ± 6.49; $p< 0.001$) decreased significantly as compared to control group of brain (257.56 ± 46.66), liver (33.60 ± 1.84), and sperm (198.78 ± 7.53), which is shown in figure 2.

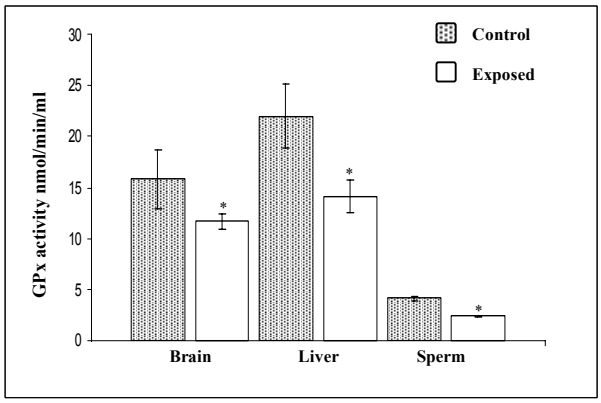

Figure 1- Antioxidant enzyme activities in rat brain, liver and sperm cells in 900MHz exposed Wistar rats. Result shows significant decrease in brain, liver and sperm GPx enzyme activity. Data is expressed as mean ± standard deviation (SD).
*P<0.05 vs control group.

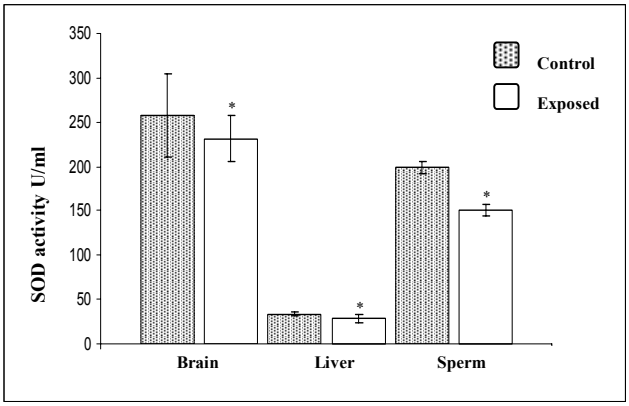

Figure 2- Antioxidant enzyme activities in rat brain, liver and sperm cells in 900MHz exposed Wistar rats. Result shows significant decrease in brain, liver and sperm SOD enzyme activity. Data is expressed as mean ± standard deviation (SD).
*P<0.05 vs control group.

3.2 Catalase activity examination

In the exposed group of animals CAT activity increased significantly in liver (22.79 ± 1.23; $p< 0.05$), brain (12.12 ± 0.75; $p< 0.001$) and sperm (9.81 ± 1.6; $p< 0.001$) as compared to control group (liver 20.45 ± 3.13; brain 8.37 ± 0.32 and sperm 6.86 ± 0.76), which is shown in figure 3.

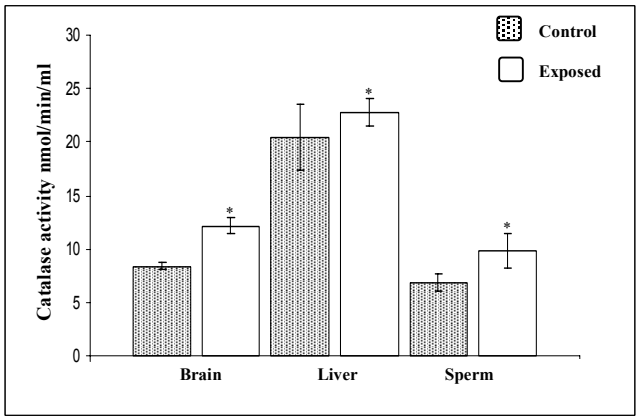

Figure 3- Antioxidant enzyme activities in rat brain, liver and sperm cells in 900MHz exposed Wistar rats. Result shows significant decrease in brain, liver and sperm catalase enzyme activity. Data is expressed as mean ± standard deviation (SD) and were analyzed by analysis of variance (One way- ANOVA).
*P<0.05 vs control group.

4. Discussion

Our findings demonstrate that the exposure of 900 MHz microwave radiations emitted by mobile phone to male rats alters enzymatic activity in brain, liver and sperm. SOD and GPx plays an important role in free radical activity detoxification. Its absence or decrease in activity may induce noxious metabolic outcomes. The hydrogen superoxide formed during the detoxification process is then illuminated by Catalase. Earlier Rotilio, [13]

demonstrated that product of SOD activity (hydrogen superoxide) inhibits the enzyme activity of SOD itself. Therefore for effective detoxification active oxygen takes place with concordant SOD CAT action. Catalase (CAT) is involved in the detoxification of hydrogen peroxide (H_2O_2) a reactive oxygen species (ROS) production.

In our study SOD and GPx activities were significantly decreased ($p< 0.05$) due to the effect of mobile phone radiations in brain, liver and sperm, whereas CAT activity was significantly increased in exposed group ($p<0.05$). Kula et al, [14] found that the activities of the antioxidant enzymes like superoxide dismutase (SOD), Catalase and GPx level of malondialdehyde increased both in the liver and kidneys of male and female rats exposed to magnetic fields. Bediz et al [15] also suggests that long term exposure to low frequency EMF increases lipid peroxidation in the brain and other body organs. The results of present study are in agreement with the findings of other workers [16].

It may be concluded that mobile phone radiation is able to influence antioxidative balance in liver, brain and sperm cells of male wistar rats. However, the precise mechanisms involved in this yet not fully elucidated and the work on these lines is in progress in our laboratory.

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