The effect of 2.45GHz Microwave Radiation on Brain Cell Apoptosis in Sprague Dawley Rats

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Abstracts

Microwave radiation is a part of non-ionizing electromagnetic radiations present in the environment and is now being perceived as health risks. The study was performed to investigate the effect of 2.45 GHz microwave radiation on brain cell apoptosis in Sprague Dawley rat. In the research done, 32 Sprague Dawley rat were used and divided into four groups; control group, G1 (1 month exposure), G2 (2 months exposure) and G3 (3 months exposure). The presence of apoptotic activity in control group was compared molecularly with exposed group through DNA ladder test. Each exposed group were irradiated in GTEM cell at frequency of 2.45GHz located at RF/MW laboratory. There was presence of necrotic instead of apoptotic activity in brain cell and increase in weight of Sprague Dawley rat. Therefore the effect of 2.45GHz microwave radiation shown no presence of apoptosis and increase in weight of Sprague Dawley rat.

Introduction

Telecommunication and wireless technology which is become very important and as part of our lives. The widespread of this technology contributed more NIR man-made sources or specifically microware radiation exposure to the human. Concerns have been raise by people using, working or living near the microwave radiation source about potential hazard to human body. The mechanisms of biological action from this radiation are not clear, but some data indicate potential hazard of low level electromagnetic irradiation (Shckorbatov et al, 2011). The innovation of technology lead to increase usage of microwave radiation among people, thus people are expose to radiation (Yakymenko, 2011). The increase usage of cellular phone also believes to have adverse effect tower human body. Study by Y.Zhu et al, show that microwave lead to significant cell death in culture and more in vivo, brain neuronal cell were stained positive for TUNNEL assay. In previous study, it was reported that extremely low frequency EMF induced tissue damage in different organs of the experimental animal (Khayyat, 2011).

The microwave radiation that form as electromagnetic field with a low frequency is believed can alter brain activity or damage to brain tissue. A study of military personnel in Poland showed a significantly increased relative risk of several nervous system tumors, including brain cancer, in persons exposed to electromagnetic fields of microwave radiation. In study by Y.G. Shckorbatov et al (2010), the influence of microwave radiation on the state of chromatin in human cells conclude that the microwave-induced condensation of chromatin in human cells. Chromatin which is important component in each cell in human body consist of DNA, RNA and other protein. The condensation of chromatin indicate the cell damage.

Apoptosis (known as programmed cell death)

Apoptosis identified by as series change of morphological features of characterized by cell shrinkage, chromatin condensation, cellular budding and rapid fragmentation (Hockenbery, 1995). The cell of multicellular organisms is highly organized cell. This number of cell in community is highly regulated, not simply controlling rate of cell division but also rate of cell death. If cell are no longer needed, they commit suicide by activating an intracellular death program. The term “Apoptosis” is derived from Greek words meaning dropping off and refer to falling of leaves (kerr et al, 1991).

Objective

The study was aim to investigate the effect of 2.45GHz microwave radiation on brain cell apoptosis in Spraque Dawley rats.

Specific objectives:

- To compare the presence of apoptotic activity between control and expose groups with different exposure duration.
- To compare weight of the rats before and after exposure according exposure duration

Materials And Apparatus

For irradiation process, GTEM Cell System were used to generate 2.45 GHz microwave radiation in shield environment at level more than 61 V/m in Nuclear Malaysia facility (RF/MW Lab).

The system consist of signal generator, amplifier and GTEM Cell. Electromagnetic field meter, PMM instrument Model 8053 attached with an isotropic electric field probe Model PMM EP-33M was used to measure the radiation inside the GTEM Cell System.

Medical laboratory of Kuala Lumpur University were used for clinical process.

Rats were euthanized by using sodium pentobarbital at intraperitoneal injection before dissect and keep in liquid nitrogen. The freezer brain tissue will go for DNA extraction before testing for DNA ladder test.

(5M NaCi, 20 ug/ml Proteinase K, Gel Loading Buffer (10X), TBE Buffer, Tris, EDTA (TE), xylene, ethidium bromide, Agarose, A Conical flask, scalpel, forceps, centrifuge, incubator, aspirate, microcentrifuge tube, micropipette, freezer, vortex, 250ml beaker, syringe, 100 mg sodium pentabarbitol, 4% paraformaldehyde, mask, gloves, DNA ladder kit, dissecting set, TBE, nanometer, Boric acid, Sodium Hydroxide)

Methodology

The study design for this research is experimental design (refer project flowchart). In the research conducted, thirty two Sprague Dawley Rats were used and divided into four groups, one control groups and three expose groups. Each group consists of 8 rats. All rats were kept at the laboratory for 7 days at a stable temperature in order to ensure adaptations of the animals to their new environment before irradiate with 2.45GHz microwave radiation using GTEM Cell.

GTEM Cell System

32 male Sprague Dawley rats

Result and Discussion

Based on Figure 1. The graph shows the weight of rats were increase directly proportional to the exposure duration. The analysis using Paired sample t-test (SPSS), shows there are significant difference between before and after exposure (the rat weight) in all group as their p value is less than α=0.05.

The psychological stress effect the eating habit of the animal. In the research study by Mary F Dallman in Stress-Induced obesity and emotional nervous system stated that in a stress mode, body system tends to activate a neural stress respone network compare to cognitive response, it increases the emotional activity and degrrades executive function. Moreover, it also induces stimulation of glucocorticoid which increases motivation for food and insulin (Mary F.D. 2010). Some of the actions of glucocorticoids help mediate the stress response by affect food intake during the sleep-wake cycle.

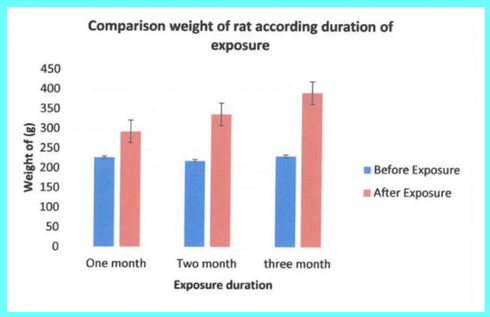


Figure 1: Comparisan mean of rat’s weight before and after according duration of exposure (Group 1: 1 month exposure, Group 2: 2 month exposure, Group 3: 3 month exposure

Project Flowchart

32 male Sprague Dawley rats (180-220g) were used

The weight of each group sample were measured before irradiated

Male Sprague Dawley rats exposed to microwave radiation

Exposed groups were irradiated in 2.45 GHz microwave radiation in GTEM cell

- Group 1: 1 month = (N=8); Rats were exposed: 7 hrs per day, 20 days per month; Total =140 hrs exposure
- Group 2: 2 month = (N=8); Rats were exposed: 7 hrs per day, 20 days per month(x2); Total =280 hrs exposure
- Group 3: 3 month = (N=8); Rats were exposed: 7 hrs per day, 20 days per month(x3); Total =420 hrs exposure

Process of dissection to obtain the brain by dissecting kid

Quantitative analysis. The weight of each exposed group were measured and compared with control group

Rats were euthanized (Intraperitoneal injection-300mg

Brain dissection

Fixation (liquid nitrogen)

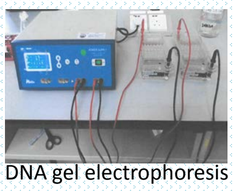
DNA extraction

Qualitative analysis. Detect presence of apoptotic activity by DNA ladder fragmentation

Data analysis

The brain mixture were centrifuge at 12000rpm at 10 minutes

The pellet from the brain



DNA gel electrophoresis

Figure 2 and 3 shows the movement of the band between control group and each expose group. The movement of the band shows no presence of apoptosis in all group. However the appearance of the band in Figure 2 in group sample 2 (G2), the presence of smear band might be due to an exceeding amount of DNA was loaded in gel. The band appears to traverse downwards fast and can disrupt the electrical field for the other bands, causing size irregularities in extreme cases (Seow V.L and Abdul R.R., 2012)

Figure 2: Agarose gel 0.9% with genomic DNA (10µl) for control and expose group (group 1, group 2, group 3), 90 mA

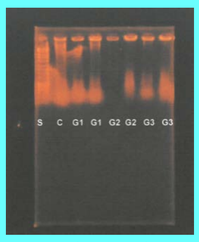


Figure 3: Agarose gel 1% with genomic DNA (10µl) for control and expose group (group 1, group 2, group 3), 90 mA

Conclusions

From the research conducted, the result had clearly shown there is no presence of apoptosis in brain cell of Sprague Dawley rat. Besides, the effect of irradiation also lead to psychological stress that result in weight gain. The release of glucocorticoid after stimulate by hypothalamus had encourage in food intake that cause increase in weight after three month exposure.

Recommendation

Further test on the brain’s rat is needed for confirmation such as reactive oxidative stress (ROS), Tunel Assay, Tumor Necrosis Factor (TNF) Test, caspase test and other test because of the brain as a vulnerable organ.