

**Kertas Asli/Original Article**

**Effects of Electromagnetic Field (EMF) on Histological Changes and Norepinephrine Levels in the Brains of Adult Male Rats**

(Kesan Medan Elektromagnet (EMF) ke atas Perubahan Histologi dan Aras Norepinefrin dalam Otak Tikus Jantan Dewasa)

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ABSTRACT

*The emergence of research about the biological effects of electromagnetic field (EMF) have growing concern among researchers. The aim of this study was to investigate the effects on the brain of rats periodically exposed to 0.1 mT EMF. Total 24 adult male Sprague Dawley rats were subdivided randomly to 4 groups: 2 control groups (C1 6 hours: 6 h/day for 5 days; C2 20 hours: 20 h/day for 5 days) and 2 treatment groups which exposed to 0.1 mT EMF (T1 6 hours: 6 h/day for 5 days; T2 20 hours: 20 h/day for 5 days). A significant decrease in the pyramidal cell number was higher as the exposure duration to EMF was extended (T1,  $p < 0.05$ ; T2,  $p < 0.001$ ). The total numbers of pyramidal cells for T1 was 15.18 % lower than of the total found in C1; and in concurring to the pattern, the number of pyramidal cells in T2 was 33.54 % lower than the total in C2. Similarly, there was a significant decrease of the Purkinje cell number as the duration exposure to EMF, extended (T1,  $p < 0.05$ ; T2,  $p < 0.001$ ). The total numbers of Purkinje cells for T1 was 11.20 % lower than C1, in T2 was 16.19 % lower than in C2. There were significant differences between the thickness of granular layer and molecular layer in the control groups and treatment groups. We also report a significant difference in the levels of norepinephrine in T2, 10.71 % higher than C2. Cumulatively, these results suggested that exposure to EMF can exert negative effect on rats brains.*

*Keywords: EMF; pyramidal cells; Purkinje cells; norepinephrine*

ABSTRAK

*Kemunculan penyelidikan tentang kesan-kesan biologi medan elektromagnet (EMF) membayangkan kebimbangan yang semakin ketara di kalangan pengkaji kini. Tujuan kajian ini adalah untuk mengkaji kesan ke atas otak tikus yang terdedah secara berkala kepada 0.1 mT EMF. Sejumlah 24 ekor tikus jantan Sprague Dawley dewasa telah dibahagikan secara rawak kepada 4 kumpulan: 2 kumpulan kawalan (C1 6 jam: 6 h / hari untuk 5 hari; C2 20 jam: 20 h / hari untuk 5 hari) dan 2 kumpulan rawatan yang terdedah kepada 0.1 mT EMF (T1 6 jam: 6 h / hari untuk 5 hari; T2 20 jam: 20 h / hari untuk 5 hari). Penurunan yang ketara dalam jumlah sel piramid adalah lebih tinggi dilaporkan apabila tempoh pendedahan kepada EMF dilanjutkan (T1,  $p < 0.05$ ; T2,  $p < 0.001$ ). Bilangan sel-sel piramid untuk T1 adalah 15.18% lebih rendah daripada jumlah yang terdapat dalam C1; pola serupa juga untuk bilangan sel-sel piramid di T2, 33.54% lebih rendah daripada jumlah keseluruhan di C2. Penurunan jumlah sel Purkinje yang signifikan diperolehi apabila tempoh pendedahan kepada EMF dilanjutkan (T1,  $p < 0.05$ ; T2,  $p < 0.001$ ). Bilangan sel Purkinje untuk T1 adalah 11.20% lebih rendah berbanding C1, manakala T2 adalah 16.19% lebih rendah berbanding C2. Terdapat perbezaan antara ketebalan lapisan bergranul dan lapisan molekular yang signifikan dalam kumpulan kawalan dan kumpulan rawatan. Kami juga melaporkan terdapat perbezaan yang signifikan dalam aras norepinephrine pada T2 yang 10.71% lebih tinggi daripada C2. Secara kumulatifnya, keputusan ini menunjukkan bahawa pendedahan kepada EMF boleh memberi kesan negatif kepada otak tikus.*

*Kata kunci: EMF; sel piramid; sel Purkinje; norepinefrin*

INTRODUCTION

The electromagnetic field (EMF) has the potential to influence the metabolism, biological process, molecular mechanisms, and cell organisms. Some energy from EMF radiation is absorbed into the body and converted into heat, this is known as thermal effects (Stavroulakis 2010). Biological effects of electromagnetic fields has

been confusing scientists since decades and until now, there is still no clear explanation. The use of mobile phones is close to the head, this has led to public concerns about potential toxic effects of harmful EMF on central nervous system. Several experimental studies indicate that the potential of radio frequency emitted by mobile phones have neurotoxic effects. Effect can be observed through electroencephalogram (EEG), sleep structure,

and cognitive processes in humans (Krause et al. 2000, Huber et al. 2000). In a study by using animals, the report shows that there is a memory disorders in rats which being exposed to radio frequency (Wang and Lai 2000) although results by studies looking at the low magnetic effects on cognitive functions in human were inconsistent in evaluating the EMF exposure health risks (Crasson, 2003, Legros, et al, 2015). However, the overall analysis of the study cannot clearly show the neurotoxic effects based on the level of study (Crasson, 2003, Wang and Lai, 2000). Only slight changes can be observed in the biochemical activity and neurotransmitters (Chung et al, 2015, Lee et al 2015).

Pyramidal cell is efferent cells located in cerebral cortex. Pyramidal cells involved in cognition, the mental processes such as awareness, perception and thinking. Reduction of pyramidal cell count will affect the function of the central nervous system (Elston 2003). Purkinje cell layer located between the molecular layers and granular layers in the cerebellum cortex. Purkinje cells and cerebellum are important for motor function of the human body. Abnormalities of Purkinje cells commonly will have a negative impact on patient movement. Purkinje cells may be influenced by genetic and disease acquisition (Leary 2006). Norepinephrine involves in fight or flight response, directly increasing heart rate, stimulates the release of glucose from energy savings and increases blood flow to skeletal muscle. In addition, norepinephrine also can resist inflammation when released from the locus coeruleus and diffuse into the brain (Heneka et al. 2010, Grudzien et al 2007). The study is designed to evaluate the effect of 0.1 mT EMF exposure on the central nervous system of rats.

## MATERIALS AND METHODS

### ANIMALS

Twenty four male Sprague Dawley rats weighed between 250-300 g (4 weeks old) were used as experimental animals, purchased from the Laboratory Animal Resource Unit, Universiti Kebangsaan Malaysia. The usage of the animals for this project has been approved by Universiti Kebangsaan Malaysia Animal Ethical Committee (approval project code: FSK/BIOMED/2011/YANTI/23 – NOVEMBER/398– NOVEMBER – 2011 – MAY – 2012). The study focused on male rats rather than females as to avoid the influence of hormonal fluctuations during the estrous cycle. The variations in the level of the female hormones would mask the actual effect of EMF and further complicate the investigation. The rats were then divided randomly into control groups and treatment groups, each group was further sub-divided into two groups (six hours and twenty hours) of six rats each, two rats per cage. The animals were left for a week to acclimatize to the animal room condition and were maintained on standard pellet diet and tap water *ad libitum*.

### LOW EMF DELIVERY

This study utilized solenoid coil to create the low EMF. Solenoid coil consisted of a rigid PVC tube (8 inch in diameter, 15 mm thickness, Malayan Industrial Plastics Sdn. Bhd) and silicone insulated copper wire (Model: 60245 IEC 03 (YG) sheath thickness 0.78mm, diameter 4.0) The solenoid coil was then attached and supplied with adjustable power supply. EMF generated with the equipment was directly monitored with a Teslameter with a hall probe (BST, Model Number: BST600). The rats in the solenoid coil were ventilated via the mesh plastic cover which enclosed the PVC tubes (solenoid) end. The treatment groups were then exposed to 0.1 mT EMF, six hours and twenty hours of each, lasted for five consecutive days. Same procedure were applied to control groups, minus the EMF exposure. At the end of the experiment, the rats were sacrificed by diethyl ether overdose. The electromagnetic delivery consistency were measured each time before the experiments were run, measured throughout the solenoid using the Teslameter probe and the acceptable tolerance was 0.1 mT difference between the centre and the ends of the solenoid.

### HISTOPATHOLOGY

For examination under light microscope, the brain samples were fixed in 4% formaldehyde solutions (Sigma-Aldrich, USA), processed and embedded in paraffin (Tissue-Tek®, USA) for sectioning. The fixed brain tissues were sectioned with a microtome (Biobase) at 5µm thickness and stained with Haematoxylin and Eosin (H & E; MasterTech, USA). On the other hand, for examination under electron microscope, the tissue samples were fixed in glutaraldehyde (Sigma-Aldrich, USA), washed in 0.1 M phosphate buffer (Bioscience, UK), and post-fixed with osmium (AgarScientific) for two hours. The tissues were then dehydrated in graded series of acetone (Primechem, Malaysia). Trimming blocks were cut on an ultramicrotome (Leica EM UC7, Malaysia) in 60nm thickness. These sections were collected on copper grids separately and stained with uranyl acetate and lead citrate (SPI Supplies) and then examined in a transmission electron microscope (Zeiss, Germany).

### STEREOLOGICAL ANALYSIS

Pyramidal cell and Purkinje cell counts Neurons from specific areas of the brain including hippocampal pyramidal cells of cerebral cortex and Purkinje cells of the cerebellar cortex were counted. Cell counts performed on tissue slides which were stained with H & E (MasterTech, USA). Tissue slides were observed under a light microscope (Zeiss, Germany).at x400 magnification. Five slides for each sample of rats was taken (a, ..., e), five areas on each slide is randomly chosen (a1, ..., a5). Then, the number of cells present in each section was calculated and the average number of cells present in the five areas was calculated

(equation 1). The average number of cells for each sample (A, ..., F) was calculated by finding the average number of cells on each slide (equation 2). The average number of cells for each group of studies, for example the control group was calculated by using equation 3.

$$a = \frac{a_1 + a_2 + a_3 + a_4 + a_5}{5} \quad (1)$$

$$A = \frac{a + b + c + d + e}{5} \quad (2)$$

$$\text{Control} = \frac{A + B + C + D + E + F}{6} \quad (3)$$

**Cerebellar layer thickness measurement** Thickness measurement of granular layer and molecular layer was carried out using a digital microscope (Zeiss, Germany).. Five slides for each sample of rats is taken (a, ..., e). Tissue slides were observed under x100 magnification. Then the tissue was observed on a computer screen and the image of tissue was captured. Method for measurement of the cerebellar layer thickness was same with the method of the cell counts. The mean of the average for the five slides were calculated to determine the average thickness of the granular layer or molecular layer on each sample (A) similar to equation 2 while the average thickness of the granular or molecular layers for each study group, for example, the control group was calculated similarly in equation 3.

**Measurement of norepinephrine** Pineal glands which preserved at -70°C were thawed and homogenized in a solution made of 0.15 M perchloric acid (SigmaAldrich) containing 0.025% each of cysteine and EDTA (AvaSupplies). The homogenates were centrifuged at 12,000 ref for 30 minutes at 4°C and the clear supernatants were used for the estimation of norepinephrine levels by high performance liquid chromatography (HPLC, Shimadzu) (Rajendra et al. 2004).

**Statistical analysis** Statistical analysis was conducted using SPSS (Statistical Package for the Social Sciences) 20.0 for Windows software. Results were expressed as mean ± S.E.M. The statistical analysis used was one-way ANOVA test and Tukey post-hoc,  $p < 0.05$  being taken for statistical significance.

## RESULTS

Microscopic examination revealed significant differences between treatment groups compared to the control groups (Figs. 1, 2, 3). The results show that the pyramidal cell counts for treatment group (6 hours) was 15:18% lower than the control group (6 hours). Besides, treatment group (20 hours) showed a 33.54% lower than the control group (20 hours). On the other hand, this study found that Purkinje cell counts for treatment group (6 hours) was 11.20% lower than the control group (6 hours) while treatment group (20 hours) showed a 16:19% lower than the control group (20 hours). Statistical analysis showed that there are no significant differences in the thickness of granular layers and molecular layer in treated groups

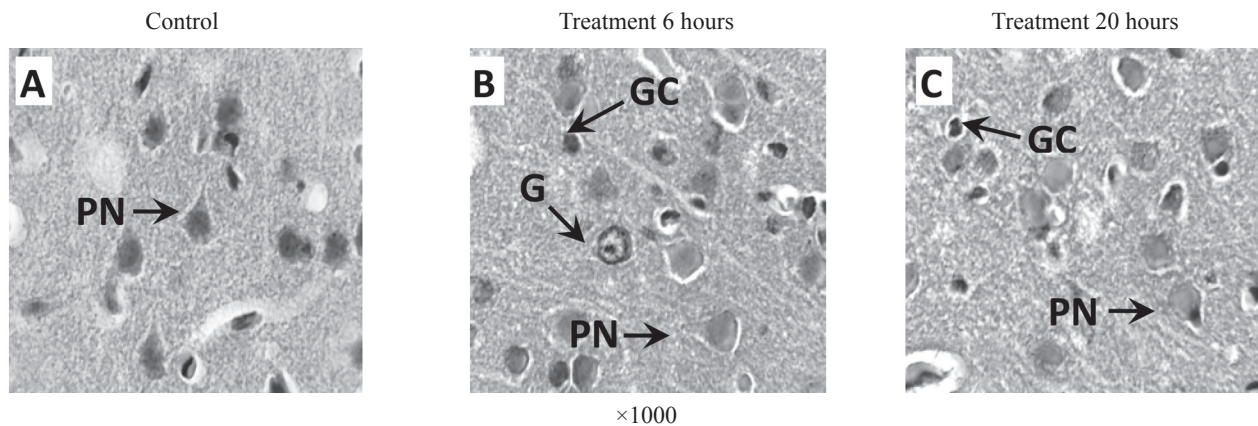


FIGURE 1. Representative photographs of cerebral cortex of the control (A) and the treated group (B) and (C).  
PC: pyramidal cell, GC: granule cell, BV: blood vessel, PN: pyramidal neuron, G: glia

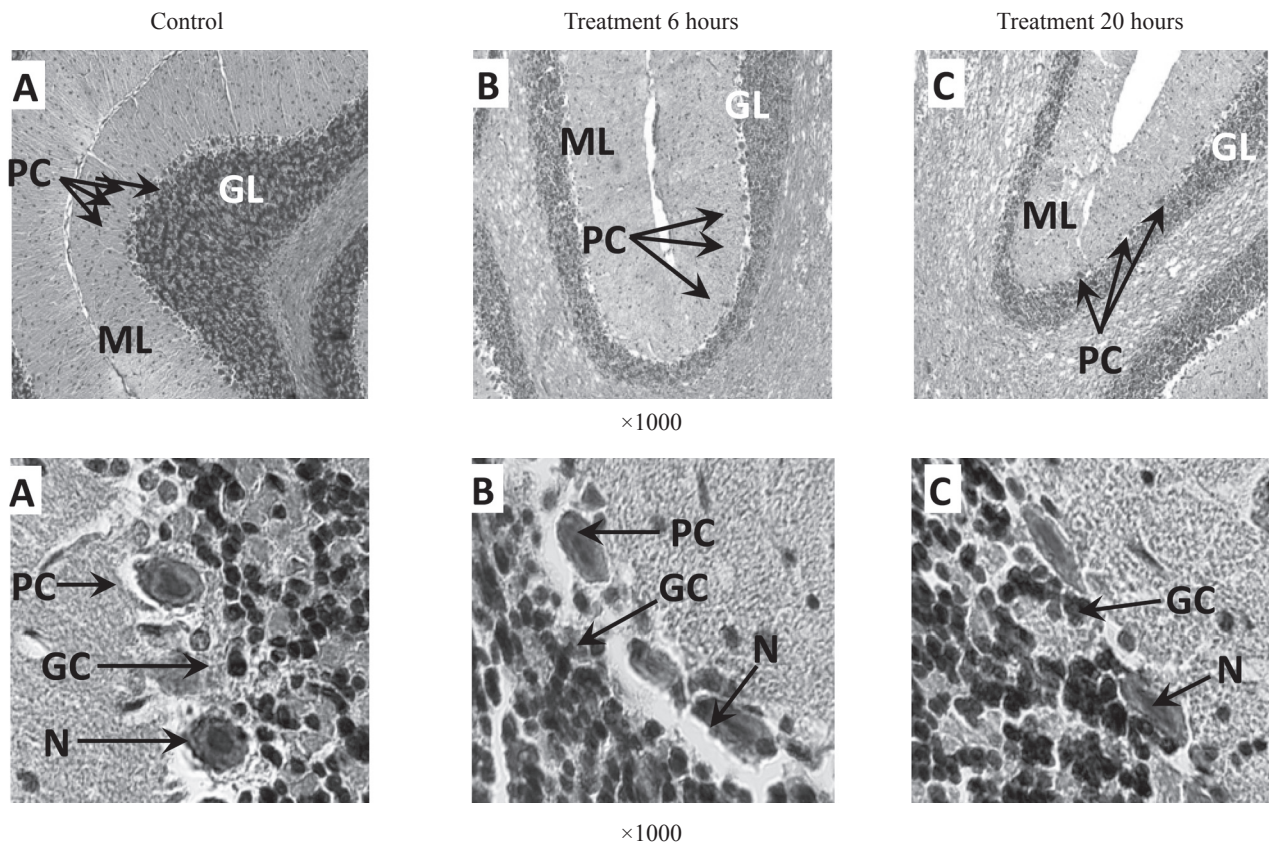


FIGURE 2. Representative photographs of cerebellar cortex of the control (A) and the treated group (B) and (C).  
PC: Purkinje cell, GC: granule cell, GL: granular layer, ML: molecular layer, N: nucleus

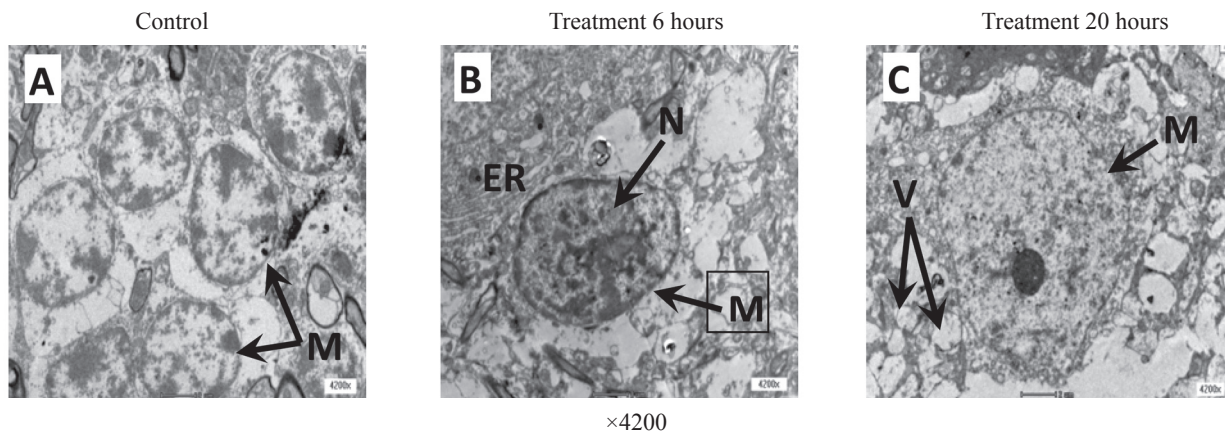


FIGURE 3. TEM Micrographs. Vacuolization can be seen around the nucleus in C. N: nucleus, M: membrane,  
ER: endoplasmic reticulum, V: vacuolization

TABLE 1. The stereological analysis and norepinephrine level result

Parameters	Control 6 hours	Treatment 6 hours	Control 20 hours	Treatment 20 hours
Pyramidal cells	11.00 ± 1.41	9.33 ± 0.82 *	11.03 ± 0.89	7.33 ± 0.82 **
Purkinje cells	16.33 ± 0.82	14.50 ± 1.05 *	16.31 ± 0.82	13.67 ± 0.82 **
Granular layer (µm)	158.14 ± 6.84	153.05 ± 12.47	163.47 ± 8.95	151.16 ± 15.34
Molecular layer (µm)	194.91 ± 21.73	182.17 ± 4.30	197.32 ± 22.64	180.36 ± 4.91
Norepinephrine level (mg/ml)	162.73 ± 7.95	174.69 ± 10.36	173.71 ± 9.40	192.31 ± 13.89 *

\*Significance level of  $p < 0.05$ , \*\* significance level of  $p < 0.001$

if compared to their respective control groups. Based on HPLC result, norepinephrine levels in treatment group (20 hours) was 10.71% higher than the norepinephrine levels in the control group (20 hours).

## DISCUSSION

The aim of this study was to investigate the effects of EMF exposure in the male rat brain. The EMF effects on the human brain is difficult and impossible to be seen, therefore this study was conducted using rat as a model because rat have the same brain cells with humans. EMF intensities used for both treatment groups were 0.1 mT. Based on the International Commission for Non-Ionizing Radiation Protection (ICNIRP 2009), the safety of EMF exposure for the general public is 0.1 mT. This limit applies to both children and adults. Thus, 0.1 mT EMF was selected in this study to determine whether 0.1 mT EMF is safe for the public or not. Parameters that have been taken into consideration in this study to determine the effects of EMF in experimental rats were light microscopic and electron microscopic observation, stereological analysis and norepinephrine levels.

Morphology of pyramidal cells can be obtained from the light microscopic observations (Figure 1), apparent differences can be found when compared between control groups and treated groups. The differences were in the arrangement of cells, shape and color of cells, which was qualitatively evaluated. Pyramidal cells of both treatment groups were widely separated, and the cell nucleus looked pale and difficult to be seen compared with the control group even all the experimental groups were followed the similar staining processing steps. These morphological abnormalities can be explained through the study of Maskey et al. (2010). This study reports that exposure to 835 MHz EMF lasted for one month has been resulted in loss of pyramidal cells in the hippocampus of rats. At the same time, there was difference in calcium binding proteins leading to changes in calcium levels in cells. These changes will negatively affect the functions of the integration and connectivity of neurons in the hippocampus. This study clearly states that EMF radiation can affect brain morphology, physiology and neuronal activity.

For histological observation of the cerebrum, there was a reduction in the pyramidal cell counts. This finding is supported by the study of Bus et al. (2009), which showed that 900 MHz EMF were induced to rats for twenty eight days resulted in significant reduction in pyramidal cells at cornu ammonis. Based on qualitative observations, the loss of cells can also be detected in the study Bas et al. (2009). As a consequence, this study proves that the toxic effects of EMF can affect the central nervous system and reduce the number of pyramidal cells in the rat's brain. According to Elston (2003), pyramidal cells involved in cognition, the mental processes such as

awareness, perception, thinking. Pyramidal cell count reduction will directly affect the central nervous system function.

Morphological alterations of Purkinje cells also can be found in this study. This is likely due to EMF emissions and thus influence brain morphology, physiology and neuronal activity (Maskey et al., 2010). This discovery was to prove the study conducted by Singh and Singh (2002), who examined the effects of magnetic water on the histology of adult rats. Based on the findings, the Purkinje cells showed irregular shape, nuclear fragmentation and shrinkage of the mass of the nucleus wall. Purkinje cell morphological changes may also be caused by decreased nuclear activities leading to reducing of cellular activities. These findings also reinforce other studies such as Sul et al. (2006) and also Manti and Darco (2010). In the study Sul et al. (2006), 2 mT EMF was induced into several cell types of cortical neurons, in vascular smooth muscle cells of aorta, fetal osteoblasts and lymphoblast B. The study proves that EMF can manipulate cell proliferation and reduce the recovery of DNA. In addition, the study of Manti and Darco (2010) discovered the potential of EMF to influence genome based on in vitro experiments. Both of the study clearly states the negative effects of EMF of cells as found in this study.

Present finding clearly demonstrated that there was a reduction in the Purkinje cell counts. The result is consistent with the previous studies (Rosli et al. 2009) and Sonmez et al. (2010). Study Sonmez et al. (2010) states that the reduction in Purkinje cell counts may be caused by toxic effects of EMF on the central nervous system of rats after induction of 900 MHz EMF. Study of Li and Wong (2000) was to examine the EMF induced apoptosis in neonatal rats and adult rats. The results showed that the reduction in Purkinje cells can caused adverse effects of EMF on the scheduling of cell death and apoptosis. 50 Hz EMF exposure on cell growth in cerebellum was studied by Lisi et al. (2005), results found that the EMF has irreversible effects on immigration cells and differentiation of cells in the cerebellum cortex. This study has proved that exposure to low intensity EMF will also result in adverse effects to the cells. Purkinje cell is important for motor function of the body (Leary, 2006). Interference of Purkinje cell usually exert a negative impact on body movement, generally associated with decreases in motor function, such as walking with an abnormal way, convulsions, body movements are not consistent and lost control of eye movements.

For thickness of granular layer and molecular layer, not much difference could be observed when compared between groups. Statistical analysis showed that there are no significant differences in the thickness of both layers if compared with the control group. This result may be due to low intensity EMF which was 0.1 mT EMF used in this study. Based on previous study (Rosli et al. 2009), a reduction in the thickness of the granular layer and molecular layer is significant, but the study was

used a high intensity EMF, that is 1.2 mT EMF. It can be concluded that high intensity EMF will cause a reduction in the thickness of the granular layer and molecular layer significantly.

Based on the transmission electron microscopic observations, membrane nucleus for the treatment group (20 hours) appeared to be broken. According to the study Maskey et al. (2010), EMF may affect the integrity of cell membrane, glycoproteins, cell activity, intracellular enzymes, cytoskeleton and nucleus. Not only damage to the membrane nucleus, vacuolization also occurs around the nucleus. At the molecular level, EMF can produce biological stress and free radicals, which cause congenital defects, damage or death of cells and tissues (Wolf et al., 2005). Vacuolization is one of stage in the process of apoptosis. Apoptosis normally occurs in growth and aging process and serves as a homeostatic mechanism to maintain the population of cells in tissues. In addition, apoptosis also acts as a defense mechanism as in the immune response or during the cell damage caused by diseases and toxic agents (Norbury & Hickson 2001). These findings prove that the effect of EMF could induce apoptosis process. This statement is supported by other parameters such as calculation of the number of pyramidal and Purkinje cells. As mentioned above, there is a reduction in the number of both types of cells.

Along with the changes elicited by EMF exposure, this study also found a significant increase in the level of norepinephrine in the pineal glands following exposure to 0.1 mT EMF. Based on the study Rajendra et al. (2004) and Chung et al (2015), EMF can result in changes of neurochemical or toxicology. Increased of norepinephrine level can be explained by the study of Rajendra et al. (2004) in chick embryos, which stated that the mechanism of increase in the level of norepinephrine can be speculated based on the regulation of the enzyme dopamine beta hydroxylase (DBH), which converts dopamine to norepinephrine. The DBH requires two copper atoms per subunit for its activity (Klinman et al. 1988). Copper is a paramagnetic metal and its role in the activity of DBH may be altered by the external EMF. As supportive evidence, a study (Binhi et al. 2001) has shown that the ion-protein complexes can rotate under static magnetic fields. Hence, the increased level of norepinephrine indicates a possibility of increased DBH activity. The exposure to 2.0mT also has been shown to increase norepinephrine production in rat brain hence supporting the findings in this study (Chung, et al, 2015).

## CONCLUSION

In summary, this study suggested that 0.1 mT EMF can exert negative effects on the central nervous system of male rat. This effect may also occur in the human central nervous system. It is undeniable that EMF may adversely affect human, thus, it is important to bring awareness to

the public about the risk of EMF exposure to the human health.

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