

Kertas Asli/Original Articles

THE EFFECT OF LOW INTENSITY ELECTROMAGNETIC FIELD (EMF) EXPOSURE ON PERICYTES IN BRAIN TISSUES AND BLOOD OXIDATIVE STRESS LEVEL IN RATS

(Kesan Pendedahan Medan Elektromagnetik Intensiti Rendah (Emf) Terhadap Perisit Tisu Otak Dan Aras Stres Oksidatif Darah Tikus)

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ABSTRACT

Studies on the potential effect of EMF exposure on permeability of the blood-brain barrier (BBB) in humans are virtually absent. This study was conducted to study the effect of EMF exposure on pericytes in brain tissues and its effect on oxidative stress level in the blood through total protein and malondialdehyde (MDA). About 16 male rats (Wistar) were used and divided into two groups which were negative control and treatment group. In negative control group, the animals were placed in a solenoid without any EMF exposure for 3 hours daily for 5 days. In the treatment group, the animals were placed in a solenoid with 0.3 mT EMF exposure for the same time duration. On day 3 and day 5, animals were sacrificed and the brain was removed for histological examination while on day 1, day 3 and day 5, the blood was collected for biochemistry analysis. Histological observation showed the presence of morphological changes in the brain tissues of rats that exposed to EMF. Statistical analysis showed that there is no significant decrease in total protein ($p > 0.05$) between negative control group and treatment group. Meanwhile, MDA level in blood showed a significant increase in treatment group ($p < 0.05$) as compared to the negative control group. The result obtained in this study, suggest that the exposure to EMF can cause changes to the morphology of pericytes in brain tissues, and can increase the MDA level in blood of rats.

Keywords: EMF; pericytes; oxidative stress; total protein; MDA

ABSTRAK

Kajian yang menilai keupayaan pendedahan EMF memberi kesan kepada ketelapan sempadan darah-otak (BBB) pada manusia hampir tidak ada dilaporkan. Kajian ini dilakukan untuk mengkaji kesan pendedahan EMF pada perisit tisu otak dan pengaruhnya terhadap aras tekanan oksidatif dalam darah melalui protein total dan malondialdehid (MDA). Sejumlah 16 tikus jantan (Wistar) digunakan dan dibahagikan kepada dua kumpulan yakni kumpulan kawalan dan rawatan negatif. Dalam kumpulan kawalan negatif, tikus dimasukkan ke dalam solenoid tanpa pendedahan EMF selama 3 jam setiap hari selama 5 hari. Dalam kumpulan rawatan, tikus tersebut ditempatkan di solenoid dengan pendedahan EMF 0.3 mT untuk jangka masa yang sama. Pada hari ke-3 dan hari ke-5, haiwan dikorbankan dan otak dikeluarkan untuk pemeriksaan histologi sementara pada hari yang sama, darah dikumpulkan untuk analisis biokimia. Pemerhatian histologi menunjukkan adanya perubahan morfologi pada tisu otak tikus yang terdedah kepada EMF. Analisis statistik menunjukkan bahawa tidak ada penurunan yang signifikan dalam jumlah protein ($p > 0.05$) antara kumpulan kawalan negatif dan kumpulan rawatan. Sementara itu, aras MDA dalam darah menunjukkan peningkatan yang signifikan dalam kumpulan rawatan ($p < 0,05$) berbanding dengan kumpulan kawalan negatif. Hasil yang diperolehi dalam kajian ini, menunjukkan bahawa pendedahan kepada EMF dapat menyebabkan perubahan morfologi perisit pada tisu otak, dan dapat meningkatkan kadar MDA dalam darah tikus.

Kata kunci: EMF; perikosit; tekanan oksidatif; jumlah protein; MDA

INTRODUCTION

The modernisation of the world has developed rapidly over the last few decades mainly in science and technology and

this development in technology is synergistically introducing electromagnetic field sources in the environment. According to Jinsheng et al. (2015) exposure to electromagnetic in public places and in workplace have

increased rapidly in the recent years. The rising presence of electromagnetic fields are inevitable due to the presence of technological innovations and in turn, more reports on the increase number vulnerable people to electromagnetic waves at higher levels than those naturally occurring in nature (SCENIHR 2015). Many studies have been conducted since aimed to study the effect of EMF on human health, studies that links electromagnetic radiation to serious illnesses such as cancer, Alzheimer's, Parkinsons, brain tumour to name a few.

EMF is widely emitted indoors mostly by the usage of electrical items around and the most common examples are by our active mobile phones that we bring almost everywhere, every day. According to WHO (2011) the electromagnetic wave produced by the mobile phones are assumed to be carcinogenic which is a possible trigger agent for cancerous growth of the living tissues. According to a research conducted by WHO (2011) under the International Agency for the International Organization for Standardization Cancer Research (IARC); it was reported that the radiation of electromagnetic waves from mobile phones increased the risk of occurrence of gliomas. This finding is suggesting that the exposure to EMF may affect the permeability of our blood brain barrier (BBB) which is part of the physiological protection mechanism (Bertil et al. 1997). The fact that we have been using our mobile phones in the past and may continue be doing so in the future and its proven effects on the BBB permeability is an unnerving.

Blood vessels were made up by two types of cells that interact with each other namely endothelial cells and perivascular cells that are also referred as pericytes. The pericyte has been defined as a perivascular cell wrapped in blood capillaries (Christian 2011). In the past, the existence and role of the pericyte was pretty much neglected but in recent years, the cell has gained increasing attention as it is recognized as an important cell regulator that are essential for development, stabilization, maturity and re-formation of blood capillaries (Annika et al., 2005). Recently pericytes were given fresh attention as a potential contributor to tumor angiogenesis function. Therefore, pericytes may possibly be involve in future antiangiogenic therapy component in which its crucial is to maintain the stability of the BBB.

Kovacic et al. (2010) expressed that exposure to EMF would affect the metabolic process which can generates oxidants and antioxidants in the cells. Low EMF intensity may have biological effects such as deep changes oxidative metabolism after various exposure (Pawel et al., 2015). Memduh & Nilgun (2012) reported that there was oxidative damage in the rat's brain tissue when exposed to 900 MHz for 10 days. The interaction between EMF and the biological system continuously lead to oxidative stress

disorder, especially in mechanisms radical pair (Claudia et al. 2012). Thus, EMF is seemed to be able to prolong free radicals life and increase their concentration in living cells. The researchers on EMF effects on the human health however, remained equivocal on their finding thus far. We have established our low intensity EMF delivery methods to rats in our lab and we have published our results from previous studies (Yanti & Teoh 2009; Yanti & Nursharmie 2014) which employed 0.6 mT and 1.2 mT of EMF intensities exposure to soft tissues like the brain, liver and kidneys. In this studies we are focussing on 0.3mT EMF exposure to pericytes of the brain as we would like to see the lowest possible intensity that the EMF exposure could affect the brain tissues.

METHODS

A total of 16 adult male rats (Wistar) were used in this study which are acquired from the Animals Unit, Kolej Tun Syed Nasir Universiti Kebangsaan Malaysia (FSK / 2016 / YANTI / 28- JAN./727-FEB.-2016-JAN.-2017). The weight of rats were between 120 g to 150 g and adapted for a week. The EMF were generated via live insulated copper wires wound around a 15cm in diameter tube represent a solenoid. The solenoid was connected to a AC/DC power box, teslameter and ammeter. Rats were divided into two groups of 8 each for the treatment group and the negative control group. As for the treatment groups, the rats were exposed to 0.3 mT EMF for 3 hours daily for 5 consecutive days. The negative control group the rats follows the same procedures, minus the EMF exposure. On the third and fifth day rats were sacrificed and the brain tissues were removed and prepared for histological observations. Pericytes counts were performed by counting the number of pericytes that stained with H and E and observed under x400 magnification using the light microscope. Three areas (a1, a2, a3) on a slide were chosen and the pericytes in each area were calculated and mean calculated (equation 1). This was repeated for b and c, then the average number of pericytes from each rat (A, B, C, D) were calculated (equation 2). The mean for each group used an equation 3.

Pericytes count equations:

$$a = \frac{a1+a2+a3}{3} \quad (1)$$

$$A = \frac{a+b+c}{3} \quad (2)$$

$$\text{Mean } (\mu) \text{ of control group} = \frac{A+B+C+D}{4} \quad (3)$$

Blood samples were taken and inserted into the tube EDTA to carry out biochemical analysis tests (total protein and MDA) on the first, third and fifth day.

RESULTS

HISTOLOGICAL OBSERVATION OF BRAIN TISSUE OF RAT

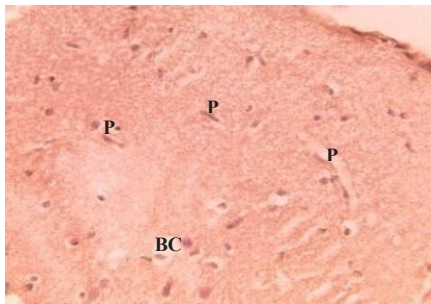


FIGURE 1. The figure shows the brain tissue of negative control rat on the third day. There are many pericytes that can be found along the blood capillaries. (BC-blood capillary, P-pericyte) (H & E, 400X)

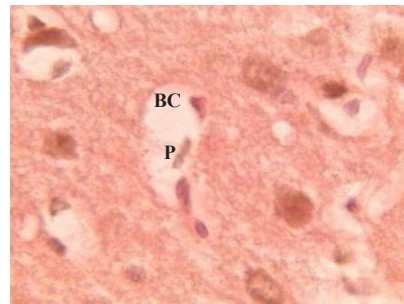


FIGURE 2. The figure shows the brain tissue of negative control rat on the third day. There are many pericytes that can be found along the blood capillaries and pericytes in oval shape which is a normal shape for pericyte. (BC-blood capillary, P-pericyte) (H & E, 1000X)

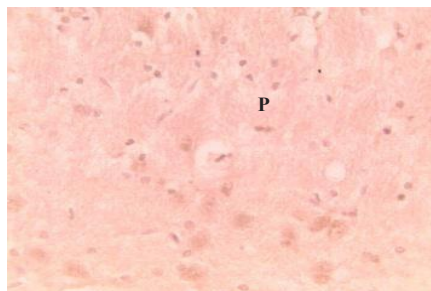


FIGURE 3. The figure shows the brain tissue of negative control rat on the fifth day. There are many pericytes that can be found along the blood capillaries. (P-pericyte) (H & E, 400X)

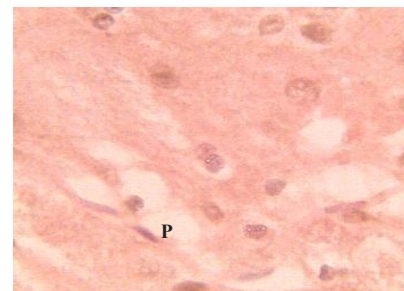


FIGURE 4. The figure shows the brain tissue of negative control rat on the fifth day. The oval-shaped pericytes and blood capillaries wall appear to be intact. (P- pericyte) (H & E, 1000X)

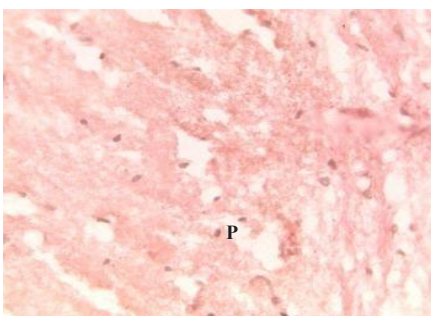


FIGURE 5. The figure shows the brain tissue of the 0.3 mT EMF treatment rat on the third day. It seems that the number of pericytes are decreasing and morphological structure of the rat is not intact. (P-pericyte) (H & E, 400X)

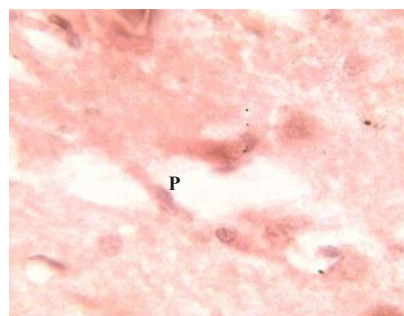


FIGURE 6. The figure shows the brain tissue of the 0.3 mT EMF treatment rat on the third day. It looks like the shape of pericyte is abnormal (not oval) and the capillaries wall are not intact. (P-pericyte) (H & E, 1000X)

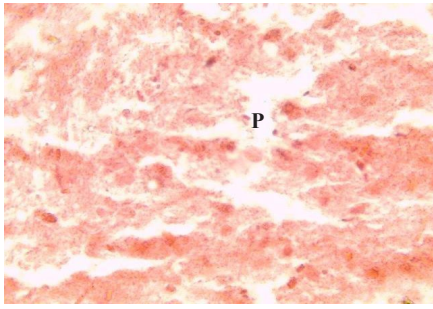


FIGURE 7. The figure shows the brain tissue of the 0.3 mT EMF treatment rat on fifth day. The number of pericyte is very low and the tissue is damaged. (P-pericyte) (H & E, 400X)

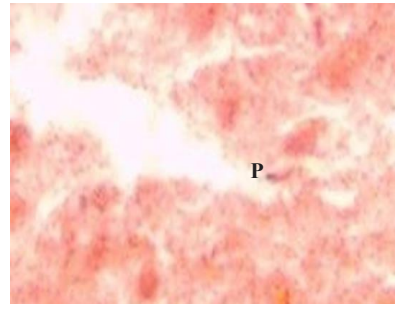


FIGURE 8. The figure shows the brain tissue of the 0.3 mT EMF treatment rat on fifth day. The shape of pericyte is abnormal. (P-pericyte) (H & E, 1000X)

THE COMPARISON OF PERICYTES PRESENCE FOLLOWING THE EMF EXPOSURES

Figure 9 shows the graph bar reporting the numbers of pericytes present on the slide according to the exposure days. A clear trend of declining number of pericytes counted on the slides from the EMF exposed rats were profound in the treatment group 0.3 mT EMF on third day (7.4 ± 0.28) and fifth day (4.9 ± 0.26) as compared to the number of pericytes on the slides from the negative control group which were more or less unchanged (D3: 10.8 ± 0.30 and D5: 10.7 ± 0.31), respectively.

TOTAL PROTEIN CONCENTRATION IN BLOOD SERUM OF RATS OF THE STUDY GROUPS

Figure 10 shows a graph of the average ratio of protein level in the rat blood serum according to the exposure period. A slight dip in the total protein concentration were observed in the EMF exposed groups with its lowest concentration shown on Day 5 of exposure can be seen (D1: 11.94 ± 0.73 mg / ml; D3: 11.84 ± 0.09 mg / ml; D5: 11.04 ± 0.15 mg / ml) compared to the negative control group total protein concentrations (D1: 12.76 ± 0.06 mg / ml; D3: 12.73 ± 1.08 mg / ml; D5: 14.04 ± 0.68 mg / ml). Statistical analysis however showed no significant difference ($p > 0.05$) between each treatment group.

BLOOD PLASMA MALONDIALDEHYDE CONCENTRATION (MDA) COMPARISONS

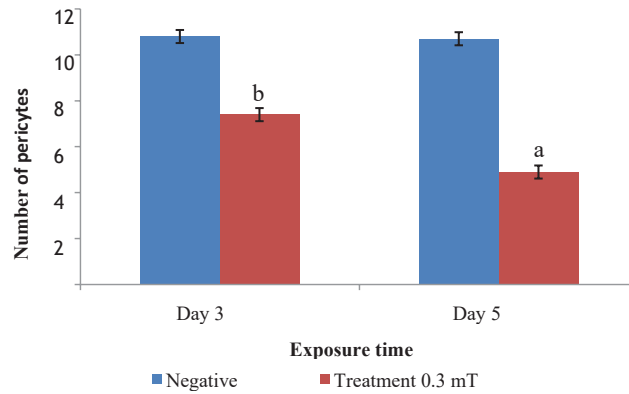
Figure 11 shows the average MDA concentration in the blood plasma according to exposure period. An obvious and significant increasing trend in MDA concentration in rats of the EMF exposed groups was evident (D1: 16.05 ± 0.99 mmol / mg protein; D3: 19.85 ± 1.26 mmol / mg protein; D5: 29.53 ± 4.48 mmol / mg protein) compared to the negative control (D1: 2.05 ± 0.38 mmol / mg protein),

D3: 2.15 ± 1.19 mmol / mg protein; D5: (2.22 ± 0.39 mmol / mg protein). In addition, the MDA level of the fifth day of treatment group was significantly higher ($p < 0.05$) compared to the MDA level of third day treatment group.

DISCUSSION

This study is an extension of the previous study (Yanti & Teoh 2009) in which it is looking for the general effects of EMF on the morphology of the cerebellum tissues and the oxidative stress levels at 0.6 mT and 1.2 mT. The results in general shows clear decline in the number of Purkinje cells and oxidative stress as the intensity increased. Various areas with thinning of the cortical layers were observed and this leads us to believe that the brain tissues are negatively affected with the EMF exposure, at least to the animals used in the study. Hence we were interested to find out what other cells in the brain that could also be impacted to EMF at an even lower EMF intensity.

As mentioned above a trend of reduced number of pericytes were recorded from the rats from the treated as compared to the negative control groups. Correspondingly, the abnormal morphology of the pericytes were also observed in which the cells were rounder or flatter as opposed to round nucleus with oval shape. Our histological findings were verified with cytologists that concurred to our observations. This abnormality in pericytes morphology is potentially the main reason for the damages in the capillaries of the BBB seen on the slides. These morphological changes of the pericytes and reduced numbers of the pericytes may in turn instigate the destabilization of the BBB capillaries wall. When there is damage or disruption to the pericytes it is likely that the structure or capillary function of the blood will also change. According to Yanti (2009), exposure to low frequency EMF causes changes in the structure of the cerebellum morphology. Measurement of protein levels can determine



The value is given in the form of an average \pm SEM, $p < 0.05$
a significantly different ($p < 0.05$) compared to the third day
b significantly different ($p < 0.05$) compared to the fifth day

FIGURE 9. Comparisons of the number of pericytes in the brain of negative control and treatment rats groups

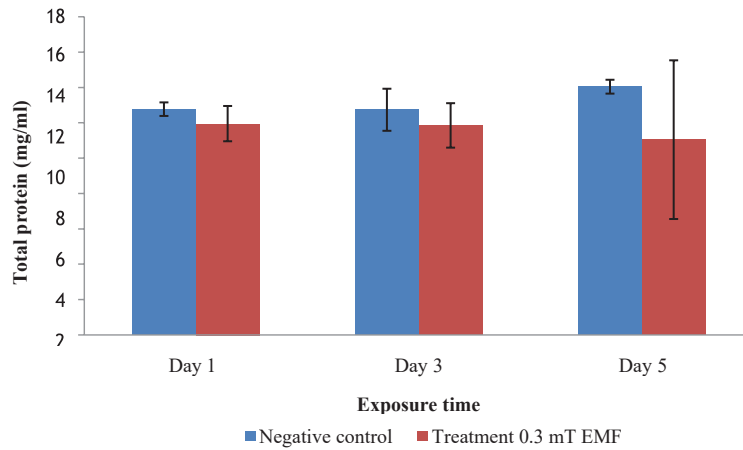
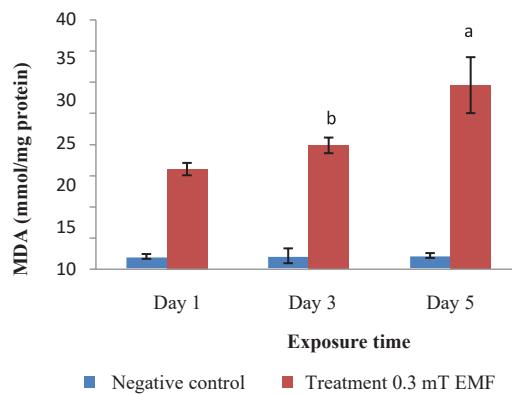


FIGURE 10. The average ratio of total protein concentration in rat blood serum between negative control group and treatment



The value is given in the form of \pm SEM, $p < 0.05$
a significantly different ($p < 0.05$) compared to 0.3mT treatment group on third day EMF
b significantly different ($p < 0.05$) compared to 0.3mT treatment group on fifth day

FIGURE 11. The average ratio of malondialdehyde concentration (MDA) in rat blood plasma between negative control and treatment group

the level of protein oxidation. ROS production from EMF exposure may cause protein fragmentation which can result in decreases of protein amount. In this study, the level of total protein in rat blood serum in the treatment group did not show significant difference compared to the negative control group. However, the pattern of protein levels decreases according to duration of exposure to EMF can be noted. Hashish et al. (2008) reported that a significant reduction in the amount of protein in the blood serum of mice after being exposed to 50 Hz ELF-EMF for 30 days, supporting to the notion that EMF exposure is able to lower the amount of protein in the blood serum. The MDA is formed from the lipid peroxidation of cell membrane reaction between free radicals with unsaturated fatty acids (PUFA) (Mudassir et al., 2012). Increase in EMF exposure can produce excessive ROS that can damage cellular components especially lipids in membranes and nucleic acids (Claudia et al. 2012). Free radical molecules resulting from EMF exposure are capable attacking membrane cells with lipid content. This will be causing lipid peroxidation to occur. MDA levels in blood plasma increase significantly after the fifth day of EMF exposure compared to the MDA level on first day. However, there was an insignificant increase of MDA level for the third day. Birsen et al. (2013) disclosed an increase in MDA levels in male rat blood plasma after the 900 MHz EMF exposure for one hour/day for 21 days, reinforcing the fact that the exposure to EMF is capable of triggering the lipid peroxidation process. This study further strengthen the results from previous studies which were equivocal initially, joining the camp of thoughts that EMF exposure even at a low intensity indeed impacts the normal cells. It is clear from this study that exposure to EMF is capable of giving negative impression on pericytes and blood serum in the study animals however, more tentative and careful interpretations on the similar negative effects to human health would require more studies to be carried out in the near future. Nevertheless, perhaps it is a good cue for us to be aware on the possible effects on making a long conversations over our mobile phones to minimize our exposure to EMF.

CONCLUSION

The exposure to low intensity of EMF had produced a negative morphological and physiological changes in the brain tissue of rats. The 0.3 mT, 3 hours daily EMF exposure for 5 consecutive days were found to significantly reduce the number of pericytes presents by nearly 50% as compared to the negative control. Furthermore, this study suggested a disorder in the oxidative system in the blood of rat after exposed to 0.3 mT EMF (3 hours / day for 5

days). Therefore, the results found in this study suggest that EMF can alter the morphology and number of pericytes and disturb the oxidative system in rats.

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