Trop J Nat Prod Res, September 2024; 8(9): 8321 – 8325 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

The Impacts of Varied Durations of 1800 MHz Mobile Phone Exposure on Oxidative Stress in the Brain and Adrenal Glands of Male Wistar Rats

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ARTICLE INFO

Article history:
Received: 04 July 2024
Revised: 05 August 2024
Accepted : 15 August 2024
Published online: 01 October 2024

ABSTRACT

The effects of prolonged and consistent use of these devices may lead to detrimental health implications due to the emission of electromagnetic radiation (EMR) by mobile phones. This research was performed to examine the impact of extended exposure to 1800 MHz mobile phone EMR on alterations in the parameters related to oxidative stress in brain tissue, focusing on malondialdehyde (MDA) levels, the thickness of zona fasciculata of the adrenal gland, and spatial memory functions. The part exposed to EMR radiation was the entire body of the rat with the exposure source placed under the cage. An 1800 MHz frequency mobile phone with a Specific Absorption Rate (SAR) of 0.897 W/kg in the GSM spectrum was used. Thirty-two eight-week-old male Wistar rats were divided into four groups: K (control), P1 (1 hour/day EMR exposure), P2 (2 hours/day EMR exposure), and P3 (3 hours/day EMR exposure) over 30 days. MDA levels were measured using a spectrophotometer, while histological analysis assessed the zona fasciculata thickness of the adrenal gland. Spatial memory was evaluated using an eight-arm radial maze. A p-value < 0.05 indicates statistical significance. The results showed that the group exposed to EMR for 3 hours/day showed high MDA levels and increased thickness in the zona fasciculata, yet no significant difference is found in spatial memory scores. These findings revealed that longer exposure to mobile phone EMR triggered oxidative stress in the brain and structural changes in the adrenal gland, but was not able to change the function of spatial memory.

***Keywords*:** Mobile Phone, Electromagnetic Radiation, Oxidative Stress, Zona Fasciculata, Spatial Memory.

Introduction

Mobile phones have become a vital necessity in today's technological era. Attractive features, ease of communication, and internet access services are now indispensable part of daily activities.[1] The number of mobile phone users in Indonesia keeps increasing and is projected by the Digital 2023 Global Overview Report to reach 5.44 billion users or 68% of the global population by 2023.[2] Unfortunately, some people lack awareness of electromagnetic radiation (EMR) emitted by mobile phones.

EMR poses thermal and non-thermal effects to users.[3]

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Citation: Farindra, I, Soetjipto, Kalanjati, VP, Rusdi, WE, Suwito, BE, Andini, A, Farmananda, IR, Jannah, RM, Arafany, F, Ramadhany, RR, Benge, WDMR. The Impacts of Varied Durations of 1800 MHz Mobile Phone Exposure on Oxidative Stress in the Brain and Adrenal Glands of Male Wistar Rats. Trop J Nat Prod Res. 2024; 8(9): 8321-8325. https://doi.org/10.26538/tjnpr/v8i9.11

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A non-thermal effect manifests during the process of free radical generation, indicating the presence of factors beyond temperature fluctuations that influence the formation of these reactive species in a system.[4] from Reactive Oxygen Species (ROS) which is associated with oxidative stress.[5] EMR triggers oxidative stress from the accumulation of free radicals that will cause DNA damages (deoxyribonucleic acid), lipid peroxidation, systemic disorders, and cell death.[6,7] The oxidative stress in the brain from exposure to mobile phones can be measured based on MDA levels. Increased levels of MDA and decreased levels of antioxidants in rat brains were found on exposure to EMR of mobile phones.[5,8]

EMR is also a factor of chronic stressor as it causes damages in the tissue.[9,10] The body adapts to stress through physiological response, where the brain will activate the HPA axis (hypothalamus-pituitary-adrenocortical) to be in homeostasis state. Long-term mobile phone EMR affects the thickness of the fasciculata layer. Radiation exposure of mobile phone with a CDMA service frequency on a 2G (second-generation technology) (900 MHz) network exposed to male Wistar rats for 6 hours/day for one and two months triggered an increase in plasma adrenocorticotropic hormone (ACTH) and cortisol concentrations increase drastically and trigger hypertrophy of the fasciculata layer in the adrenal cortex.[3,9]

Exposure to EMR has the potential to induce symptoms such as headaches, fatigue, compromised learning processes, diminished spatial

memory, and impaired cognitive functions.[11] The decreases might occur due to EMR that inhibits the release of neurons in the hippocampal CA1 region, resulting in changes in the structure and function of global transfer which causes a decrease in memory and learning abilities.[12]Unfortunately, research that discuss the duration of EMR 1800 MHz exposure to oxidative stress parameters of brain tissue (MDA), the thickness of the adrenal gland zona fasciculata, and the spatial memory of male Wistar rats (*Rattus norvegicus*) were still limited. Therefore, this study includes a series of experiments with different EMR exposure durations at a frequency of 1800 MHz and a SAR of 0.897 W/kg. The aim is to investigate and assess the possible impacts or changes in brain tissue and adrenal gland histology structure as the duration of EMR exposure increases.

Materials and Methods

Experimental animal and ethical clearance

Thirty-two male Wistar rats (8 weeks old, 130-150 grams) were used in the experiment conducted at Faculty of Medicine, Universitas Airlangga, Indonesia. The rats were acclimatized for 7 days and housed at standard temperature and humidity with free access to food and water *ad libitum*, with a 12-hour light and 12-hour dark cycle. Ethical approval of this research was granted by the Ethical Committee on Health Research, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya (No. 094/EC/KEPK/UNUSA/2021).

Experimental Design

Rats were randomly assigned into 4 groups, each consisting of 8 animals: Group K served as the control group with no exposure to EMR, while Group P1 was subjected to EMR for 1 hour per day for 30 days. Similarly, Groups P2 and P3 had EMR exposure for 2 hours and 3 hours per day, respectively, for 30 days. Rats in each group were kept in a cage measuring 58 x 45 x 16 cm with harmonica wire fencing. The design of the cage allowed the animals to move around within the confined space to resemble their natural environment. Within the cage, a GSM mobile phone was positioned at the base and shielded by a plastic bag (Fig 1). The EMR exposure was set at the frequency of 1800 MHz (characteristic of 4G technology) and with a SAR of 0.897 W/kg. Unrestricted access to the internet was provided by a GSM card. Before each exposure session, the mobile phone had been fully charged, and the rats were provided with internet access to watch YouTube videos in silent mode throughout the duration of exposure.

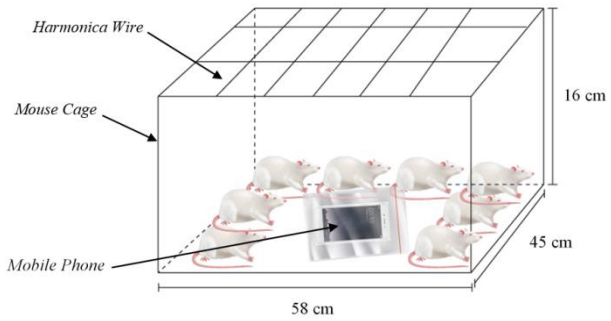

Figure 1: Exposure cage. All rats in a group were placed in cages, with mobile phone in active mode placed at the bottom of the cage (exposed group). Rats were exposed in the morning (08:00 a.m. – 11:00 a.m.) for 30 days. Rats were free to move in the cage.

Brain tissue processing

On the 31st day of the experiment, all the rats were anesthesized by ethylchloride. Subsequently, the brain specimens were carefully excised and placed in a sterilized container and taken to the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga. The examination of MDA levels in this study was conducted with precision and adherence to the standardized procedures set forth in the seminal work by Farindra and colleagues in 2017.[13] This investigation was primarily performed to examine the impact of EMR on the levels of oxidative stress manifesting in the brains of Wistar rats using MDA formation as a biomarker, which can be quantified by employing a spectrophotometer utilizing the thiobarbituric acid reactive substances (TBARS) technique. Precisely 500 mg of brain tissue specimens were weighed and subsequently subjected to homogenization with 4.5 cc of Phosphate-buffered saline (PBS). After that, the homogenates were subjected to centrifugation at a rate of 5000 RPM for a duration of 15 minutes, yielding 4 cc of supernatant. Subsequently, 1 cc of Trichloroacetic acid (TCA) at a concentration of 15% was introduced to the homogenized mixture for further processing and 1 cc of Thiobarbituric acid (TBA) dissolved in a Hydrochloric acid (HCL) solution at a concentration of 0.25 N was added to the mixture for continued homogenization. The solution was then subjected to a controlled heating process within a water bath set at a temperature of 80°C for a duration of 15 minutes, followed by a cooling period at ambient temperature lasting 60 minutes. The homogenates were subsequently subjected to centrifugation at 3000 RPM for 15 minutes, with the supernatants being carefully collected for the quantification of MDA levels. The absorbance of the samples at a wavelength of 532 nm was quantified utilizing a UV-VIS spectrophotometer (Hitachi U-2810 Model: 122-000 No: 1819-011a, Japan). The data obtained from this experiment were carefully analyzed to determine the mean value of each experimental group.

Adrenal gland tissue processing

On the 31st day, surgery was performed on the rats, including stomach and brain tissue processing. The adrenal gland tissue was then excised, placed in a pot containing formalin solution, and sent to the Anatomical Pathology Laboratory at the Faculty of Medicine, Universitas Airlangga, Indonesia. The adrenal glands were extracted and then fixed overnight at 4 °C in 4% paraformaldehyde (PFA) solution. After being inactivated through a series of graded alcohol concentrations, the tissue underwent a clearing process by incubation in xylene before being embedded in paraffin and sectioned. Serial coronal sections (6 μm) were produced using a microtome. The paraffin-embedded sections were then used for hematoxylin and eosin (H&E) staining.[9] The thickness of the zona fasciculata was measured by making 5 dotted lines at the end of the glomerulosa zone to the beginning of the reticularis zone for each preparation. Observations were performed using a light microscope with magnification of × 100, then photos were taken using the Cellseen application (Olympus, Japan) and measured using image-J software (NIH, USA). The fasciculi zone which constitutes 65% to 80% of the cortex histologically consists of one or two long rows of polyhedral cells between the glomerular and reticular zones, separated by open sinus-like capillaries. These cells were filled with lipid droplets and appeared as vacuoles or foams. The average thickness of the zona fasciculata for each group was then calculated.

Spatial memory test

The spatial memory level of the rats was measured using an eight-arm radial maze on the 30th day. The eight-arm radial maze is in the form of octagonal arms connected by eight doors with central diameter of 34 cm made of transparent plexiglass and the maze arms are made of opaque polyvinylchloride measuring 38 x 10 x 20 cm. The end of the eight-arm radial maze is fed (pellets) weighing 4 grams and put in a cup (2.5 cm in diameter and 1 cm high). The pellets were wrapped in perforated plastic and placed in cups to prevent the rats from seeing whether the bait in the arm was still present. The rats were placed in the center of the instrument in the opposite direction to the researcher and left with the maze door closed for 30 seconds in order to adapt. After that, the door was opened to allow movement. Observations were terminated when the rats had eaten the pellets all over the arm or after the time had run out (10 min). The memory score of each rat was calculated using the following formula:

$$\text{Memory score} = \frac{\text{(correct number of arms – wrong number of arms)}}{\text{(correct number of arms + wrong number of arms)}}$$

The maximum memory score that could be obtained was 1. Value closer to 1 shows higher level of memory. The research data were analyzed by measuring the mean spatial memory score of each group.

Statistical analysis

The data were presented as the mean ± standard deviation (SD). Statistical differences were analyzed using SPSS 25.0 software at a significance level of $p< 0.05$. The Shapiro-Wilk test was performed to determine whether the data were normally distributed, while the Levene test was conducted to determine the homogeneity of the data. If the data were parametric, the one-way ANOVA test would be employed, while the Kruskal Wallis test would be performed for non-parametric data in order to see any significant difference between groups. Following the variance analysis, post hoc Tukey tests (for one-way ANOVA) and post hoc Mann-Whitney tests (for Kruskal-Wallis) were conducted to identify the specific groups that differed in terms of variables.

Results and Discussion

Oxidative stress on brain samples

This research found that MDA levels increased with longer gadget exposure, peaking at three hours, indicating oxidative stress. MDA, an indicator of lipid peroxidation, reflects oxidative stress in the central nervous system and other organs.[14,15] Higher MDA levels were observed in the brain and significant differences were observed in all groups ($p< 0.05$). The longest exposure group (P3with EMR 3 hr/days) had the highest average MDA level, whereas the control groups (K or non-EMR) had the lowest (Fig 2). The longer exposure resulted in increased brain MDA levels.Neuronal dysfunction or death is linked to oxidative stress from excessive ROS production or reduced antioxidant defenses.[16] The brain is especially sensitive to EMR due to its high metabolic rate, weak oxidative defense, PUFA-rich membranes (polyunsaturated fatty acid), and low cell turnover.[17,18]

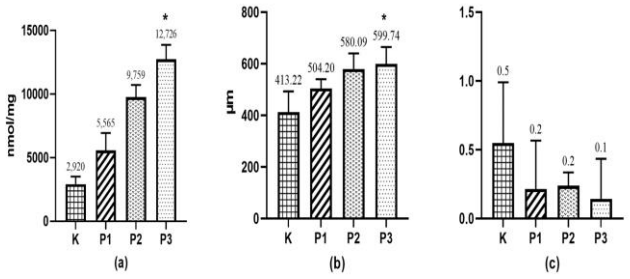

Figure 2: (a) MDA Level, (b) Zona fasciculata Thickness, (c) Spatial Memory score. a) Brain MDA levels increased with increasing duration of EMR exposure ($p< 0.05$). b) The thickness of the zona fasciculata in groups P2 and P3 increased significantly compared to the other groups, as the duration of exposure increase, the zona fasciculata became thicker. c) the spatial memory score did not show significant results, but a decrease in spatial memory was seen in all treatment groups compared to the control. *Statistical analysis showed a significant difference.

The results of this research corroborate with that of Farindra and colleague's study in 2017 who found higher MDA levels and lipid peroxidation in the brains of male Wistar rats exposed to EMR at a frequency of 2100 MHz for 30 days. In the research, significant differences were found between the groups exposed and not exposed to EMR devices, as indicated by differences in mean MDA levels between the treatment and control groups.[13]Study by Singh and colleagues in 2020 showed a significant increase in hippocampal ROS concentration in the group exposed to EMF radiation (2 h/day, 16 weeks) on brain tissue of Wistar rats with a SAR value of 0.36 W/Kg.[13] In addition, the research also showed increased lipid peroxidation, such as ROS, protein oxidation, and antioxidant levels, which changed significantly in the exposed group.[19]

Reactive free radicals trigger lipid peroxidation.[20,21] Both direct and indirect impacts of highly reactive lipid peroxidation induce harm to neurons.[16] The immediate impacts include the loss of fluidity, a decrease in fluidity, the depression of membrane protein mobility, and enhanced phospholipid exchange across the membrane bilayers.[22] The neuron membrane may leak, allowing enzymes to penetrate and potentially compromise its function as a barrier. In the worst-case scenario, membrane breakdown can lead to the inactivation of membrane-bound enzymes and loss of compartmentalization. In addition to impairing Ca^{2+} ion compartments, lipid peroxidation also causes a loss of ionic equilibrium, eventually resulting in the loss of neuronal cell metabolic regulation.[20,23,24] The indirect effects of lipid peroxidation occur through the activation of other mediators,[25] resulting in production of MDA which has biological activity.[26]

Zona Fasciculata thickness

Figure 2 present the data on the thickness of the zona fasciculata that increased as the duration of EMR exposure increased. Significant differences were also observed in groups P2 (EMR 2 hr/days) and P3 (EMR 3 hr/days) ($p < 0.05$), in which group P3 had the highest average value and the control group obtained the lowest average (Fig 2).In this research, male Wistar rats exposed to EMR for 3 hours per day showed an increased thickness of the fasciculata layer in the adrenal glands compared to the control group. This finding collaborate with Shahabi and colleague's research in 2018 which found similar thickening of the fasciculata layer after exposure to 900 MHz gadgets for 6 hours per day over 1 to 2 months. The duration of EMR exposure in our research was also observed to influence changes in the adrenal glands fasciculata layer thickness.[9] However, our results differ from Gupta and colleague'sresearch, which investigated the effects of 900 MHz cell phone radiation over 6 months and did not observe significant histological or physiological changes in the adrenal glands.[27]

Stress has been major factor of health problems. The EMR emitted by cell phones can trigger chronic stress.[3] Stress occurs through the HPA axis activation mechanism which leads to an increase in ACTH and cortisol.[28,29] Prolonged stimulation of the ACTH hormone due to EMR exposure results in increased adrenal gland activity and increased cortisol secretion, leading to thickening zona fasciculata layer.[9] In this research, ACTH and cortisol levels were not observed as research parameters, yet the thickening fasciculata was observed in this research. A thicker zona fasciculata occurs due to hypertrophy of spongiocyte cells, as confirmed by Shahabi who found a larger cell perimeter in the group exposed to EMR 900 MHz yet stable number of spongiocyte cells.[9,30] Research on the increase in fasciculata thickness due to EMR exposure primarily focuses on cell phones operating at a frequency of 900 MHz, with limited discussion on frequencies of 1800 MHz.

Spatial memory

Figure 2 shows that the difference between the groups in spatial memory variance is not statistically significant ($p > 0.05$). The average score of spatial memory in the control group (K) was higher than the P1, P2, and P3. All treatment groups experienced a decrease in spatial memory compared to controls.Frequent use of mobile phones can lead to general brain damage, particularly on the hippocampus which is a sensitive region in the temporal lobe that regulates spatial learning, working memory, and cognitive function. The spatial memory of male Wistar rats exposed to mobile phone radiation at 1800 MHz for 1 hour, 2 hours, and 3 hours per day was examined using an eight-arm radial in this research. The results did not show significant decrease in spatial memory scores despite the varying exposure time across groups. The results of this research are in line with Zheng and colleague's study in 2023, in which exposure to cellphone radiation for 28 days did not significantly change the subject's spatial memory.[31] This decrease in memory score may occur due to the radiation-exposed hippocampal CA3 neurons experiencing nuclear degeneration with the formation of vacuoles in their cytoplasm, resulting in lower number of hippocampal pyramidal cells.[12,32] Hippocampal CA3 neuron damage can occur due to oxidative stress that triggers DNA damage. The death of neurons

results in changes in the structure and function of the hippocampus which are one of the factors that can change or affect spatial learning and memory.[33] Although this research observed a decrease in spatial memory in the treatment group compared to the control, the analysis between the treatment groups did not yield significant results.

Conclusion

This research shows that EMR exposure from a mobile phone can increase the MDA levels of brain and the thickness of the fasciculata layer of the adrenal glands of male Wistar rats. This condition is due to the oxidative stress generated by EMR from cell phones. This research also demonstrates a relative change in spatial memory scores and indicates that the duration of radiation exposure does not significantly affect the scores of the research subjects.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

We would like to thank the Universitas Nahdlatul Ulama Surabaya for their assistance, which enable this research to be done.

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