ORIGINAL ARTICLE

BLOOD-BRAIN BARRIER DYSFUNCTION IN WISTAR RATS EXPOSED TO MULTI-TRANSCEIVER MOBILE RADIOFREQUENCY, SOUND, AND VIBRATIONS

*Jamil D.U.[1,3], Umar M.I.[2] and Fasanmade A.A.[3]

[1]Department of Human Physiology, Faculty of Basic Medical Sciences, Federal University Dutse, Nigeria

[2]Department of Human Anatomy, Faculty of Basic Medical Sciences, Federal University Dutse, Nigeria

[3]Department of Physiology, College of Medicine, University of Ibadan, Nigeria, Nigeria

ABSTRACT

This study looked at the functional changes in the blood-brain barrier (BBB) in Wistar rats after exposure to electromagnetic field, vibration, and ringtone from a multi-transceiver mobile phone. Twenty-five (25) male Wistar rats were randomly divided into five groups (n=5). For six weeks, group A (control) and test groups were exposed to mobile phone electromagnetic field through a 10-minute calls/day from a Tecno 900/1800 MHz in various modalities. Viz: groups B – E in silent-only, vibration-only, ringtone-only and ringtone with vibration, respectively. BBB in various regions of the brain using evans blue dye tracer technique and brain TNF-α was studied at the end of the sixth week of exposure. There were significant (α0.05) decrease in BBB in the cerebellum, cerebrum, and the two hemispheres of the brain and insignificant increases in the levels of brain TNF-α across all groups of animals exposed to phones in various modalities. These findings suggest that exposure to electromagnetic fields, vibration, and sound from multi-transceiver phones may be a risk factor for loss of BBB integrity.

Keywords: *Evans blue dye, brain, blood-brain barrier, mobile phone, electromagnetic field, vibrations, ringtone*

*Corresponding Author
Dauda Usman JAMIL; Department of Human Physiology, Faculty of Basic Medical Sciences, Federal University Dutse, Nigeria, jamso85@yahoo.com

Citing this article
Jamil D.U., Umar M.I. and Fasanmade A.A.. BLOOD-BRAIN BARRIER DYSFUNCTION IN WISTAR RATS EXPOSED TO MULTI-TRANSCEIVER MOBILE RADIOFREQUENCY, SOUND, AND VIBRATIONS. KIU J. Health Sci, 2023: 3(1);

Conflict of Interest: None is declared

INTRODUCTION

Mobile phones have completely transformed the telecommunications industry, to the point where the same device that was originally designed to make calls and send SMS is now used to connect to the internet. It is more widely used than traditional computers all over the world [1]. To communicate with a base station, they use a radio transmitter that emits electromagnetic waves. Phones use wireless technology, with the majority operating on a frequency range of about 900 - 1800 MHz, with a pulse frequency of 217 Hz, a pulse width of 577 s, and a duty cycle of 12.5% [1]. Only recently has the frequency risen above 2100 MHz [2].

Many people have recently expressed concern about the effects of mobile phone emissions on body systems, particularly the brain as well as the heart [3, 4]. The raised concerns were likely due to the proximity mobile phones to major organs or systems in the body with vital physiological responsibilities during usage. Because of the high metabolic rate and lipid composition of organs in the cardiovascular and nervous systems [5], respectively, they are a very sensitive target for oxidative stress response to electromagnetic field exposure during both physiological and pathological processes, such as anxiety, hypertension, and neurodegeneration [2]. Despite the insufficient data on the underlying mechanism for biological system reponse to electromagnetic field exposure, oxidative stress is been implicated [2, 3]. Consales et al. Jamil et al. and Srav and Seyhan reported that, radiofrequency energy emissions similar to mobile phone magnetic fields can alter biological responses through oxidative stress [2, 3, 6]. Electromagnetic fields from multi-transceiver mobile phones have recently been shown to cause oxidative stress in blood, brain, and heart tissue of animals [2, 7]. Due to their increased SIM capacity, multi-transceiver phones give consumers more freedom than traditional mobile phones with a single subscriber identity module (SIM) [2, 7, 8]. Mobile phones with multiple transceivers (MTrMP) are known to emit more electromagnetic energy than single transceiver prototypes [7, 8, 9]. Shehu et al. discovered that using a cell phone causes anxiety in rodents, but their findings did not link cell phone activity exposure to blood-brain barrier responses [8].

The blood brain barrier is a specialized selective vascular tissue that prevents unwanted substances from entering the central nervous system [10]. It is made up of endothelial cell tight junctions, basal lamina, and glial processes that separate circulating blood from cerebrospinal fluid and have a low permeability to ionized water [11, 12]. A typical brain injury can be associated with reduced intergrity of the blood-brain membrane barrier, which can lead to vasogenic brain oedema, which is a potentially fatal event [13]. One of the primary goals of neuroprotective therapies is to

www.kjhs.kiu.ac.ug https://doi.org/10.59568/KJHS-2023-3-1-04 KJHS 3(1); 2023. Page (33-47)

protect this membrane barrier [14].

In this study, the blood-brain barrier integrity assessment is important for identifying some of the neuropathologies or complaints that may be associated with its pathophysiology or disruption. The most widely used method for assessing blood brain barrier integrity is the Evans blue staining technique, which involves staining plasma serum albumin with Evans blue dye. After being injected into the bloodstream, the dye has the property of binding with serum albumin. Because plasma albumin does not pass through the BBB under normal conditions, its spectrophotometric analysis from the brain can provide an easy and reliable measure of the blood-brain barrier's integrity [15]. The level of quantified Evans blue dye increases with increased permeability, which is a measure of the development of some blood-brain barrier pathology such as blood-brain barrier inflammation, perforation, and vasogenic oedema [15]. There are several methods for assessing blood-brain barrier permeability, including the use of magnetic resonance imaging and functional magnetic resonance imaging technique. However, these methods are complex, and some rely on radioactive tracers [16]. These tracers are expensive and dangerous to human use due to potential health effects, as well as challenges in handling and administration [16]. Due to the paucity of information on the potential effects of mobile phone usage on the BBB, the current study investigates the effects of phone radiofrequency, sound, and vibration exposure on blood-brain barrier integrity in rats.

MATERIALS AND METHODS

Experimental protocol

This study used twenty-five male Wistar rats (140-180g) from our breed. They were kept in plastic boxes for two weeks acclimatization in a controlled environment (temperature 252°C, 12-hour dark/light cycle) with the phone turned off. They were fed a standard laboratory meal and had unrestricted access to water. At random, the Wistar rats were divided into five groups (n=5): For six weeks, the group A (control without phone exposure) and test groups were continuously exposed to an electromagnetic field via 10-minute calls per day from a Tecno MTrMP 900/1800 MHz in various alerting modalities. Viz: groups B-E in silent, vibration, ringtone, and ringtone + vibration, respectively.

Ethical approval

Throughout the animal handling and protocol of the investigations, the ethics and guidelines of animal care and use for research (UI - ACUREC, 2018), University of Ibadan, and rules governing the care and use of experimental animals (NIH Pub. No. 85-23 amended 1999) were strictly followed.

Evans blue dye assay

At the end of sixth-week of exposure, the Evans blue dye tracer technique was used to examine the blood-brain barrier integrity. The Evans blue dye injected

intravenously or intraperitoneally, bind to serum albumin and the amount of dye was determined quantitatively from the brain tissue [17]. 4 mL/kg body weight of 1 percent Evan Blue dye was given intraperitoneally to the animals and allowed to circulate through the whole body for 3 hours after which the chest of the animals was opened under intraperitoneal injection of 0.1mL/100g body weight of xylazine (40mg/mL) mixed with ketamine (25mg/mL) (1:1) anaesthesia. Intracardiac perfusion with normal saline solution was done for about 15 minutes until saline fluid that passed through the left ventricle of the heart to the right atrium become colourless. At the end, the animals were sacrificed humanely and their brain tissues were dissected out, weighed, then homogenised in 2.5 mL of phosphate buffer. 2.5 mL of trichloroacetic acid was dropped into the homogenised solution and mixed with the aid of a vortex mixer to precipitate the protein molecule in the solution. The homogenate was later centrifuged for 30 minutes at 5000 rpm for quantitative analysis of the dye using a spectrophotometer at 620 nm. The dye concentration was calculated as µg/g of brain tissue against the standard [17].

Tumour necrotic factor alpha assay

Tumour necrotic factor alpha (TNF-α) assay of the brain tissue homogenate was done using enzyme-linked immunosorbent assay (ELISA) technique. The labelling and recording of the sample/standard microwells layout were done, while all reagents, samples and standards were prepared according to the kits' description. 100 microliters of sample or standard was introduced to the wells and incubated for 1 hour at 37 degrees Celsius. The wells were aspirated and 100 µL of detection reagent A specific for TNF-α made from monoclonal antibody conjugated with biotin was added and further incubated at 37 degrees Celsius for another 60 minutes. The reaction mixture was aspirated again the wells were washed 3 times. 100 µL of another detection reagent B made from Horseradish Peroxidase conjugated with avidin was introduced and incubated at 37 oC for 30 minutes. The wells were also aspirated, washed 5 more times and dry using absorbent paper to remove the unbound Avidin conjugated HRP, after which 90 microliters of substrate solution (3, 3' 5, 5' tetramethyl-enzidine) was introduced and incubated at 37 oC for 10-20 minutes. The biotin and enzyme conjugated molecules caused a colour change. 50µL stop solution (sulphuric acid) was introduced, after which ELISA spectrophotometric absorbance readings were recorded at 450 nm immediately. TNF-α concentration in pg/mL was calculated against a standard curve [18].

RESULTS

The results in Figure 1- 6 show the concentration of the Evan blue dye in the serum and various sub-regions of the brain i.e. right and left hemisphere, cerebrum, right and left cerebrum, cerebellum, right and left cerebellum and the whole brain of Wistar rats exposed to multi-

transceiver mobile phone (MTrMP) activity (electromagnetic emission), sound (Rg) and vibration (Vr) exposure) in the final phase of this study.

Evans blue dye concentration was observed to increase significantly in all the animals whole brain ($P < 0.01$) and decrease significantly in the serum ($P < 0.01$) across all the animals of the treatment group exposed to double transceivers or subscriber identification module phone activity in all modalities use for the present work compared with the control (Figure 1 and 5). The whole cerebral Evans blue dye concentration increased significantly ($P < 0.01$) in animals of group E (MTrMP +Vr+Rg) (Figure 2). Differential Evans blue dye concentration study of two cerebral hemispheres showed a significant increase in the levels of Evans blue dye concentration only in the right cerebrum of animals of group D (MTrMP +Rg) ($P = 0.01$) and E (MTrMP+Vr+Rg) ($P < 0.01$) when compared with the control (Figure 2). No significant differences were observed in the levels of Evans blue dye concentration in the two hemispheres of the cerebrum when compared with each other across the groups (B - E). The levels of Evan blue dye concentration in the whole cerebellum ($P < 0.01$) (Figure 3) and the left part of the cerebellum ($P < 0.01$) (Figure 4), increased significantly across all the treatment groups (B - E) compare with their controls. The right cerebellum Evans blue dye concentration was observed to significantly increase in the animals of groups D (MTrMP+Rg) ($P < 0.01$) and E (MTrMP +Vr+Rg) ($P < 0.01$) (Figure 4) compare with the control.

No significant differences were observed in the two halves of the cerebellum when compared with each other across the groups (B - E). (Figure 4). The increased levels of Evan blue dye concentration observed in various regions of the brain were an indication of the increased loss of blood-brain barrier integrity.

The level of TNF-α ($P = 0.09$) in the animals' brains were not significant. However, the groups exposed to phone activity in either ringtone (Rg) or vibration (Vr) expressed higher level of this proinflammatory cytokine compared to control (Table 1).

DISCUSSION

Radiofrequency electromagnetic wave from mobile phone, in addition to sound and vibration, was shown to reduce the integrity of the blood-brain barrier. This is demonstrated by the higher levels of Evans blue dye found in several brain areas in the current investigation. The concentration of Evans blue dye increased significantly in all of the animals' entire brains and decreased significantly in the serum across all of the animals exposed to multi-transceiver mobile phone activity in all of the modalities used in the current study. The most substantial alterations were detected in rats exposed to multi-transceiver mobile phone activity, either in vibration or sound, indicating that vibration or

sound created stresses that induce the blood-brain barrier to break down. This disruption in the integrity of the blood-brain barrier can be linked to oxidative stress, which caused elevated levels of inflammations induced impaired blood-brain barrier, thereby allowing the Evans blue dye to enter the brain. This observation is supported by the decrease in the level of some brain antioxidant enzymes, increased levels of nitric oxide activity [7] and tumour necrotic factor alpha (Figure 8), in the animals' brain tissue homogenate after being exposed to multi-transceiver mobile phone activity. This finding is consistent with the reports of Friedman et al., who observed that radiofrequency waves triggered the production of reactive oxygen species, which stimulated the extracellular signal-regulated kinase cascade, which caused transcription and other cellular processes that in turn caused inflammation to the blood-brain barrier membranes [19]. The perforated blood-brain barrier membrane reduces its functional integrity by providing passage of unwanted substances through it into the brain. The current reports are also consistent with the findings of Simko and Mattsson, who reported that radiofrequency exposure directly caused an inflammatory response of the blood-brain barrier [20].

The significant increase in the levels of Evans blue dye in the brain of animals exposed to phone activity in vibration and ringtone mode could also be due to enhanced sympathetic tone induced oxidative stress as evidenced by decreasing HRV observed in our previous study [7]. This assertion is consistent with previous studies on mice, which found that sound caused non-invasive infrasonic or ultrasound pulses induce stress, which can impair blood brain barrier integrity by forming microbubbles from lipids. The formed microbubbles are then used to produce sonoporation which mediates cell membrane permeability of the BBB [21]. The uptake of Evans blue dyes by the brain increases significantly in the right cerebrum, and right cerebellum in rats exposed to mobile phone activity either vibration or sound alert, while in the left region of the cerebellum, it increased significantly across all treatment groups when compared to controls. The above mention area of the brain, is probably a potential area close to the membrane lining of circumventricular organs that is vulnerable to sonoporation. Microbubbles vibrate during sonoporation, causing various forms of deformation that lead to implosion. The implosive oscillations caused by acoustic emissions (from ringtone sound and vibration alert) generate shock waves that perforate the weak section of the BBB cell membrane, which is likely near to the lining of the circumventricular organs [22]. It could also be that sound or vibration caused oxidative stress, which triggered an inflammatory response that triggered the loss in the integrity of the blood-brain barrier [23]. The is evidenced by increasing levels of inflammatory agents like Nitric oxide (NO) and tumour necrosis factor alpha across all the phone activity exposed animals of the previous study [2].

The current observation on sound and vibration also agrees with reports of Fei, et al. that reported that infrasound of 8Hz frequency and above 70 dB intensity, can cause damage to right frontal cortex histology, cerebral ultrastructure, and BBB, which in turn allows undesired substances to pass across the BBB into the brain tissue [24]. The current findings on the effect of sound and vibration agree with those of Semyachkina et al., who discovered that loud music/sound with a frequency of more than 70 dB, as easily produced by MP3/MP4 players, or vibration of kitchen appliances and loudspeakers at concerts, opens the BBB to unwanted molecules [21, 25]. Arvanitis et al. also presented a supporting evidence suggesting that prolonged exposure to music or sound above 70 dB may result in an increased level of sonication, which increased microbubble concentration to be the initial factor triggering BBB leakage [26]. Thus, increase in sound induced microbubble concentration may increase BBB permeability via an increase in the extension of cracks in tight contacts of endothelial cells with a change in the ultrastructure of the brain, thereby causing an increased transport and pinocytotic activity of endothelial cells [27, 28].

Sound at the hearing threshold is also known to modify neural activity in numerous brain areas, some of which are known to be engaged in auditory processing, while others are thought to be important actors in emotional and vascular autonomic control in humans [21]

Our findings also suggest that the ringtone sound of 72 dB, caused an increase in sympathetic activity via a likely increase in epinephrine levels, as previously reported by Shehu et al. [8]. The ringtone sound causes stress-induced epinephrine elevation, that could have produces high levels of microbubble concentration that alters the cerebral ultrastructure, which could be the initial mechanism driving BBB leakage.

CONCLUSION

The current findings suggest that the 72 dB ringtone sound, vibration, and magnetic fields from a multi-transceiver phone can alter the cerebral ultrastructure, which could be the initial mechanism driving BBB leakage.

Acknowledgments

The authors thank the members of the neurophysiology and cardio-respiratory unit of the physiology department, Small Animal Clinic of the Veterinary Medicine Department, University of Ibadan, for their technical assistance.

References

1. Hamblin, D. L., & Wood, A. W. (2002). Effects of mobile phone emissions on human brain activity and sleep variables. International journal of radiation biology, 78(8), 659-669.
2. Usman, J. D., Isyaku, U. M., Magaji, R. A., & Fasanmade, A. A. (2020). Assessment of electromagnetic fields, vibration and sound exposure effects from multiple transceiver mobile phones on oxidative stress levels in

serum, brain and heart tissue. Scientific African, 7, e00271.

3. Consales, C., Merla, C., Marino, C., & Benassi, B. (2012). Electromagnetic fields, oxidative stress, and neurodegeneration. International journal of cell biology, 2012.
4. International Agency for Research on Cancer. (2013). IARC working group on the evaluation of carcinogenic risks to humans. IARC Monogr Eval Carcinog Risks Hum. 61, 45-119.
5. Hansson, H. A., & Johansson, B. B. (1980). Induction of pinocytosis in cerebral vessels by acute hypertension and by hyperosmolar solutions. Journal of neuroscience research, 5(3), 183-190.
6. Sırav, B., & Seyhan, N. (2016). Effects of GSM modulated radio-frequency electromagnetic radiation on permeability of blood–brain barrier in male & female rats. Journal of Chemical Neuroanatomy, 75, 123-127.
7. Dauda Usman, J., Umar Isyaku, M., & Fasanmade, A. A. (2020). Evaluation of heart rate variability, blood pressure and lipid profile alterations from dual transceiver mobile phone radiation exposure. Journal of Basic and Clinical Physiology and Pharmacology, 32(5), 951-957.
8. Shehu, A., Mohammed, A., Magaji, R. A., & Muhammad, M. S. (2016). Exposure to mobile phone electromagnetic field radiation, ringtone and vibration affects anxiety-like behaviour and oxidative stress biomarkers in albino wistar rats. Metabolic brain disease, 31, 355-362.
9. Hyland, G. J. (2000). Physics and biology of mobile telephony. The Lancet, 356(9244), 1833-1836.
10. Kaya, M., Gurses, C., Kalayci, R., Ekizoglu, O., Ahishali, B., Orhan, N., ... & Kemikler, G. (2008). Morphological and functional changes of blood–brain barrier in kindled rats with cortical dysplasia. Brain research, 1208, 181-191.
11. Rubin, L. L., & Staddon, J. M. (1999). The cell biology of the blood-brain barrier. Annual review of neuroscience, 22(1), 11-28.
12. Lochhead, J. J., Yang, J., Ronaldson, P. T., & Davis, T. P. (2020). Structure, function, and regulation of the blood-brain barrier tight junction in central nervous system disorders. Frontiers in physiology, 11, 914.
13. Smith, B. C., Tinkey, R. A., Shaw, B. C., & Williams, J. L. (2022). Targetability of the neurovascular unit in inflammatory diseases of the central nervous system. Immunological Reviews, 311(1), 39-49.
14. Xu, S., Zhou, Z., Zhang, L., Yu, Z., Zhang, W., Wang, Y., ... & Zhong, M. (2010). Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain research, 1311,

189-196.

15. Manaenko, A., Chen, H., Kammer, J., Zhang, J. H., & Tang, J. (2011). Comparison Evans Blue injection routes: Intravenous versus intraperitoneal, for measurement of blood–brain barrier in a mice hemorrhage model. Journal of neuroscience methods, 195(2), 206-210.
16. MacLellan, C. L., Silasi, G., Poon, C. C., Edmundson, C. L., Buist, R., Peeling, J., & Colbourne, F. (2008). Intracerebral hemorrhage models in rat: comparing collagenase to blood infusion. Journal of Cerebral Blood Flow & Metabolism, 28(3), 516-525.
17. Sirav, B., & Seyhan, N. (2016). Effects of radio-frequency radiation on the permeability of blood-brain barrier. Medical Science and Discovery, 3(5), 206-12.
18. Lathe, R. (2001). Hormones and the hippocampus. Journal of Endocrinology, 169(2), 205-232.
19. Friedman, J., Kraus, S., Hauptman, Y., Schiff, Y., & Seger, R. (2007). Mechanism of short-term ERK activation by electromagnetic fields at mobile phone frequencies. Biochemical Journal, 405(3), 559-568.
20. Simkó, M., & Mattsson, M. O. (2004). Extremely low frequency electromagnetic fields as effectors of cellular responses in vitro: possible immune cell activation. Journal of cellular biochemistry, 93(1), 83-92.
21. Semyachkina-Glushkovskaya, O., Esmat, A., Bragin, D., Bragina, O., Shirokov, A. A., Navolokin, N., ... & Kurths, J. (2020). Phenomenon of music-induced opening of the blood-brain barrier in healthy mice. Proceedings of the Royal Society B, 287(1941), 20202337.
22. Shepherd, D., McBride, D., Welch, D., Dirks, K. N., & Hill, E. M. (2011). Evaluating the impact of wind turbine noise on health-related quality of life. Noise and Health, 13(54), 333.
23. Liu, Y., Gong, Y., Xie, W., Huang, A., Yuan, X., Zhou, H., ... & Qin, X. (2020). Microbubbles in combination with focused ultrasound for the delivery of quercetin-modified sulfur nanoparticles through the blood brain barrier into the brain parenchyma and relief of endoplasmic reticulum stress to treat Alzheimer's disease. Nanoscale, 12(11), 6498-6511.
24. Fei, Z., Zhang, X., Wang, X. F., Li, Z. G., Lu, P. L., Liu, X. Z., ... & Jia, K. Y. (2000). The changes of rat blood-brain barrier permeability and expression of heat shock protein 70 after infrasonic damage. Journal of low frequency noise, vibration and active control, 19(2), 93-99.
25. Salmina, A. B., Komleva, Y. K., Malinovskaya, N. A., Morgun, A. V., Teplyashina, E. A., Lopatina, O. L., ... & Shuvaev, A. N. (2021). Blood–brain barrier breakdown in stress and neurodegeneration: biochemical mechanisms and

new models for translational research. Biochemistry (Moscow), 86(6), 746-760.

26. Arvanitis, C. D., Livingstone, M. S., Vykhodtseva, N., & McDannold, N. (2012). Controlled Ultrasound-Induced Blood-Brain Barrier Disruption Using Passive Acoustic Emissions Monitoring. PLoS ONE. 7(9), e45783.
27. Urayama, A., Grubb, J. H., Banks, W. A., & Sly, W. S. (2007). Epinephrine enhances lysosomal enzyme delivery across the blood–brain barrier by up-regulation of the mannose 6-phosphate receptor. Proceedings of the National Academy of Sciences, 104(31), 12873-12878.
28. Sachdeva, S., Persaud, S., Patel, M., Popard, P., Colverson, A., & Doré, S. (2022). Effects of Sound Interventions on the Permeability of the Blood–Brain Barrier and Meningeal Lymphatic Clearance. Brain Sciences, 12(6), 742.

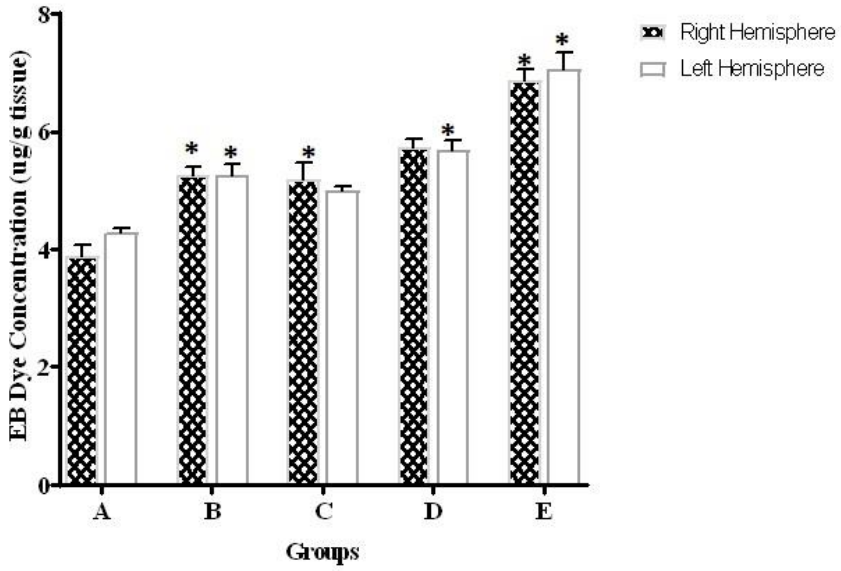

Figure 1. The concentration of EB Dye in both right and left-brain hemisphere tissue homogenate results of Wistar rats exposed to multi-transceivers phone activity

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C: MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5; *P < 0.05 (A vs B, C, D, E).

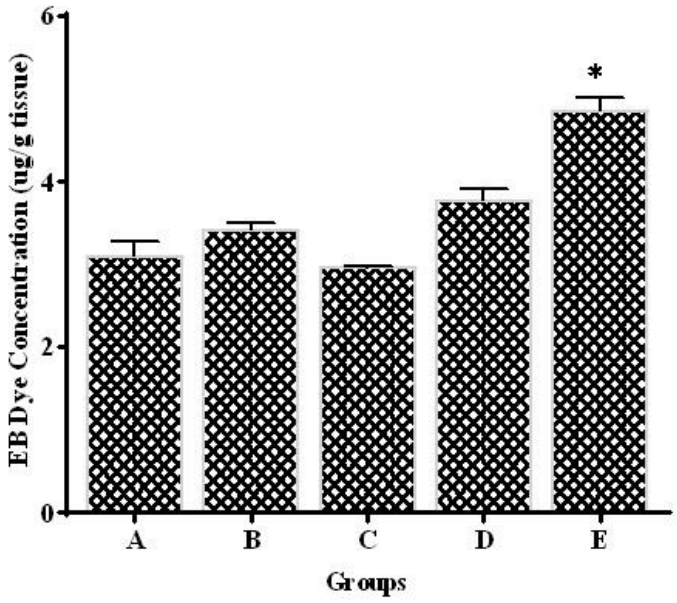

Figure 2. Cerebrum EB Dye concentration of Wistar rats exposed to calls from mobile phone with double transceivers

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C: MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5; *P < 0.05 (A vs B, C, D, E).

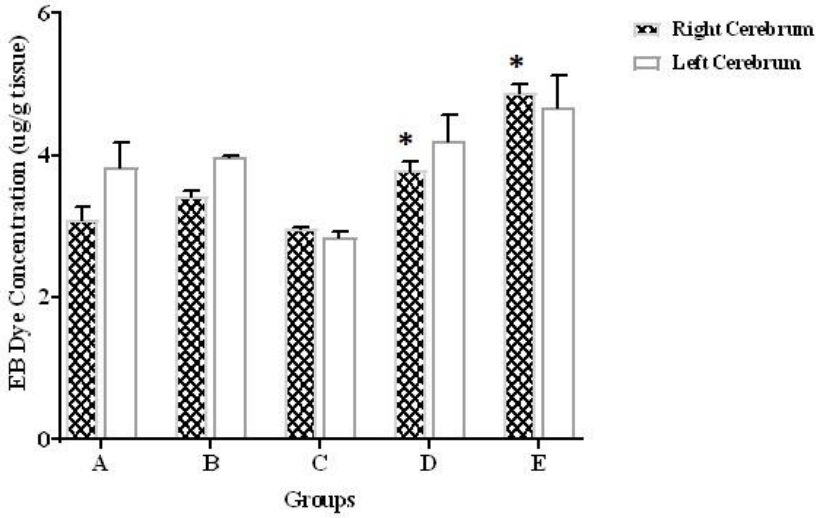

Figure 3. Right and left cerebrum EB Dye concentration of Wistar rats exposed to calls from mobile phone with double transceivers

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C: MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5; *P < 0.05 (A vs B, C, D, E).

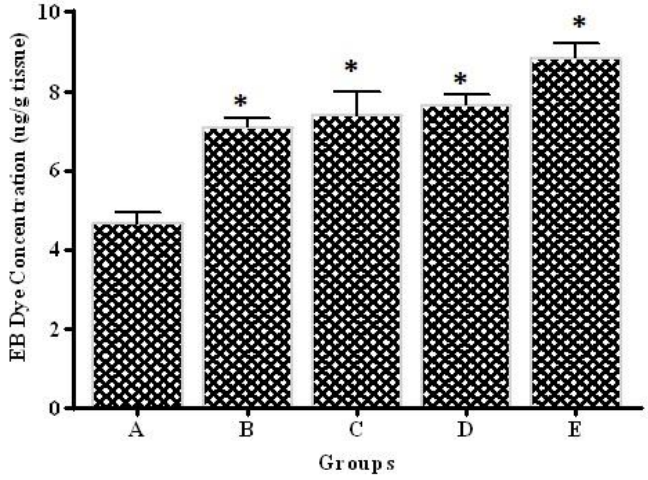

Figure 4. Cerebellum tissue EB Dye concentration of Wistar rats exposed to calls from mobile phone with double transceivers

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C: MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5; *P < 0.05 (A vs B, C, D, E).

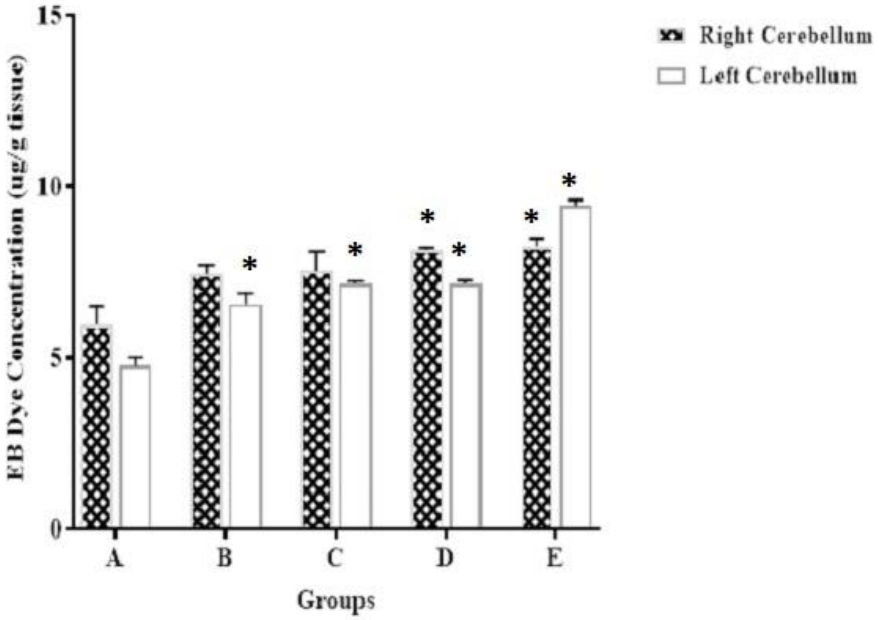

Figure 5. The concentration of EB Dye in both right and left cerebellum tissue homogenate of Wistar rats exposed to calls from mobile phone with double transceivers

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C: MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5; *P < 0.05 (A vs B, C, D, E).

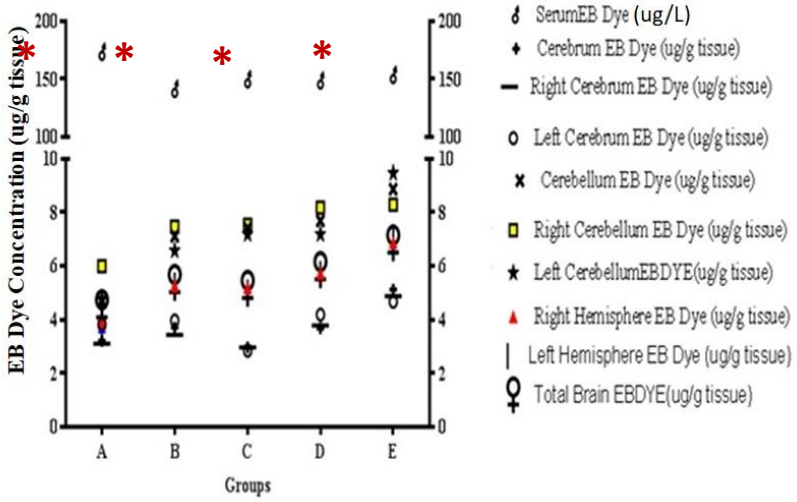

Figure 6. Summary of distribution of EB dye concentration in both brain and serum of Wistar rats exposed to calls from mobile phone with double transceivers

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C:

MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5; *P < 0.05 (A vs B, C, D, E).

Table 1. Nitric oxide and TNF alpha results of Wistar rats after six weeks of exposure to multi-transceiver mobile phone activities in various modality (silence, vibration and sound)

Groups	TNF ALPHA (pg/ml)
A(Control)	868.62 ± 125.49
B(MTrMP**)**	888.71 ± 92.94
C(MTrMP+**Vr)**	887.85 ± 120.70
D(MTrMP+**Rg)**	871.53 ± 103.32
E(MTrMP+**Vr+Rg)**	907.82±54.30

MTrMP: Electromagnetic radiation during calls from multi-transceiver mobile phone (silence mode); Vr: vibrations; Rg: ringtone or sound; A(control): no phone; B: MTrMP only; C: MTrMP+Vr; D: MTrMP+Rg; E: MTrMP+Vr+Rg; n = 5.