

Mobile Phonerays versus Wi-Fi Waves on Brain Structure, Motor and Behaviour development of Infant Rabbits: Experimental Study

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ABSTRACT

Background: Electro-magnetic radiations have an effect on brain structure and motor development of infant rabbits. **The aim:** This study aimed to compare between different duration exposure to cell phone rays and Wi-Fi waves on brain structure and motor behavior development of infant rabbits. **Methods:** From a Rabbitry of 300 mothers' capacity, 120 rabbits were selected to participate in the current study. They were divided into six homogeneous comparable experimental groups (20 in each) (Study groups 1, 2 and 3 were exposed to mobile phoneradiation, 900 MHZ for 2, 4 and 6 hours daily respectively, Study groups 4, 5 and 6 were exposed to Wi-Fi radiation, 2500 MHZ for 2, 4 and 6 hours daily respectively). The Experiments were lasted 6 weeks, a week for preparation the field work and to make all mothers nurse only five infant rabbits. Rabbit mothers with litter size at birth more than five bunnies (newborns) were still adopting another mother, whereas the mothers with litter size at birth less than five bunnies (newborns) were adopting other bunnies. The actual duration of experimental work represented in exposing animals to different electro-magnetic radiations and collecting data were five weeks. In this study motor development was measured by hindlimb motor function; locomotion and circular motion, and brain histology regarding neural apoptosis was assessed, respectively in both groups after each time exposure. **The results:** Before the study, there was no significant difference in the mean values of all variables among the six groups. After study there were significantly ($P \leq 0.5$ or 0.1) harmful in each parameters studied represented in values of each of (**hindlimb motor functions; locomotion and circular motion**). WiFi groups recorded the worst value in both parameters studied (motor development and neural stress revealed by apoptosis in histological examination), compared by mobile phone. The harmful effects of electro-magnetic radiation were increased by increasing exposure time in all studied groups (mobile phone and WIFI groups). **Conclusion:** Electro-magnetic radiations result in marked disturbances of motor development and brain structure in infant rabbits that result from exposure to cell phone and Wi-Fi radiation at different duration interval. WiFi radiation recorded bad effects more than those of mobile phone radiation, in motor function and brain structure.

Key Words: Electro-magnetic radiation, Cell phone rays, Wi-Fi waves, Brain structure, Motor development, Infant rabbits.

INTRODUCTION

The mobile phone is one of the fastest growing technological developments that have become popular and necessary in modern life. The fourth generation (4G) is the latest application of mobile phone technologies. The 3G & 4G mobile telecommunication system is developed for a variety of services such as voice and high-speed data transmission, Internet and multimedia applications which require high speed and bandwidth. The 3G & 4G-launched applications can emit continuous electromagnetic radiation to the environment [1; 2; 3]. Side effects of mobile phone usage increased the concerns on health and quality of life. Potential consequences after electromagnetic radiation exposure have been reported in many studies and brain is one of the most commonly discussed tissues [3 & 4]. Neuronal damage and oxidative stress have been shown by several studies [4; 5 & 6]. Another device that's being used on a daily basis in every home or institute all over the world is the Wi-Fi device which is used to connect many computers in an institute or for transmission of internet by a wireless way [6].

Electromagnetic waves that are emitted from cell phones ranges from 900 MHz to 1.9 GHz while that emitted from Wi-Fi devices are of 2.4 GHz. These waves have thermal and non-thermal effects [3 & 7]. Using mobile phones and Wi-Fi have become an essential part of our daily living activities. All these devices are working by emitting electromagnetic waves which vary in their wave lengths and intensity. Many researchers have been carried out to investigate the effect of these waves on the brain. Many studies approved the negative effects on the brain tissues and oxidative capacity [3 & 4]., but none of them was conducted to compare the structural and behavior changes that result from exposure to cell phone and Wi-Fi at different duration interval.

There are some studies on the health effects on people living near cell phone towers [8]. Though cell technology has been in existence since the late 1980s, the first study of populations near cell tower base stations was only conducted by Santini, et al [8]. It was prompted in part by complaints of adverse effects experienced by residents living near cell base stations throughout the world and increased activism by

citizens[9],[10 & 11]were looking to identify neurobehavioral deficits in people living near cell phone base stations in Egypt. Their results found a prevalence of neuropsychiatric complaints: headaches, memory changes, dizziness, tremors, depressive symptoms, and sleep disturbance were significantly higher among exposed inhabitants. A study showed that when people used a cell phone for 50 minutes, brain tissues metabolized more glucose. This may cause negative health outcomes on brain tissues. [12]reported histological changes in the different visceral organs including heart, lung, liver and kidney of rats exposed to radio frequency radiation for 4 weeks (1h/day). Pre-school children may also be considered to be more sensitive to any negative health effects than adults because of the greatest absorption of mobile phone radiation. Furthermore, depending on the size of a child's head, there can be the head resonance effect and greater ease in penetration of Global System for Mobile Telecommunication radiation to the thinner skull of an infant. Particularly in the developmental stages (from conception to adulthood), their brain tissues, with high content of water and ions, absorb more electromagnetic radiation

at mobile phone frequencies [13]. Increased intensity and extended duration of exposure to RF radiation during these stages may lead to inherited disorders by altering the conformation of structural molecules.

MATERIALS AND METHODS

1. Subject:

This Experimental work was conducted at a Commercial Rabbitry (300 mothers capacity), Mansoura, Dakahlia Governorate, Egypt, while the Laboratory investigation was carried out at Animal Health Research Institute, (AHRI), Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Dokki, Giza, Egypt.

All rabbits were comparable experimental homogeneous animals (No: P.T.REC/012/002109).

The animals were eligible to participate in the study under the following:

Inclusion criteria:

- Average age 1 week.
- Without any congenital anomalies.
- Physically normal
- Both genders were involved

2. Procedures:

All animals were housed in a specialized rabbit cage with controlled temperature and lighting (12 Hrs light/dark cycle). Rabbits were allowed to access to food and water.

The study groups were established as follows:

Animals were divided into six homogeneous comparable experimental groups (20 in each) as follow:-

- Study groups 1, 2 and 3 were exposed to mobile phoneradiations, 900 MHZ for 2, 4 and 6 hours daily respectively.
- Study groups 4, 5 and 6 were exposed to Wi-Fi radiations 2500 MHZ for 2, 4 and 6 hours daily respectively.

The Experiments were lasted 6 weeks. It occurred 6 hours daily, mobile phone & Wi-Fi device were placed in an intermediate position between the groups to be studied.

Instruments for evaluation

Assessment of motor behavior:

-It was done by using Hindlimb motor function scoring system of rabbit [14].

Motor function was evaluated by a five-point scale of motor impairment ranging from normal function to total paralysis of the hind legs. Animals were placed one at a time on a (0.7 × 0.9 m) paper-covered mattress on a table.

The first motor score was calculated by observing each animal for 1 min in the open field.

- 0- There are no noticeable movements at all.
- 1- There are barely visible movements at any hind limb joints (hip, knee or ankle).
- 2- There are obvious movements of one or more hind limb joints (hip, knee or ankle) in one or both limbs, but no co-ordination, alternate stepping movements or weight bearing were observed. This included movements ranging from only one joint to movements in all the joints of the lower limbs.
- 3- If there are alternate stepping and forward propulsive movements of the hind-limbs, but no weight bearing. In this case the hind-limbs will be externally rotated and the

animal will use the hind limbs for forward propulsion. There will be no plantar placement of the feet. Rabbits scoring 3 will use the tips (nails) of the middle three toes for forward movement.

4- If they are able to bear weight on their hind-limbs and can walk with some deficit. There will be plantar placement of the feet. Deficit will include slight external rotation of one or both limbs and/or hip instability.

5- If it is not possible to differentiate the injured from a normal animal except reduced mobility they will be scored 5.

Rabbits demonstrated normal movement (score 5) were then assessed using steel bars (50 cm long and 3 mm thick) with decreasing widths: 15cm, 12cm, 8cm, 6 cm and 4 cm. The rabbits were required to walk on the bars and the narrowest bar they can traverse without any slips, in at least two trials, were recorded. If the rabbits, instead of walking, tried to climb under the bar it was considered as a failure, i.e., an inability to walk on the bar.

6- If the animals can walk on the 15-cm bar

7 - Can walk on a 12-cm-wide-bar

8 - Can walk on a 8-cm-wide-bar

9 - Can walk on a 6-cm-wide-bar

10 - Can walk on a 4-cm-wide-bar [14].

- Locomotion

Locomotion on a flat surface was assessed by grading the amount of spontaneous movement of the head, trunk, and limbs.

0- No movement and Moves head

1- Slight movement and Moves front legs

2- Distinct movement, showing good range of motion and Moves back legs

3- Rapid movement, entire range of motion [14& 15]

- Circular motion

0-No movement, Straight line forward motion

- 1- Slight movement, slight jump, Jumping and jerky motions
- 2- Good range of motion, maintains for one to two steps, occasional jump
- 3- Entire range of motion, maintains for at least three 3 steps [14; 15& 16]

Tissue Analysis

At the end of the 6th week, all rabbits will be sacrificed by general anesthesia, The brains were removed from the skull by nontraumatic technique (resection of bone structures at the skull base, followed by amidline incision from the foramen magnum to the nose) after an extended in situ postmortem fixation time of 30 min [17].

Brain samples were taken from four rabbits in each group directly after slaughtering and fixed in 10% neutralized formalin. The tissues were then dehydrated with increasing concentrations of ethyl alcohol (50, 75, 98 and 100%), cleared in xylene and embedded in paraffin wax. Each brain was sectioned coronally in 50 μ m-thick and stained with hematoxylin, eosin and cresyl violet then examined morphometrically under a light microscope (Nikon Eclipse E-200) with attached camera (Nikon, 1103473, Japan) [18; 19; 20& 21].

Dark stained neurons indicated neuronal damage.

Statistical analysis

Data were subjected to analysis of variance according to [22] using the General Linear Model Program of [23]. Duncan's new multiple range tests were used to test the significance of the differences among means [24]. Data presented as percentages were

transformed to the corresponding arcsine values [25] before being statistically analyzed.

RESULTS

1. Motor Development:

Hindlimb motor functions; locomotion and circular motion significantly ($P \leq 0.5$ or 0.1) decreased in descending order due to exposure to EMR of mobile phone and WiFi, respectively at the three different time exposure (2, 4 and 6 hours). The highest ($P \leq 0.5$ or 0.1) values indicated hindlimb motor functions; locomotion and circular motion of infant NZW rabbits exposed to different sources of EMR (mobile phone and WiFi) were recorded in the 4th week of age, while the lowest ($P \leq 0.5$ or 0.1) values were recorded in the 2nd week of age. Values of hindlimb motor functions; locomotion and circular motion of infant NZW rabbits recorded in the 5th week of age were insignificantly higher than those obtained during the 3rd week.

There were no significant differences on values of each of hindlimb motor functions; locomotion and circular motion of infant NZW rabbits up to weaning, due to the interaction among sources of EMR and times of exposure (Table 1, and Figures 4; 5& 6).

Brain Histology:

In our current study (Figures 1; 2& 3), the (hematoxylin&eosin) and cresyl violet staining of the hippocampal region clearly showed interspersed, deeply stained, shrunken cells, which clearly indicates the degenerative changes in these areas. Dark staining was more prominent in groups exposed to WiFi than those exposed to

mobile phone radiations. The exact mechanism responsible for this degeneration has to be investigated; perhaps the mechanism is through reactive oxygen species.

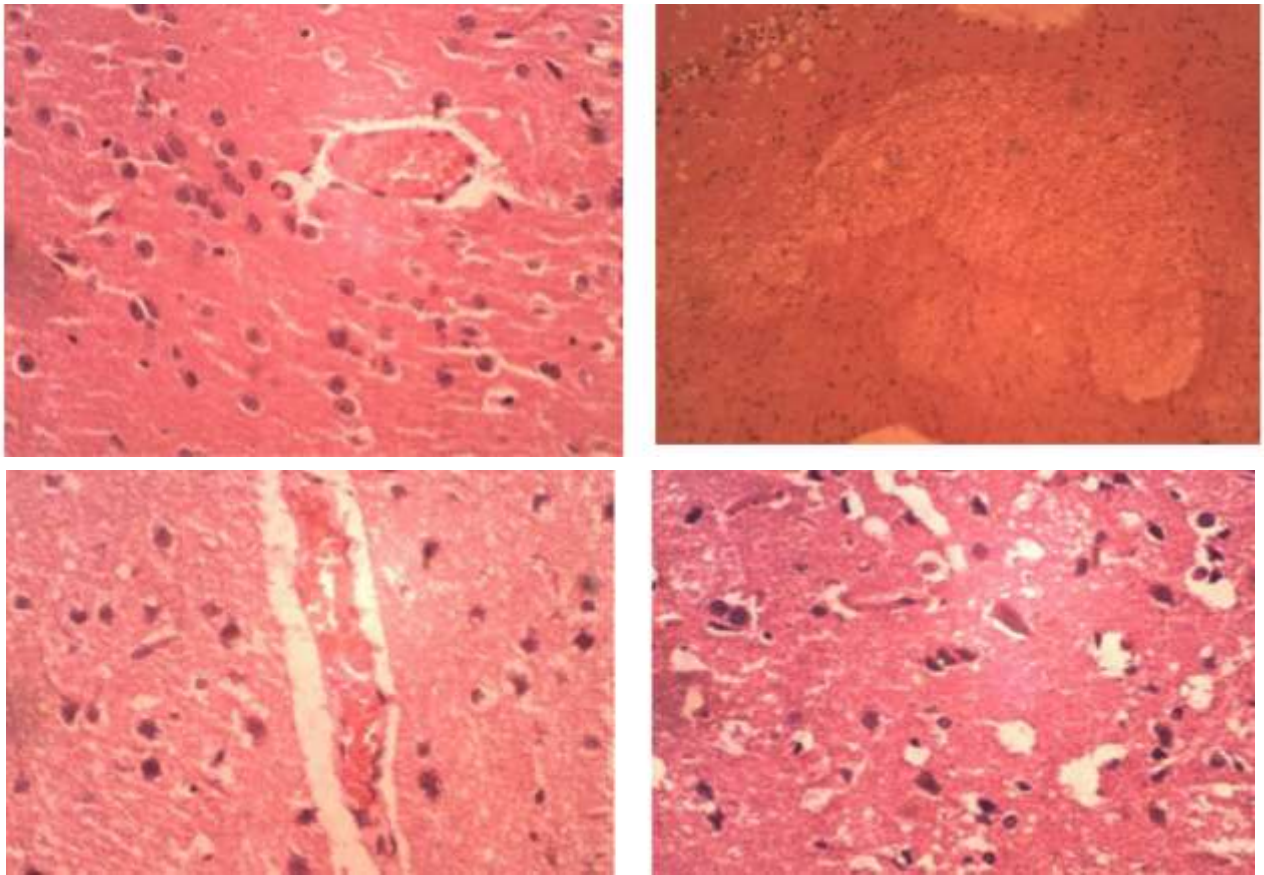
Marked morphological changes were detected in the CA3 region of the hippocampus of the -EMR-exposed rabbits. The hippocampus of RF-EMR-exposed rabbits showed shrunken, darkly stained neurons.

The cresyl violet and H & E staining revealed scattered and grouped dark neurons, which were often shrunken and darkly stained, homogenized with loss of discernible internal cell structures. Here are some slides with description beneath each slide to show difference in effect between exposure to mobile and WiFi radiations.

Table 1. Effect of different time exposure to different electro-magnetic radiations (EMR), on hindlimb motor functions, locomotion and circular motion of infant New-Zealand White rabbits, up to weaning, (Means \pm SE).

Age (week)	Time of Exposure to EMW (Hrs/Day)	Treatments & Source of Exposure to EMR								
		Hindlimb Motor Functions			Locomotion			Circular Motion		
		Mobile (M) 900 MHZ	Wi Fi (W) 2500 MHZ	Means \pm SE	Mobile (M) 900 MHZ	Wi Fi (W) 2.5 GHZ	Means \pm SE	Mobile (M) 900 MHZ	Wi Fi (W) 2.5 GHZ	Means \pm SE
2 nd week	2	4.52 \pm 0.04	4.40 \pm 0.02	4.46 \pm 0.01 ^A	3.97 \pm 0.02	3.69 \pm 0.04	3.83 \pm 0.02 ^A	1.17 \pm 0.02	1.13 \pm 0.02	1.15 \pm 0.01 ^A
	4	4.46 \pm 0.02	4.37 \pm 0.03	4.42 \pm 0.02 ^B	3.89 \pm 0.02	3.54 \pm 0.03	3.72 \pm 0.02 ^B	1.10 \pm 0.01	1.09 \pm 0.03	1.10 \pm 0.01 ^B
	6	4.43 \pm 0.02	4.30 \pm 0.02	4.37 \pm 0.01 ^C	3.87 \pm 0.04	3.50 \pm 0.02	3.69 \pm 0.01 ^C	1.07 \pm 0.01	1.02 \pm 0.01	1.05 \pm 0.01 ^C
	Means \pm SE	4.47 \pm 0.02 ^a	4.36 \pm 0.02 ^b	4.42 \pm 0.01	3.91 \pm 0.02 ^a	3.58 \pm 0.03 ^b	3.75 \pm 0.01	1.11 \pm 0.01 ^a	1.08 \pm 0.02 ^b	1.10 \pm 0.01
3 rd weeks	2	4.49 \pm 0.05	4.39 \pm 0.04	4.44 \pm 0.03 ^A	3.95 \pm 0.03	3.61 \pm 0.03	3.78 \pm 0.02 ^A	1.79 \pm 0.03	1.63 \pm 0.03	1.71 \pm 0.02 ^A
	4	4.41 \pm 0.02	4.30 \pm 0.04	4.36 \pm 0.03 ^B	3.80 \pm 0.03	3.58 \pm 0.03	3.69 \pm 0.03 ^B	1.68 \pm 0.02	1.61 \pm 0.02	1.65 \pm 0.01 ^B
	6	4.40 \pm 0.03	4.28 \pm 0.05	4.34 \pm 0.02 ^C	3.72 \pm 0.03	3.49 \pm 0.04	3.61 \pm 0.03 ^C	1.62 \pm 0.03	1.56 \pm 0.02	1.59 \pm 0.03 ^C
	Means \pm SE	4.43 \pm 0.02 ^a	4.32 \pm 0.03 ^b	4.38 \pm 0.02	3.82 \pm 0.02 ^a	3.56 \pm 0.02 ^b	3.69 \pm 0.01	1.70 \pm 0.03 ^a	1.60 \pm 0.03 ^b	1.65 \pm 0.02
4 th weeks	2	5.99 \pm 0.07	5.81 \pm 0.09	5.90 \pm 0.05 ^A	3.72 \pm 0.04	3.53 \pm 0.03	3.63 \pm 0.02 ^A	2.04 \pm 0.03	1.77 \pm 0.04	1.91 \pm 0.02 ^A
	4	5.81 \pm 0.05	5.69 \pm 0.04	5.75 \pm 0.03 ^B	3.64 \pm 0.03	3.50 \pm 0.03	3.57 \pm 0.01 ^B	2.01 \pm 0.04	1.69 \pm 0.02	1.85 \pm 0.02 ^B
	6	5.76 \pm 0.05	5.61 \pm 0.05	5.69 \pm 0.03 ^C	3.61 \pm 0.05	3.41 \pm 0.05	3.51 \pm 0.03 ^C	1.94 \pm 0.03	1.65 \pm 0.02	1.80 \pm 0.02 ^C
	Means \pm SE	5.85 \pm 0.04 ^a	5.70 \pm 0.04 ^b	5.78 \pm 0.02	3.66 \pm 0.03 ^a	3.48 \pm 0.02 ^b	3.57 \pm 0.02	2.00 \pm 0.04 ^a	1.70 \pm 0.02 ^b	1.85 \pm 0.02
5 th weeks	2	5.93 \pm 0.08	5.71 \pm 0.04	5.82 \pm 0.04 ^A	3.30 \pm 0.03	3.24 \pm 0.02	3.27 \pm 0.02 ^A	2.34 \pm 0.04	1.94 \pm 0.02	2.14 \pm 0.03 ^A
	4	5.80 \pm 0.06	5.59 \pm 0.04	5.70 \pm 0.04 ^B	3.24 \pm 0.05	3.22 \pm 0.04	3.23 \pm 0.03 ^B	2.18 \pm 0.02	1.82 \pm 0.04	2.00 \pm 0.02 ^B
	6	5.71 \pm 0.04	5.49 \pm 0.05	5.60 \pm 0.04 ^C	3.16 \pm 0.02	3.10 \pm 0.03	3.13 \pm 0.02 ^C	2.13 \pm 0.03	1.78 \pm 0.03	1.96 \pm 0.02 ^C
	Means \pm SE	5.81 \pm 0.04 ^a	5.60 \pm 0.04 ^b	5.71 \pm 0.03	3.23 \pm 0.02 ^a	3.19 \pm 0.02 ^b	3.21 \pm 0.01	2.22 \pm 0.03 ^a	1.85 \pm 0.02 ^b	2.04 \pm 0.01
Overall mean \pm SE		5.14 \pm 0.04 ^a	5.00 \pm 0.04 ^b	5.07 \pm 0.02	3.66 \pm 0.03 ^a	3.45 \pm 0.02 ^b	3.56 \pm 0.02	1.76 \pm 0.02 ^a	1.56 \pm 0.02 ^b	1.66 \pm 0.01

Means with the different superscripts in the same row (a, b) or in the same column (A, B, C) are significantly ($P \leq 0.05$, or 0.01) different.



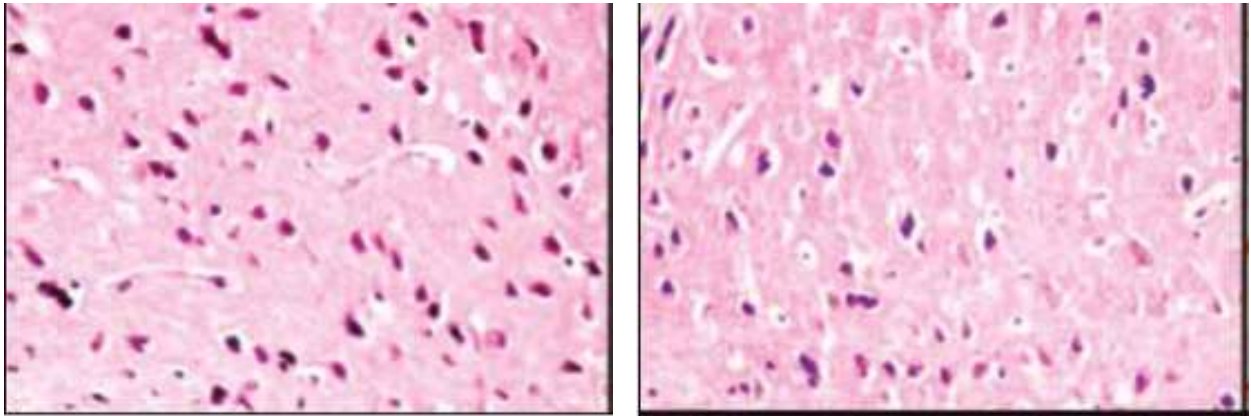
(A)

Fig. 1

(B)

The (A) photomicrograph of rabbit's brain exposed to 6 hours WiFi radiations showed a marked congestion of the cerebral blood vessels and presence of numerous spongiform vacuoles in the neuropil.

The (B) photomicrograph of rabbits' brain exposed to 6 hours mobile phone radiations showed moderate congestion of the blood vessels along side with moderate locally extensive gliosis and multiple foci of spongiform vacuolation.

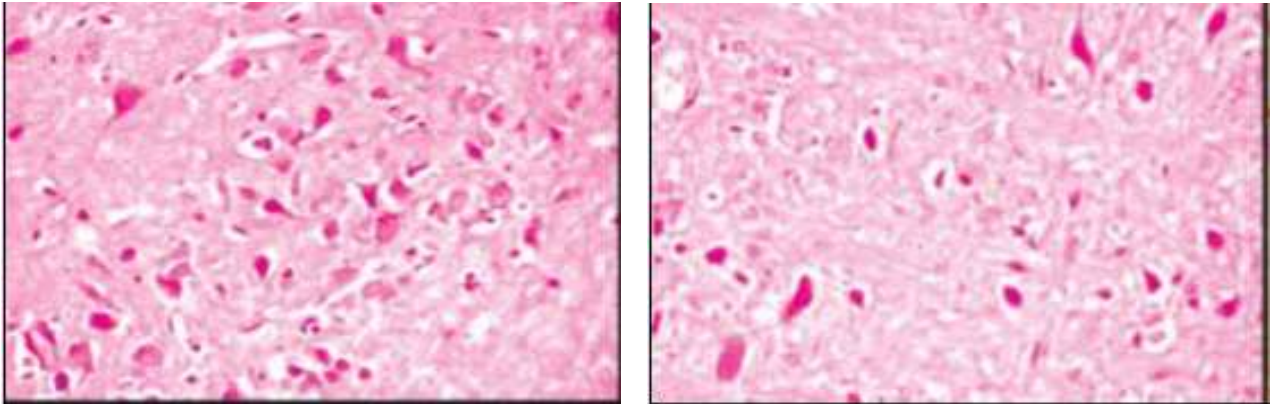


(A)

Fig. 2

(B)

The (A) Representative light microphotograph showing the morphology of the frontal cortex tissue in mobile phone 6 hrs (A) and WiFi 6 hrs (B) groups by hematoxylin-eosin, 50 μm). severe degenerative changes, shrunken cytoplasm, and extensively dark pyknotic nuclei is observed. The intensity of neuronal changes in mobile phone group is less than in the WiFi group. The number of neurons in the frontal cortex of the WiFi group was also significantly less than in the mobile phone group.



(A)

Fig. 3

(B)

Representative light microphotograph showing the morphology of the brain stem tissue in mobile phone 6 hrs (A) and WiFi 6 hrs (B) groups by hematoxylin-eosin, 50 μm).severe degenerative changes, shrunken cytoplasm, and extensively dark pyknotic nuclei is observed. The intensity of neuronal changes in mobile phone group is less than in the WiFi group. The number of neurons in the brain stem of the WiFi group was also significantly less than in the mobile phone group.

DISCUSSION

biological effects of acute exposure to Electro-magnetic waves result in marked disturbances of motor development and brain structure in infant rabbits that result from exposure to cell phone and Wi-Fi at different duration interval.

The present study comes in agreement with [25] who reported that electromagnetic field exposure change protein activity both inside and/or outside the cell leading to marked deformities.

Marked decreased disturbances of motor development and brain structure in this study may attributed to the biological effects of electromagnetic fields may lead to exposure and alternation in biological molecules structure [26].

Disturbances of brain structure in this study also may attributed to a strong relationship between radiation and cholinergic system. Acetylcholine (ACh) is a neuro-transmitter of the cholinergic system and plays a vital role in many functions of both the peripheral and central nervous systems acting in the learning and memory processes as well as locomotor control and cerebral blood flow [27].

This study also comes in accordance with [28] who stated that WiFi exposure inhibited acetylcholine esterase (AChE) activity when compared with control. This will result in over-activation of AChE in the CNS and thus lead to neurotoxicity in exposed rabbits.

In line with the present study, a study done by [29] who concluded that mobile phone radiation could modify body weight produce observable histological changes in the brain tissue of exposed rabbits. However, these changes may not be associated with a concomitant DNA damage.

The biological effects of electromagnetic radiation are associated with the induction of electric fields in the body. Strong electric fields lead to damage to neuronal functions, depending on the frequency involved. The magnitude of the photon energy of radiofrequency is one-millionth of the ionization energy and one-thousandth of the thermal energy [13].

However, non-thermal effects on biological systems of radio frequency electromagnetic field at low levels are controversial. The photon energy of electromagnetic field emitted from mobile phone

breaks the weak non-covalent bonds of DNA strands and induces chemical reactions and changes [4].

Several potential reaction mechanisms have been reported such as oscillating resonances, reactive oxygen species (ROS)-mediated mechanisms, which induced dipole moments [29].

Cellular response to electromagnetic field exposure: signaling pathways Alterations in the calcium signaling pathways have been reported in response to the effects of electromagnetic field exposure, calcium channels and receptors on the cell membrane, which affects the response of mitochondrial calcium reaction as the energy source of the cell. Additionally, there is an increase in intracellular Calcium levels as a result of the cellular effects of electromagnetic field exposure. Alterations in voltage-gated calcium channels have been investigated, and studies have shown enhanced activity of voltage-gated calcium channels due to direct effects of electromagnetic field exposure in many cell types. In particular, voltage-gated calcium channels play a crucial role in the response to electromagnetic field. In the microwave, electromagnetic field activation of voltage-gated calcium channels causes a rapid increase in intracellular calcium, nitric oxide, and

peroxynitrite. While the pathophysiological effects of electromagnetic field are related to the Calcium/nitric oxide/peroxynitrite pathway at the cellular level, its therapeutic effects are related to the Calcium/nitric oxide/cGMP/ protein kinase G pathway [30& 31] exposed Sprague Dawley rabbits to microwave radiation with a frequency of 591 MHz at an average power density of 13.8 mW/cm², which induced a reduced availability of adenosine triphosphate resulting in brain energy metabolism disorders [31].

Acetylcholine (ACh) is a neurotransmitter with an important role in many functions of both the peripheral and central nervous systems acting in the learning and memory processes as well as locomotor control and cerebral blood flow [32; 33& 34].

In the present study, WiFi and mobile phone exposure alters some neuro-behavioral function associated with neurological diseases. WiFi and mobile phone exposure affect motor coordination and alter exploratory motor function in exposed rabbits. This is in agreement with previous studies where high radiofrequency electromagnetic radiation exposure induces cognitive impairment and stress-related behaviors in rats [35; 36& 37].

Acute exposure of rabbits to continuous, pulsed, and amplitude modulated 591 MHz microwaves (2.48– 5.8 W/kg) resulted in marked disturbances of energy metabolism in the brain, as reflected by a 40% decrease in adenosine triphosphate and creatine phosphate concentration, and a 12.5% increase in reduced nicotinamide adenine dinucleotide content]31& 37[.

CONCLUSION

Based on the findings of this study

It could be concluded that, electro-magnetic waves result in marked disturbances of motor development and brain structure of infant rabbits that result from exposure to cell phone and Wi-Fi at different duration interval with more negative effects with increased duration of exposure. Despite the time factor WiFi radiations have deleteriously effects more than recorded due to mobile phone radiations. More studies and investigations are still required to evaluate the bad effect of electro-magnetic radiations on human health, especially young developing children.

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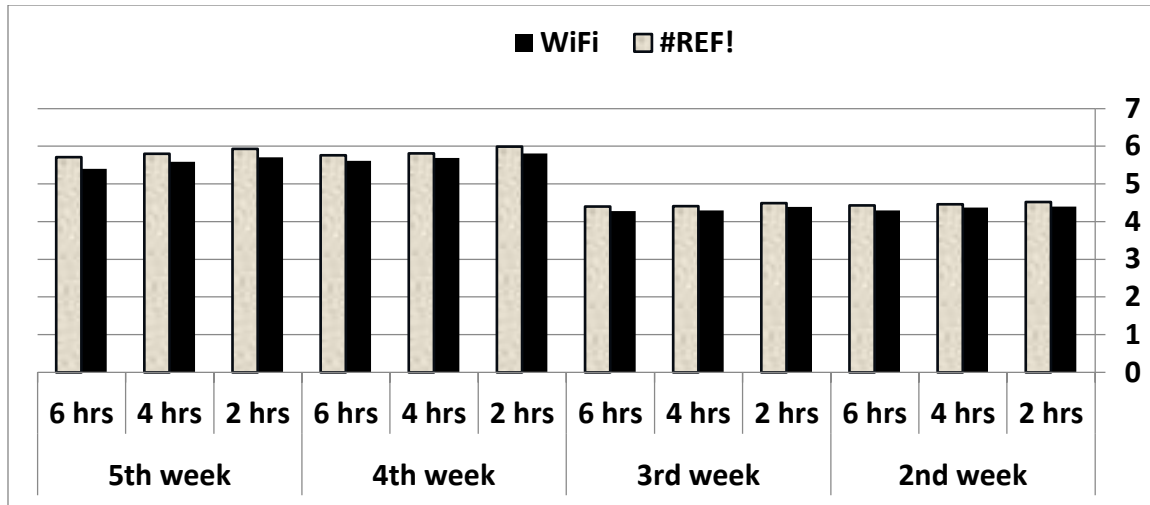


Figure4. Effect of different time exposure to different electro-magnetic radiations (EMR), on hindlimb motor functions of infant New-Zealand White rabbits, up to weaning, (score 0-10).

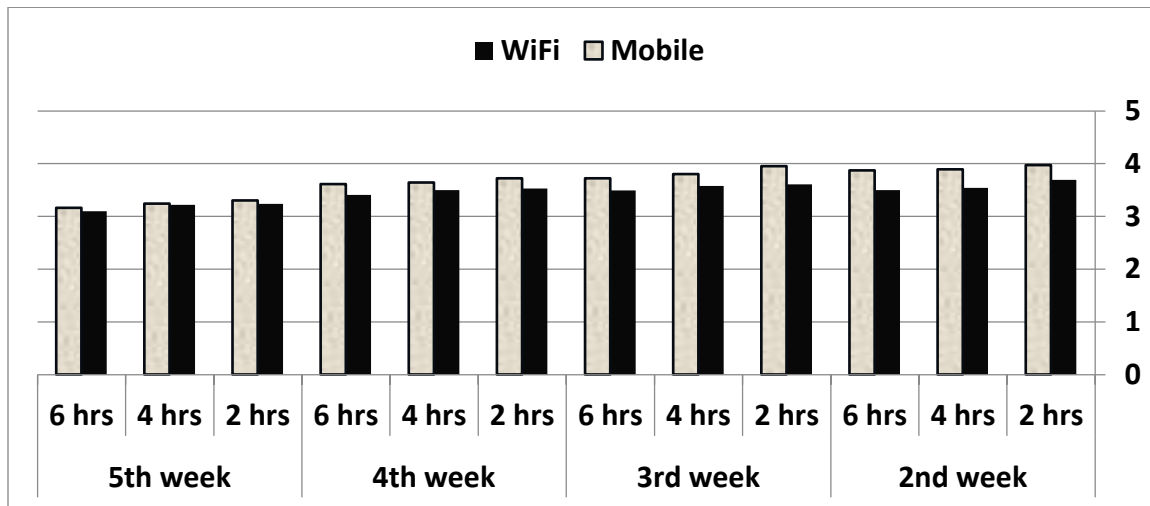


Figure 5. Effect of different time exposure to different electro-magnetic radiations (EMR), on locomotion of infant New-Zealand White rabbits, up to weaning, (score 0-5).

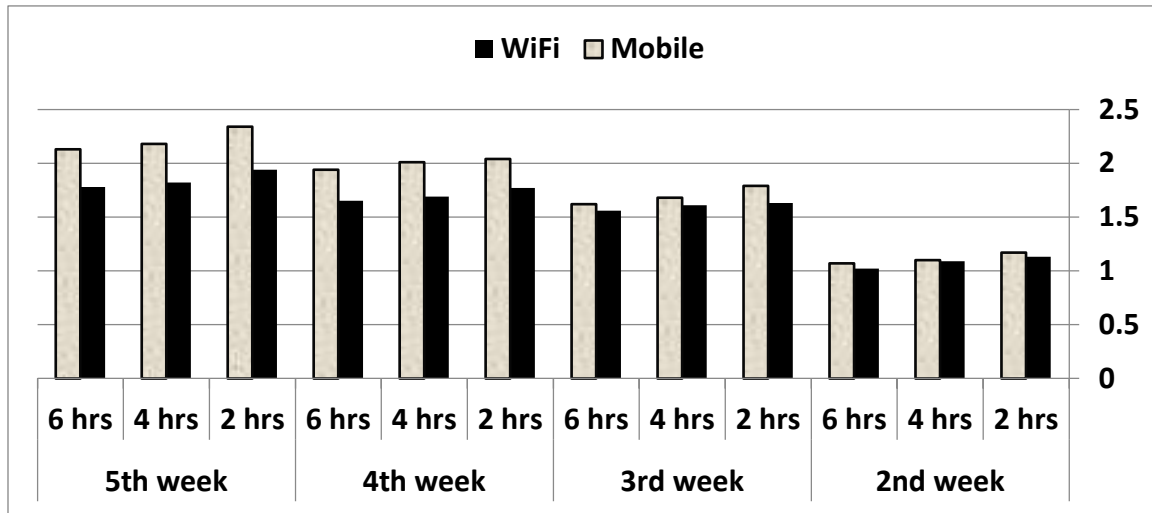


Figure 6. Effect of different time exposure to different electro-magnetic radiations (EMR), on circular motion of infant New-Zealand White rabbits, up to weaning, (score 0-5).