



Biochemical and histological studies on adverse effects of mobile phone radiation on rat's brain



Shaymaa Hussein^a, Abdel-Aleem El-Saba^a, Mona K. Galal^{b,*}

^a Department of Cytology and Histology, Faculty of Veterinary Medicine, Cairo University, Egypt

^b Department of Biochemistry and Chemistry of Nutrition, Faculty of Veterinary Medicine, Cairo University, Egypt

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ABSTRACT

With the rapid development of electronic technologies, the public concern about the potential health hazards induced by radiofrequency (RF) radiation has been grown. To investigate the effect of 1800 MHz RF radiation emitted from mobile phone on the rat's brain, the present study was performed. Forty male rats were randomly divided into two equal groups; control and exposed group. The later one exposed to 1800 MHz emitted from mobile phone with an SAR value of 0.6 W/kg for two hours/day for three months. The brain tissues were collected at the end of the experimental period and separated into hippocampus and cerebellum for subsequent biochemical, histological, immunohistochemical and electron microscopic investigations. The rats that were exposed to RF- radiation had a significant elevation in MDA content and a significant reduction in antioxidant parameters (glutathione, super oxide dismutase and glutathione peroxidase) in both regions. Degenerative changes were observed in the hippocampus pyramidal cells, dark cells and cerebellar Purkinje cells with vascular congestion. In addition a significant DNA fragmentation and over expression of cyclooxygenase-2 apoptotic gene was detected. Those results suggested that, direct chronic exposure to mobile phone caused severe biochemical and histopathological changes in the brain.

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1. Introduction

The mobile phone is one of the major inventions which have changed the way of communication in today's world. There is accumulating evidence reported that exposure to the radio-frequency (RF) radiation emitted from mobile phones and or their base stations could affect people's health (Hao et al., 2015). People generally hold their mobile phones close to the head in the talking mode and this causes a higher exposure of the brain to the RF-radiation than other parts of the body. The brain tissue is a major potential route for the absorption of hazardous materials encountered in the environmental place (Irmak et al., 2002). In addition the brain is one of the most sensitive target organs of RF-radiation, where the mitochondrial injury occurs earlier and more severely than in other organs (Hao et al., 2015). The radiation emitted from mobile phone could be absorbed by neural tissue more than other tissues (Irmak et al., 2002). RF-radiation could affect individuals by increasing free radical production, which enhances the lipid peroxidation (LPO) leading to oxidative damage

(Ozben, 2007). RF-radiation might disturb reactive oxygen species (ROS) production by decreasing antioxidant enzymes activity or elevating ROS production. It has been reported that RF-radiation generates ROS by stimulating cell membrane nicotinamide adenine dinucleotide (NADH) oxidase and causes production of extracellular superoxide leading to oxidative stress and subsequent cellular damage (Consaes et al., 2012). The continuous produced ROS are scavenged by different antioxidant enzymes such as SOD, GPX, and catalase (Ozguner et al., 2005). Under some circumstances, the endogenous antioxidant defenses are likely to be perturbed due to overproduction of oxygen radicals, inactivation of detoxification systems, consumption of antioxidants, and failure to adequately replenish antioxidants in tissue (Kovacic and Somanathan, 2010). Moreover, the over ROS production can harm cells by depleting enzymatic and/or non-enzymatic antioxidants (Kong and Lin, 2010). The high metabolic rate and the composition rich in polyunsaturated fatty acids, the target for ROS, make the brain more sensitive to oxidative damage (Ozmen et al., 2007). RF-radiation is known to induce oxidative stress, which in turn activates the apoptotic pathway (Ozben, 2007). The hippocampus is the valuable part of the brain cerebrum that controls the behavioral and cognitive functions, including spatial learning and memory (Fortin et al., 2002). Bolla (2015) shown that mobile

* Corresponding author.

E-mail address: monagalal12@gmail.com (M.K. Galal).

phone radiation can cause damage to hippocampus leading to hyperactivity and difficulty learning. Based on subsequent data presented here the present study was designed to investigate the effect of chronic RF-radiation exposure (frequency 1800 MHz, specific absorption rate 0.6 W/kg) emitted from the mobile phone on oxidative stress and apoptosis through the biochemical, histological, immunohistochemical, electron microscopy in hippocampus and cerebellum of adult rats.

2. Materials and methods

2.1. Animals used

Forty apparently healthy adult male rats (130–150 g) of relatively equal age obtained from the International Institute of Oncology, Cairo University was used in the present study. The rats were kept in the same environmental condition (temperature, 24–26 °C; humidity, 55–60%, on a 12:12-h light/darkness, away from any external noise, fed standard food pellet and water ad libitum) in the Faculty of Veterinary Medicine, Cairo University. All rats were carried out in accordance with the guide to the Care and Use of Laboratory Animals published by the Material Institute of the Health and approved by the Animal Experimental Local Ethics Committee at Cairo University. The rats were divided randomly into two equal groups. The group I: Control group was placed in four cages (five rats per cage) in a separate room away from any mobile phones. Group II: Exposed group was placed also in four cages (five rats per cage). One mobile phone was placed in the bottom of each cage at the center under a wire mesh to give maximum exposure near the brain. At the end of the experiment, the animals sacrificed by cervical dislocation and the brain were carefully dissected and separated into hippocampus and cerebellum for subsequent analysis.

2.2. Exposure device

The Radiation for this study (1800 MHz) was provided by mobile phone with specific absorption rate (SAR) value of 0.6 W/kg (according to the user guide of the mobile phone, and with electric field 0.87 mV/cm² at 5 cm away from the mobile phone) estimated by Radiofrequency meter. During the experimental period, the rats were exposed to RF-radiation emitted by a mobile phone continuously for 120 min/day for three months.

2.3. Oxidative stress parameter measurements

Specimens from brain tissue (hippocampus and cerebellum separately) were weighted and homogenized with Teflon tissue Homogenizer. The samples were homogenized in cold phosphate buffered saline (pH 7.4) using Teflon Homogenizer. The homogenates were centrifuged at 14,000 × g for 15 min at 4 °C. The supernatant was used to measure the neuronal MDA (Ohkawa et al., 1979), glutathione peroxidase (GPX) activity (Rotruck et al., 1973), superoxide dismutase (SOD) activity (Marklund and Marklund, 1974), reduced glutathione (GSH) concentration (Ellman, 1959) and estimation of protein content (Bradford, 1976).

2.4. General histological and histochemical study

After sacrificing of the rats, the different parts of the brain (hippocampus and cerebellum) were immediately dissected out and sectioned into small pieces. These specimens were fixed in neutral buffered formalin. They were processed by dehydration in ascending graded of alcohol, xylene and embedded in paraffin. Serial and step-serial sections of 5–6 µm thick were obtained and stained with Haematoxylin and Eosin (H&E) and Mallory's

phosphotungstic acid-Haematoxylin. The later stain neurons-salmon and Myelin & glial nerve fiber-blue as adopted by (Bancroft and Gamble, 2008).

2.5. Immunohistochemistry for cyclooxygenase-2 (COX2) protein

Different Brain region sections were deparaffinized in xylene and rehydrated in graded alcohol. Drops of Hydrogen Peroxide Block (Thermo Scientific, USA) were added to block the endogenous peroxidase activity. The tissues were pretreated with 10 mM citrate buffer, pH 6.0 in the microwave oven at 500 W for 10 min for antigenic retrieval. The slides were washed with PBS, and blocked with ultra V Blocking solution (Thermo scientific, USA) for 5 min. Sections were incubated overnight at 4 °C in a humidified chamber with the following primary antibodies rabbit anti-COX2 polyclonal antiserum (Cayman Chemical, Ann Harbor, MI) at a 1:50 dilution. The sections were rinsed again with PBS, then incubated with a biotinylated goat anti-rabbit antibody (Thermo Scientific, USA) for 10 min. The sections were rinsed again with PBS. Finally, sections were incubated with Streptavidin peroxidase (Thermo scientific, USA). To visualize the reaction, slides were incubated for 10 min with 3, 3' diaminobenzidine tetrahydrochloride (DAB, Sigma). The slides were counterstained with hematoxylin then dehydrated and mounted. Primary antibodies were omitted and replaced by PBS for negative controls.

2.6. Transmission electron microscopy

Small tissue blocks from the different parts of the brain (hippocampus and cerebellum) were fixed in paraformaldehyde-glutaraldehyde in phosphate buffer (Karnovsky, 1965). Specimens were post-fixed in 1% osmium tetroxide for one hour, washed in 0.1 M phosphate buffer (pH 7.3), then dehydrated in graded ethanol and embedded in an open Araldite mixture (Mollenhauer, 1964). Semi-thin sections (1 µm) were cut, stained with Toluidine Blue (Richardson et al., 1960) and examined with the light microscope. Ultra-thin sections were cut and stained with uranyl acetate and lead citrate. The sections were examined with a JEOL 1010 transmission electron microscope at Regional Center for the Mycology and Biotechnology (RCMB) Al-Azhar University, Cairo, Egypt.

2.7. Genomic DNA fragmentation

Quantitative DNA fragmentation percentage assay and DNA laddering were determined calorimetrically by diphenylamine assay and agarose gel electrophoresis as previously described by Ogaly et al. (2015). Hippocampus and cerebellum were lysed separately in hypotonic lysis buffer pH 8.0. Lysates were centrifuged for 10 min. The supernatant containing small DNA fragments was separated from the pellet of intact DNA and divided into two portions one for electrophoretic analysis using agarose gel electrophoresis for DNA laddering. Another portion and pellet were precipitated with trichloroacetic acid and then were centrifuged. After centrifugation, the supernatant was resuspended in two volumes of diphenylamine solution. Samples were stored at 4 °C for 48 h and measured spectrophotometry at 578 nm.

2.8. Statistical analysis

The different analytical determinations in the biological samples were carried out in duplicate and results are expressed as the mean ± SE. Student's *t*-tests were performed to determine whether differences between the two groups were statistically significant (*p* < 0.05) using SPSS version 16 packages for Windows.

3. Results

3.1. Oxidative stress parameters

The concentration of GSH, MDA, and activity of SOD and GPX in different brain regions homogenate of experimental rats were recorded in Figs. 1 and 2 (hippocampus, cerebellum respectively) in comparison to the control group. It was obvious that MDA, the indicated marker for LPO, showed a significant elevation in the exposed group for both parts compared to control. Under normal condition, the over ROS production was neutralized by the antioxidant defense mechanisms. GSH is an important non-enzymatic antioxidant that plays a crucial role in the detoxification of ROS. SOD and GPX enzymes are the first line of cellular defense against oxidative injury. In the current study, the exposed of adult rats to radiation emitted from mobile phone led to a significant reduction in GSH concentration by 33.45%, SOD by 63.44% and GPX by 45%, GSH concentration by 34.3%, SOD by 45.9% and GPX by 64.6% from activities compared to the control in hippocampus and cerebellum respectively.

3.2. General histological, histochemical and electron microscopy study

The brain tissue of the control rat showed general normal appearance and normal arrangement of nerve cells. The histological structure of the hippocampus region showed that it is formed of three layers; molecular layer, pyramidal cell layer and polymorphic layer (Fig. 3A). The molecular layer contained few small astrocytes, other neuroglia cells, and blood vessels. While the pyramidal cell layer formed mainly of dense columns of pyramidal cell. These cells appeared rounded with large spherical shaped nuclei. Their nuclei were vesicular with prominent nucleoli and thin cytoplasm. Dark cells wedged between the pyramidal cell layer (Fig. 4A) and also in the region under the pyramidal cell layer. The polymorphic layer contained various types of cells including pyramidal cells, astrocytes, other neuroglia cells, and blood vessels. The pyramidal cells were the main one and it consisted of regularly arranged pyramidal cells, each had a triangular cell body and large rounded, vesicular nuclei with prominent nucleoli. At the ultrastructural level the pyramidal cells showed large euchromatic nuclei and clear nucleoli with well-developed nuclear envelop. The cytoplasm contained numerous rough endoplasmic reticulum and regular arranged mitochondria (Fig. 5A). Dark cells detected between the

pyramidal cells (Fig. 6A). The axons appeared normal with healthy myelin sheath (Figs. 7A and 8A). There were some pathological changes in the brain of rats, which exposed to mobile phone radiation when compared to the control ones. Pyramidal cell layer appeared degenerated and decreased in their diameter. Some of these cells showed degeneration in the form of deeply stained nuclei in some cells, partial decrease of the nuclear basophilia with intra nuclear vacuolation in other cells. The dark cells which wedged between the pyramidal cell layer in control group degenerated and decreased in number (Fig. 4B). The pyramidal cells in the polymorphic layer lost their triangular shape and surrounded with pericellular haloes. Some of them had intra nuclear vacuolation (Fig. 5B). At the level of electron microscopic examination, some of these cells appeared shrunken with pyknotic nuclei. The nuclei became ovoid in shape. The mitochondria were swollen with fewer cristae having a less regular arrangement (Fig. 6B). The dark cells were decreased in between the pyramidal cells. Additionally congestion of the cerebral blood vessels, increased perivascular space and presence of cellular mononuclear were observed (Fig. 5C). The axons appeared with less or absence of myelin sheath (Fig. 7B). The gray matter of the cerebellum of control group convoluted with many distinctive folds consisted of three layers; outer molecular layer, middle Purkinje and inner granular layer (Fig. 8A). Outer molecular layer constituted of scattered small neurons, nerve fibers and blood vessels. Middle Purkinje was formed by a single row of large fusiform shaped Purkinje cells (Fig. 9A). These Purkinje cells revealed around lightly stained nuclei and deeply stained cytoplasm. The inner layer was closely packed with small neurons (Fig. 10A). Few astrocytes scattered among the three layers. Purkinje cells of control group whose multiple branching dendrites ramify throughout the molecular layer and axons pass through the granular to join tracts in the molecular layer (Fig. 11A). Normal myelin sheath observed around the axons by electron microscopy (Fig. 12A). While irregularity of the cerebellar cortex observed in the exposed group (Fig. 8B), disorganized in Purkinje cell population (Fig. 9B). Cerebellar Purkinje cells appeared shrunken deeply basophilic with serrated edges and deformity, irregularity, pyknotic or lysed nuclei, additionally, increase number of astrocytes, gliosis, was observed (Fig. 10B). The processes of Purkinje cells decreased in number and increase perineural spaces (Fig. 11B), another population of the Purkinje cells revealed vacuolization in the cytoplasm and swollen of the nucleus (Fig. 11C). Clear apoptosis

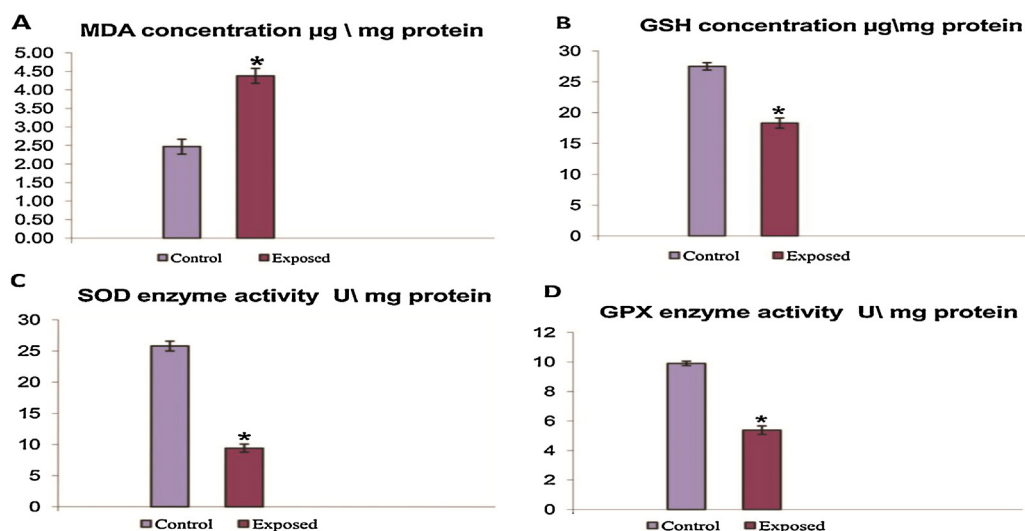


Fig. 1. A–D Effect of mobile phone exposure on the oxidative stress parameters in rat's hippocampus. Values are expressed as mean $\pm$ S.E. *Asterisk means significant difference.

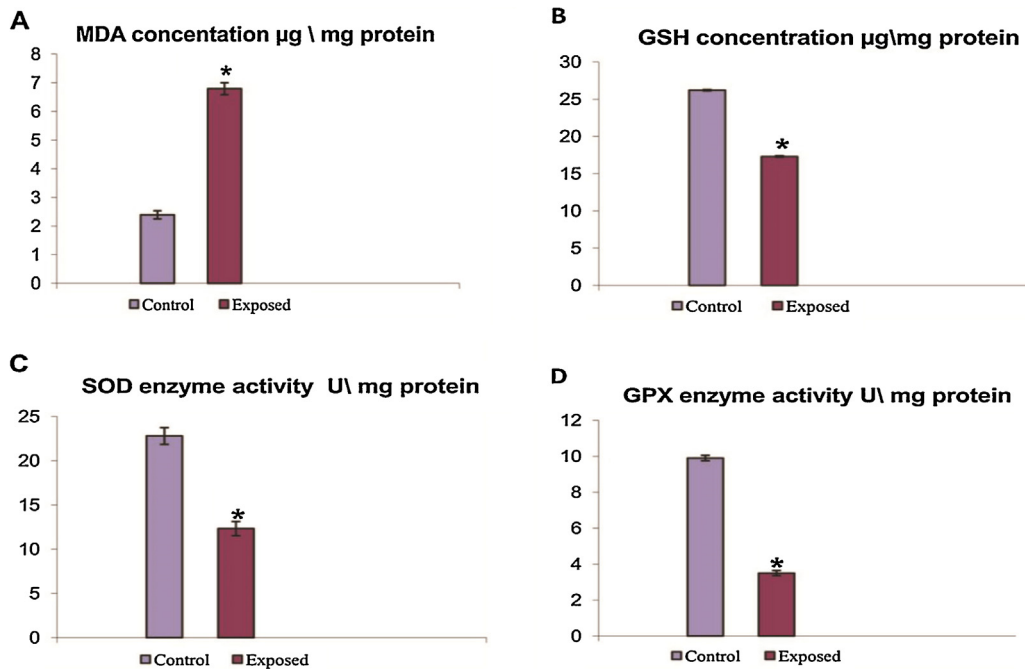


Fig. 2. A–D Effect of mobile phone exposure on the oxidative stress parameters in rat cerebellum. Values are expressed as mean ± S.E. *Asterisk means significant difference.

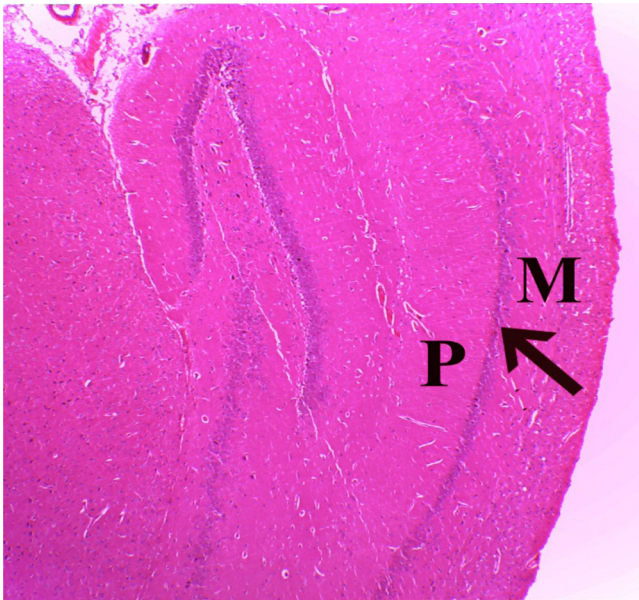


Fig. 3. The brain tissue of the control rat showing general normal histological structure of the hippocampus region; three layers; molecular layer (M), pyramidal cell layer (arrow) and polymorphic layer (P). H&E X40.

and degenerative changes in the form of central chromatolysis, neuronal swelling and lysing of cell membrane detected in many neurons (Fig. 11D). At the electron microscopy level leakage and decrease of the myelin sheath around the axons, irregular shrunken heterochromatic nucleus and increase perinuclear space of Purkinje cell of experimental group in comparison to control group observed (Fig. 12B).

3.3. Immunohistochemistry of COX2 protein

COX2 immunoreactivity was characteristically cytoplasmic apoptotic factor. The cytoplasm stained dark brown color

considered a positive response. COX2 was significantly elevated in the neurons of exposed group than in the control group in hippocampus region and cerebellum respectively (Fig. 13A–D).

3.4. Genomic DNA fragmentation

Effect of RF-radiation emitted from mobile phone on genomic DNA damage was evaluated by measuring the level of genomic DNA fragmentation percentage using the diphenylamine assay and by detecting DNA ladders on agarose gel electrophoresis (Fig. 14). Compared to the control group, exposure to mobile phone radiation induced significant elevation in the DNA fragmentation percentage in both hippocampus and cerebellum. As well as marked DNA laddering was shown on agarose gel electrophoresis in both regions in the exposed group compared to control one.

4. Discussion

Exposure to environmental contaminants such as mobile radiation involves many complex processes which can be evaluated by antioxidant enzymes activity as well as LPO measurement (Koc et al., 2013). Evaluation of oxidative stress in the brain involved measurement of MDA content, a product of LPO, and measurement of antioxidant defense systems, including GSH, SOD, and GPX activities were reported in the present study. The obtained data represented in (Figs. 1 and 2) revealed that the exposure of rats to mobile phone radiation could induce a significant elevation in MDA level as well as a significant reduction in GSH content and significant inhibition in the activities of SOD and GPX in hippocampus and cerebellum. Exposure to RF-radiation emitted from mobile phone mostly associated with overproduction of ROS and altering antioxidant activities (Shehu et al., 2016; Sahin et al., 2016; Eser et al., 2013). These results suggested that chronic exposure to the mobile phone radiation could induce oxidative damage to the brain tissue of rats. In our study, we detected similar response to mobile phone radiation in hippocampus and cerebellum with a different percentage. The exposure to RF-radiation can lead to increase in the activity of NADH oxidase enzyme, which increases ROS production (Friedman et al., 2007).

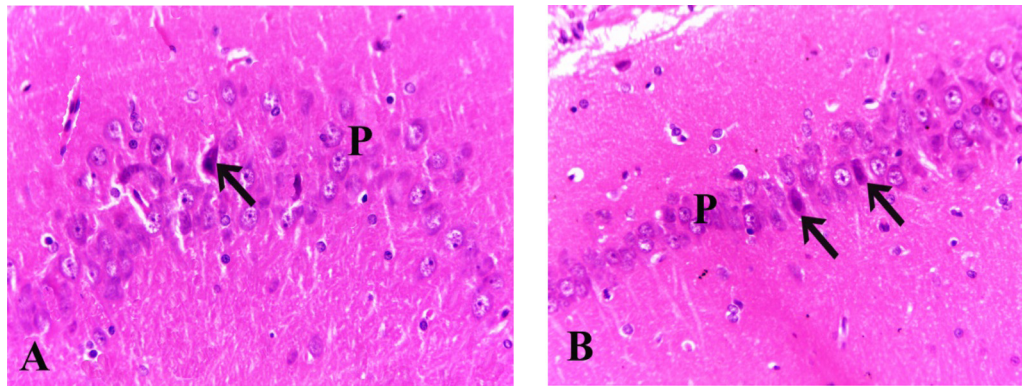


Fig. 4. A: The pyramidal cell layer of control group was formed of dense columns of pyramidal cells (P) and normal dark cells (arrow). B: degeneration and decreasing in diameter of pyramidal cells layer (P) of exposed group. Degeneration in the dark cells (arrow). **H&E X400.**

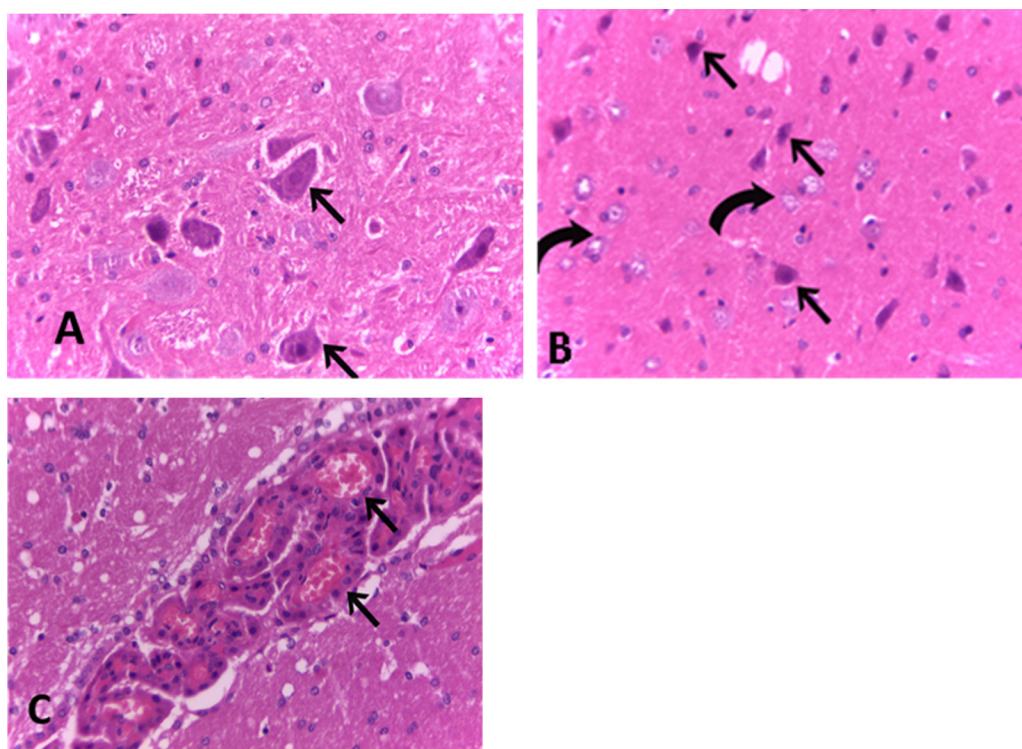


Fig. 5. Photomicrograph of a longitudinal section of the hippocampus region showing A. Pyramidal cells of control group (arrow). B. Pyramidal cells in the polymorphic layer lost their triangular shape (arrow) and some of them had intranuclear vacuolation (curved arrow) of exposed group. C. Congestion of the cerebral blood vessels, increased perivascular space and presence of cellular mononuclear (arrow). **H&E X400.**

In the same consequence, the over ROS production decreased the serum levels of melatonin, which is an efficient free radical scavenger and also potent stimulant for the activity or gene expression of several important endogenous antioxidant enzymes, this could be the main cause of decreasing its activities and suppression of the total antioxidant capacity in chronic exposure to RF radiation (Kesari et al., 2013). The change in enzymes activity and depletion of GSH may be regarded as an indicator of increased ROS production occurring during the exposure period and may reflect the pathophysiological process of the exposure (Dogan et al., 2012).

The present study revealed some pathological changes as results of mobile phone exposure representing in degenerative changes in many neurons and some pyramidal cells which appeared deeply stained nuclei with intranuclear vacuolation and some of the pyramidal cells changed to the irregular shape

with ovoid nuclei. Those changes were in the line with Usikalu et al. (2012), Afeefy et al. (2013) and Faridi and Khan (2013). As well as congestion and signs of hemorrhage with an enlarged perivascular space detected in our study was in agreement with Faridi and Khan (2013). In addition to the reduction of dark cells in the exposed group in the present study was previously reported by Afeefy et al. (2013). The RF- radiation can affect the brain cellular structure through many ways, mainly through ROS overproduction which causes oxidative stress at the cellular level interfering with protein synthesis (Faridi and Khan, 2013), in addition, degeneration of the neurons, causing changes in the permeability of the blood brain barrier leading to leakage of albumin (Stam, 2010). Albumin acts as a shield, protecting the brain against many harmful substances, and its disruption might account for the damage of pyramidal neurons (Nittby et al., 2009). In the same consequence, the reduction of dark cell might be due to their

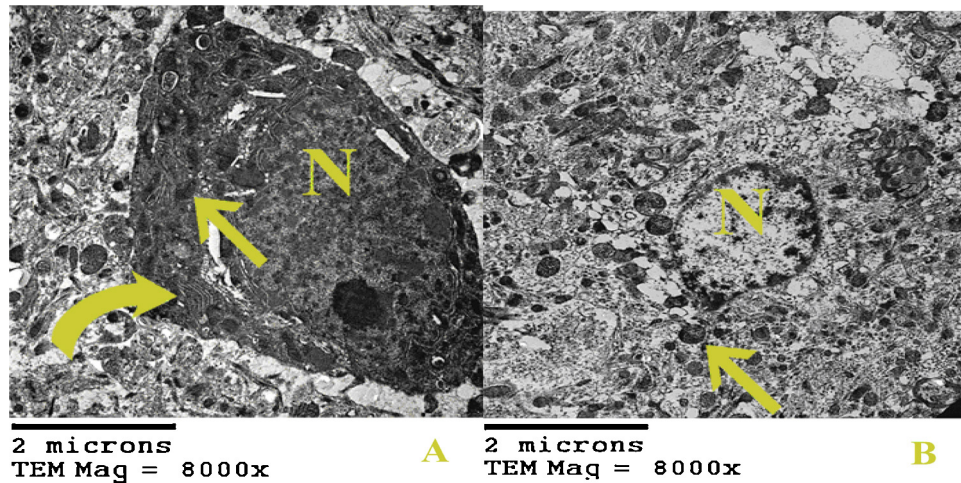


Fig. 6. Electron micrograph of the hippocampus region showing A. Pyramidal cells of the control group had a large achromatic nucleus (N) and clear nucleoli, well-developed nuclear envelope, numerous rough endoplasmic reticulum (curved arrow) and regular arranged mitochondria (arrow). B. Pyramidal cells of exposed group appeared shrunken with Pyknotic ovoid nuclei (N) and swollen mitochondria (arrow) with fewer Cristae. **Uranyl acetate and lead citrateX8000.**

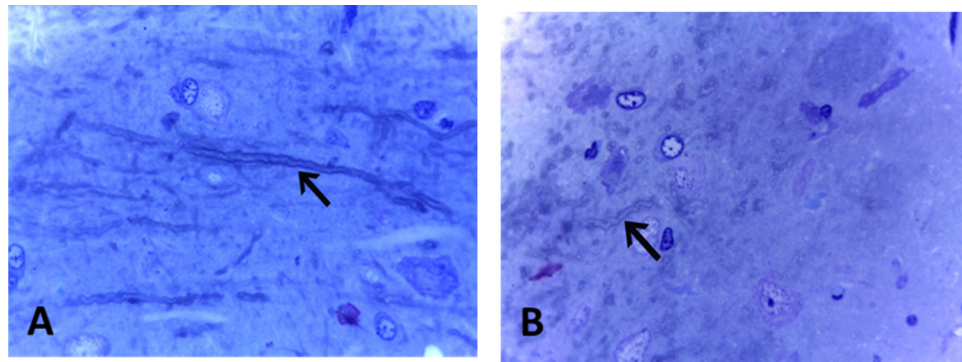


Fig. 7. Photomicrograph of a Semi thin section of the hippocampus region showing A. Axons appeared normal with healthy myelin sheath in the control group. B. The axons appeared with less or absence of myelin sheath thin exposed group (arrow).**Toluidine Blue X1000.**

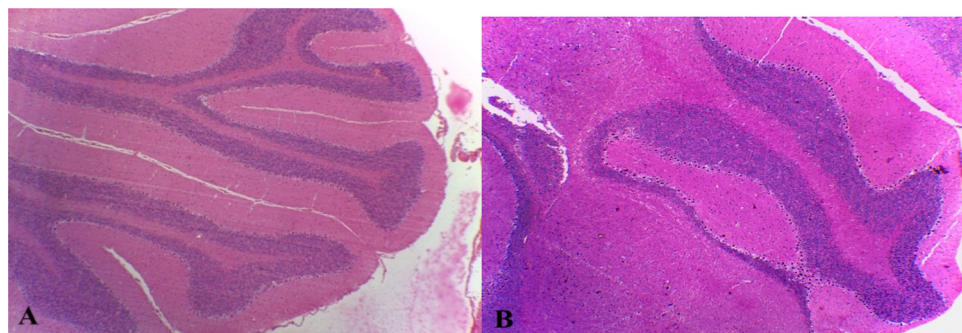


Fig. 8. Photomicrograph of a longitudinal section of the cerebellum showing A. Normal histological picture in control group; B. Irregularity in the cerebellar cortex of the exposed group. **H&E X40.**

differentiation into granule cells, in order to compensate their degeneration (Afeefy et al., 2013). In this investigation, the increase in astrocytes was noticed in agreement with Azmy and Abd Allah (2013). Reactive gliosis is a phenomenon describing endogenous CNS tissue responses to injury that referred to the accumulation of enlarged glial cells, notably microglia and astrocytes (Kim et al., 2008). The ultra structure showed through the electron microscopy, some of neurons appeared shrunken with Pyknotic nuclei, also the mitochondria appeared swollen with fewer Cristae having less

regular arrangement this leading to decrease energy which needed to the cellular function (Hao et al., 2015; Faridi and Kahn, 2013). Those results concluded that the radiation decreased the activity of the mitochondrial electron transport system, leading to energy metabolism disorders which play an important role during the process of RF-radiation-induced brain damage (Hao et al., 2015). In this study, cerebellar Purkinje cells of exposed rats appeared shrunken, deeply basophilic with serrated edges and deformity, irregularity, Pyknotic or lysed nuclei also another population of

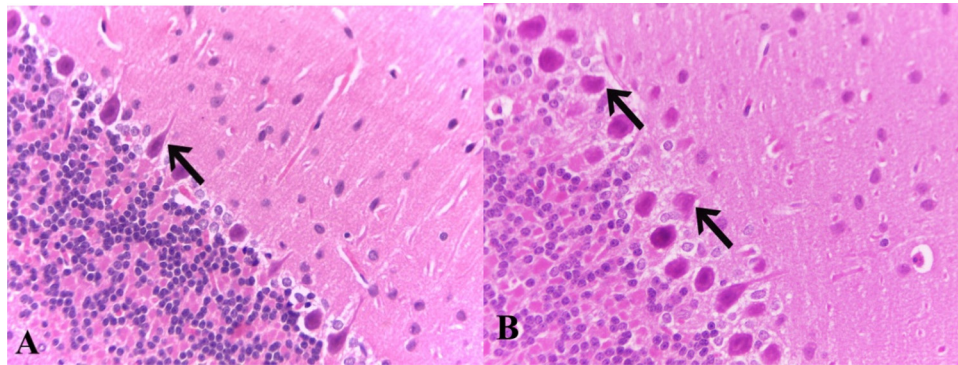


Fig. 9. Photomicrograph of a longitudinal section of the cerebellum showing A. Middle Purkinje was formed of single row of Purkinje cells (arrow) in control group. B. Disorganization of Purkinje cells (arrow) in exposed group. **H&E X400.**

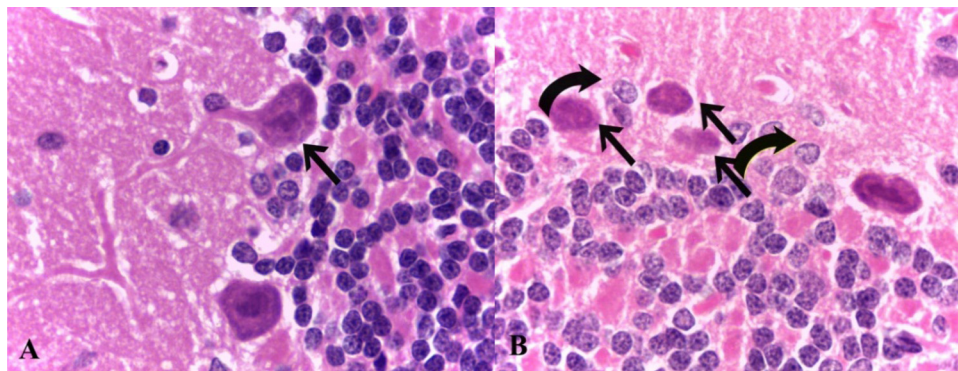


Fig. 10. Photomicrograph of a longitudinal section of the cerebellum showing A. Purkinje cells (arrow) in the control group revealed round lightly stained nuclei and deeply stained cytoplasm. B. Purkinje cells (arrow) in the exposed group appeared shrunken deeply basophilic with serrated edges and deformity, irregularity, Pyknotic or lysed nuclei. Noticed an increase in flow cells (curved arrow) **H&E X1000.**

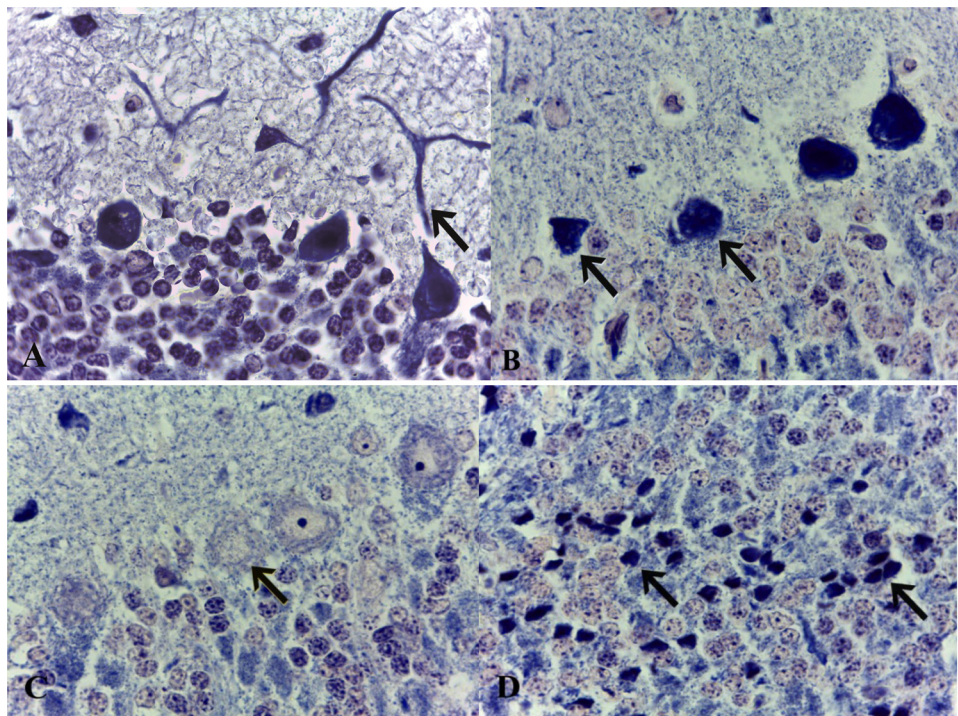


Fig. 11. Photomicrograph of a longitudinal section of the cerebellum showing A. Purkinje cells in the control group with multiple branching processes (arrow). B. Decreased number of processes and increase perineurial spaces (arrow) in Purkinje cells in exposed group. C. Vacuolization in the cytoplasm and swollen of the nucleus of Purkinje cells (arrow) in exposed group. D. Apoptosis and degenerative changes in many neurons (arrow) in exposed group. **Mallory's phosphotungstic acid-haematoxylin stains X1000.**

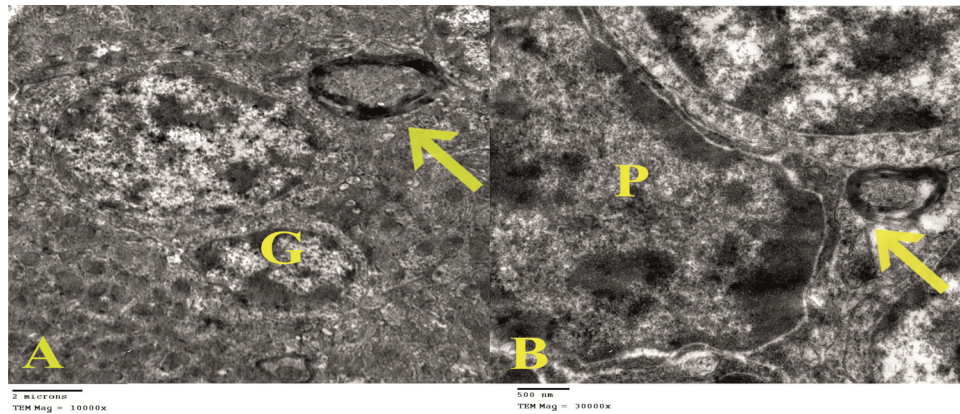


Fig. 12. Electron micrograph of the cerebellum showing A. The normal myelin sheath around axons (arrow) and granular cell (G) in the control group. **X10000.** B: Leakage of myelin sheath (arrow) with irregular shrunken heterochromatic nucleus and increase perinuclear space of Purkinje cell (P) in exposed group. **Uranyl acetate and lead citrate X30000.**

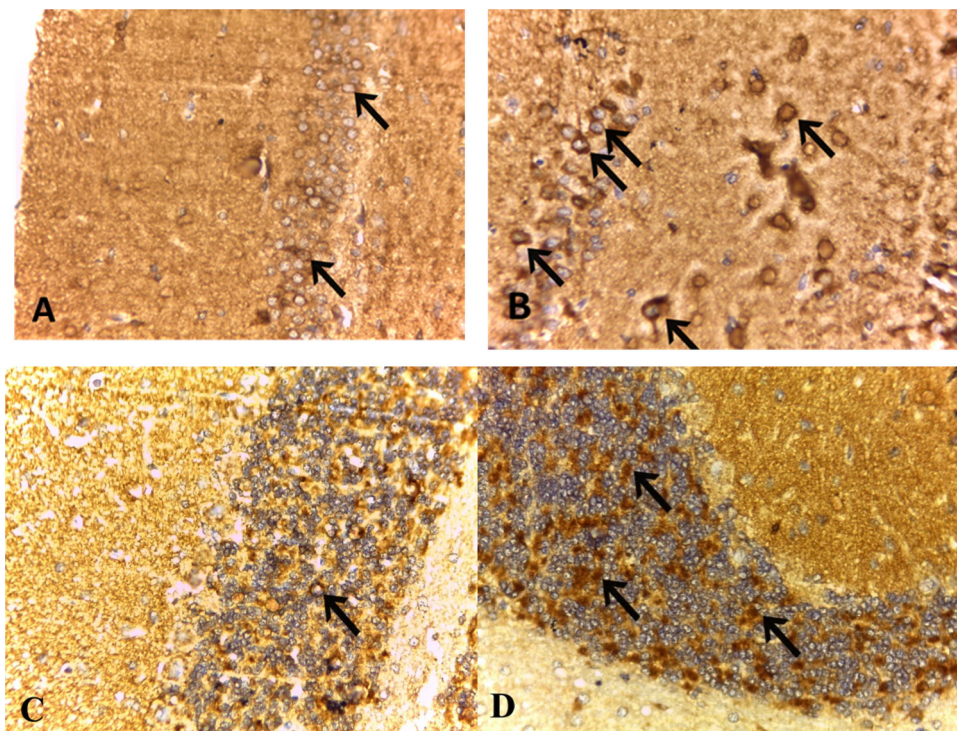


Fig. 13. Photomicrograph for COX2 immunohistochemistry X400: A. Low or little COX2 immunoreactivity (arrow) in cytoplasm of most neurons of hippocampus region in control group. B. Intense positive response in the neurons of hippocampus region in exposed group. C. Low or little COX2 immunoreactivity in cytoplasm of most neurons of the cerebellum in control group. D. Intense positive response in the neurons of the cerebellum in exposed group.

cells revealed vacuolization of cytoplasm in the Purkinje cells and disorganization was occurred rather than arranged in one layer in the control group as recorded by [Khalil et al. \(2012\)](#) and [Azmy and Abd Allah \(2013\)](#). This phenomenon was explained by [Saad El-Dien et al. \(2010\)](#) as prolonged exposure to neuronal insult could lead to an adaptive response in the form of crowding of Purkinje cells, That's in a trial to re-establish the synaptic contact with other neurons in order to perform their function.

Chronic exposure to RF-radiation increases cells apoptosis and induces functional disorders in many cell types, which even could be cause for the development of cancer ([Atasoy et al., 2009](#)). During the process of RF-radiation induced brain damage, apoptosis is one of the final outcomes of damaged cells. Apoptosis plays a

fundamental role in normal tissue homeostasis of the multi-cellular organism. In the current study, a clear apoptosis and degenerative changes detected in many neurons were clarified on a genetic level through assessed of protein expression of an apoptotic COX2 gene and genomic DNA fragmentation. In the CNS, the apoptotic gene (COX2) is expressed under normal conditions ([Minghetti, 2004](#)). COX2 is a key regulatory enzyme in the biosynthesis of prostaglandins. Overexpression of COX2 is aroused to be both a marker and an effector of neural damage ([Strauss, 2008](#)). According to the current study, overexpression of COX2 protein was detected in the exposed group. In addition, a significant elevation in DNA fragmentation percentage was also detected with over fragmentation in hippocampus region.

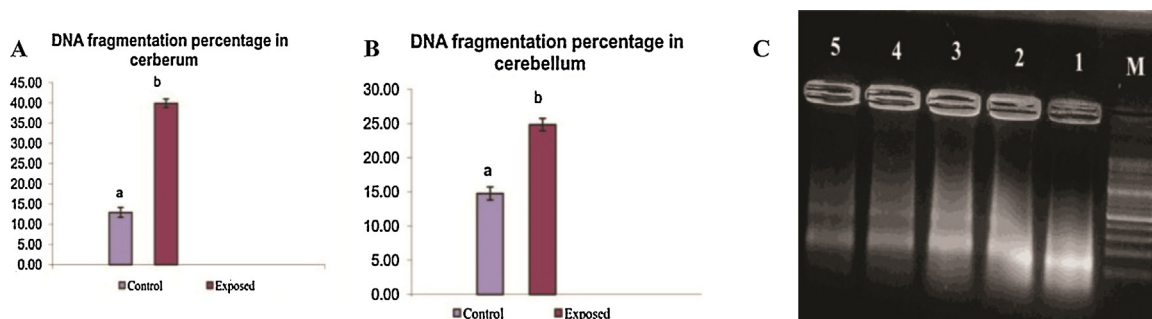


Fig. 14. Effects of continuous exposure of mobile phone radiation on the percentage of genomic DNA fragmentation (A) DNA fragmentation percentage in hippocampus, (B) DNA fragmentation percentage in cerebellum. Values are expressed as mean $\pm$ S.E. Different superscript letters are significantly different. (C) Representative results of DNA laddering assay brain tissue induced by mobile phone exposure, lane (5) control for hippocampus, (4) control for cerebellum, (3) exposed group cerebellum, (1, 2) exposed group hippocampus and (M) 100 bp DNA ladder.

RF-radiation induced the neural cell apoptosis via the classical mitochondria-dependent caspase-3 pathway. RF-radiation causes the loss of mitochondrial membrane potential and down-regulation of Bcl-2 and up-regulation of Bax, triggering cytochrome c release from mitochondria to the cytosol, subsequent caspase-3 activation and finally leading to induction of neuronal apoptosis (Zuo et al., 2014). Another mechanism, included the RF-radiation, acting especially on calcium ions, it induced variation in its ionic homeostasis. Perturbation of calcium ions through its uptake by mitochondria initiates (release from the endoplasmic reticulum). This change in calcium homeostasis results in the release of cytochrome c from mitochondria, activation of caspase 9 and consequently, of the effectors caspases 3, 6 and 7 and finally cell death through apoptosis (Kesari et al., 2013). Zuo et al. (2014) reported that exposure of rats to RF-radiation resulted in chromatin condensation, apoptotic body formation in neural cells, a significant elevation in DNA fragmentation, and alteration in the expression of several apoptotic genes. In addition, Kesari et al. (2014) showed that, exposure of rats to mobile phone for two months significantly induced DNA strand breaks in the brain, and significant increases in micronuclei and apoptosis. Excessive levels of ROS produced during RF-radiation exposure were closely related to neural cell apoptosis, as previously described by Kesari et al. (2014) and Kesari et al. (2011).

Our study can be distinguished from the studies of the literature since we evaluated oxidative damage induced by RF-radiation in different brain regions (hippocampus and cerebellum) rather than in the whole brain as different brain regions may respond differently to oxidative stress (Sandhir et al., 1994).

5. Conclusion

RF-radiation emitted by mobile phones significantly lead to oxidative stress and apoptosis to brain tissue. In this context, mobile phones should keep in the calling mode for short periods and should usually be kept away from the body. Finally, more advanced technologies with fewer biological effects should be developed.

Competing interests

"The authors declare that they have no competing interests to disclose."

Authors' contributions

Dr. Shaymaa Hussein and Abdel-Aleem El-Saba participated in the study design and histological analysis. Dr. Mona Khames

evaluated the biochemical parameters and drafted the manuscript. All authors read and approved the final manuscript.

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