

Mobile Phone Radiation Effects on Brain with Reference to the Protective Role of *Moringa oleifera*: Sonographic and Histological Study

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Abstract

Background: Concerns regarding potential negative impacts on human health are growing as cell phone use dramatically expands worldwide. Due to the anatomical position of the brain, cell phones emit nonionizing radiofrequency waves that may have an impact on it. **Objectives:** The study aims to examine the effect of mobile phone radiation in male rats on the histological structure, and sonographic imaging of the brain of rats after mobile phone radiation exposure and investigate the ameliorating role of moringa. **Materials and Methods:** In the study, 44 male Albino rats were used. The rats were randomly divided into six groups and exposed to cell phone radiation (in the data or calling modes) for 10 weeks. The 200 mg/kg of body weight of moringa extract was administered to evaluate the protective role of the rats for 10 weeks. **Results:** A sonographic and histological analysis of the rat brain showed that rats exposed to mobile phone radiation suffered serious damage to their neurons in several parts of the brain. Meanwhile, the rats given moringa extract suffered less harm. **Conclusion:** Rats given moringa extract had less damage to neuronal architecture in their brains.

Keywords: Brain, cell phone, moringa, sonographic

INTRODUCTION

Today, it is impossible to imagine living without a mobile phone because they have transformed modern society. In the past 20 years, the number of people using mobile phones has grown significantly throughout the world.^[1]

The human body absorbs damaging radiation from mobile phones, and the rate of absorption varies depending on the part of the body. For instance, studies have shown that the head and hand, which were in contact while using the phone, absorbed roughly 40% of the radiation released from mobile phones.^[2] Therefore, for mobile phone users, holding their smartphones to their heads when making voice calls causes the greatest amount of exposure to the brain.^[3]

Radiofrequency-electromagnetic frequencies (RF-EMFs) from mobile phones, in particular, are absorbed into the brain to the point where they can

influence neuronal activity.^[4] According to National Institutes of Health Research, RF-EMFs from mobile phones stimulate the brain's metabolic functions.^[5] The brains of those who were exposed to RF-EMF experienced a sharp increase in glucose metabolism. This demonstrated that the brain is sensitive to the effects of RF-EMF exposure.^[6] RF-EMF exposure has been linked to an increase in neuropsychiatric and behavioral disorders.^[7]

The established thermal effects of microwave radiation are heat generation by the rotation of polar molecules

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induced by RF-EMF. Some degree of temperature elevation can be lowered partially by blood circulation in the brain.^[8]

The brain's neurons are the information-processing cells.^[9] Neurons occur in a wide variety of types, each with a unique size, shape, and function.^[10] To create the information-processing networks that underlie all of our ideas, feelings, and actions, neurons link with one another. The adult brain is thought to have more than 60 trillion neuronal connections since each neuron can interact with more than 1000 additional neurons.^[11] The neocortex, a layer of cells on the surface of the brain, and the subcortical nuclei, which transmit and receive information from the neocortex, are part of the largest and most significant brain information-processing networks.^[12] The subcortical nuclei are collections of neurons that act as relay centers for signals traveling between various regions of the cortex and the rest of the body.^[13]

The plant *Moringa oleifera* (MO) Lam (Family: Moringaceae), which is primarily farmed in tropical and subtropical regions.^[14] With more than ninety nutrients, forty-six antioxidants, and eighteen amino acids, including eight essential amino acids, moringa is one of the most nutrient-rich plants. Moringa is the cheapest source of minerals, proteins, enzymes, and vital vitamins.^[15] The leaves have been reported to have a radio-protective effect in addition to being a good source of carotene, riboflavin, nicotinic acid, folic acid, pyridoxine, protein, vitamins A, B, C, and E, amino acids, various phenolic compounds, sterols, alkaloids, glycosides, flavonoids, and minerals.^[16]

The aims of this study were to evaluate the brain damage produced by cell phone usage and the potential benefits of using moringa to prevent it.

Aims of the study

The objective of this study is to detect cell damage that occurs in the brain as a result of mobile phone use and find strategies to stop cell damage that can interfere with organ function and different normal processes, especially the brain.

MATERIALS AND METHODS

The experimental design

Sampling

This research used forty-two healthy albino male rats "1 month "old. They were obtained from the Faculty of Veterinary Animal House at Mosul University, Iraq. All rats were kept at a room temperature of $22 \pm 2^\circ\text{C}$ with 12h of light and darkness, unrestricted access to food and water and *ad libitum*. All procedures followed the guidelines of the Faculty of Dentistry's Institutional Animal Research Ethics Committee in the College of Dentistry, University of Mosul, Iraq code (UoM.Dent/A.84/22).

Mobile phone radiation exposure protocol

Animals were exposed to the electromagnetic radiation (EMR) that a widely available GSM (Global System for Mobile communication) cell phone with a 900 MHz to 1800 MHz frequency was emitting.^[17] Animals' cages were covered up with a cell phone to prevent damage, and aluminum foil was used to enclose the cages to concentrate waves and keep EMF inside the enclosures. In a different room, the groups that had not been exposed were kept apart.^[18,19]

Medication

Moringa leaves ethanolic extract was prepared according to Mubeen *et al.*^[20] and 200mg/kg body weight was administered orally by gavage once daily, for a total of 10 weeks.^[21]

Experimental design

The 42 rats will be divided into the following six groups at random:

- Animals in group I (negative control) received normal saline without any EMR management or ethanolic extract from moringa leaves.
- Animals in group II received moringa leaves ethanolic extract once daily by gavage needle orally, 200mg/kg body weight, and were not exposed to any EMR application for a total of 10 weeks.^[21]

Animals in group III (mobile data) were subjected to mobile data mode for 2h each day for a total of 10 weeks.

- Animals in group IV (calling) were subjected to calling mode for 2h each day for a total of 10 weeks.
- Group V (mobile data + moringa): Animals received 200mg/kg body weight of moringa leaves ethanolic extract should be taken orally by gavage needle once daily and exposed to 2h per day of mobile data exposure for a total of 10 weeks,
- Animals in group VI (calling + moringa) received 200mg/kg of body weight of the ethanolic extract from moringa leaves once daily by gavage needle for a total of 10 weeks while being subjected to calling mode for 2h each day.

Sonographic assessment of rat's brain

At the end of the experiments, the rats were euthanized with ketamine (35mg/kg) and xylazine (5mg/ kg) given I/M.^[22] After removal brain from the skull, the brain is washed with deionized water to remove blood and then placed in a container filled with deionized water to reduce the impurities that the effect the clarity of the images, the deionized water acts as a media for examination,^[23] the prop placed 2cm above the specimen.^[24]

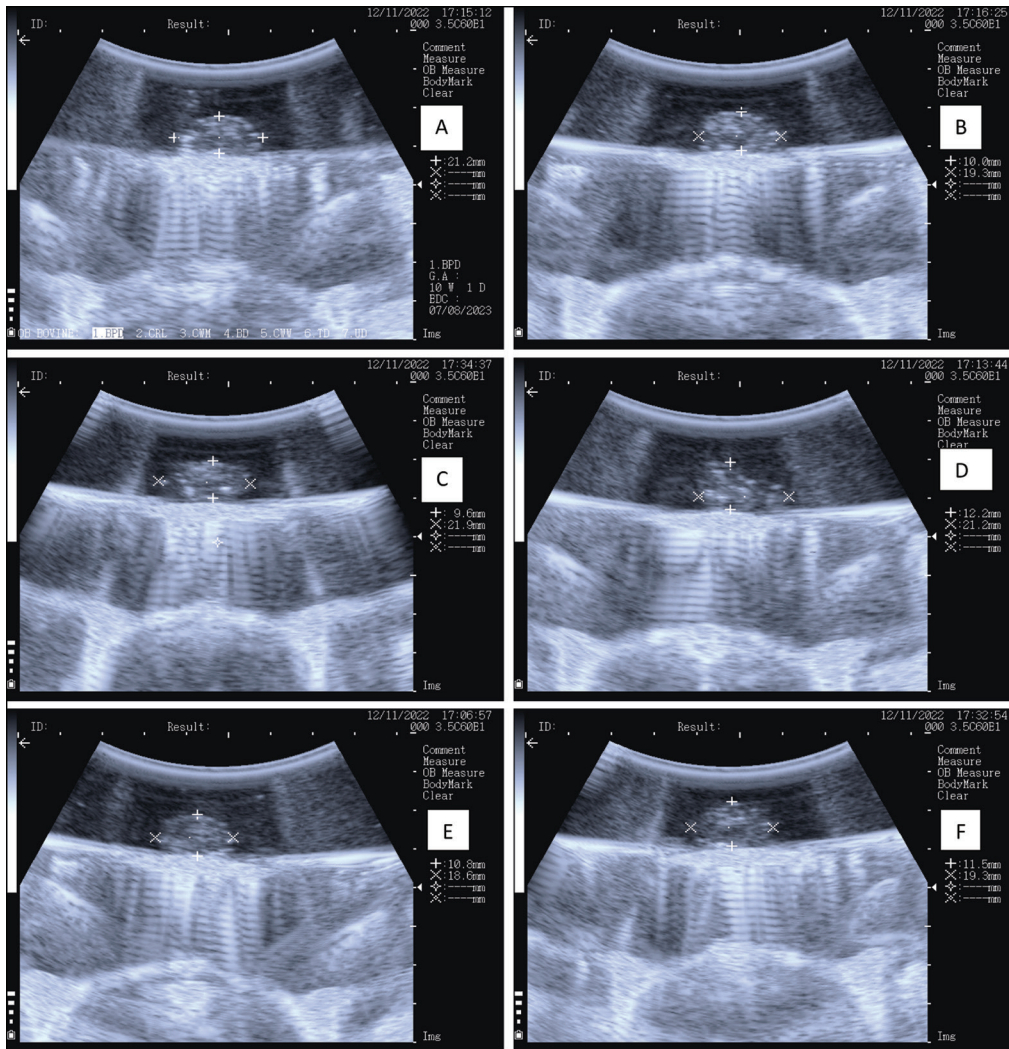


Figure 1: Brain sonogram (a) control group, (b) moringa group both showed homogenous texture with normal echogenicity. (c) mobile data mode group showed irregular outline, heterogeneous echogenicity multiple echogenic spots. (d) Calling mode group showed irregular outline, heterogeneous echogenicity, change the shape of the specimen (shrunk), multiple echogenic foci and echo free spots. (e) mobile data mode with moringa group showed homogenous texture and with normal echogenicity. (f) Calling mode with moringa group shows less heterogeneity and less irregular outline edges of the brain

Histological study

At the end of the experiments, the rats were euthanized brains were dissected out and fixed in 10% buffered formalin for 2 weeks. Specimens were washed and rinsed with tap water. Dehydration (by serial dilution of ethyl alcohol), clearing (by xylol), and paraffin embedding of specimens were performed. Sections of 5 μ m were taken for all blocks to be ready for H&E staining to be ready for blinded examination under a Bright field Olympus light microscope (Japan) light microscope. The photomicrographs of structural changes were obtained by a digital camera attached via plan apochromatic objectives.^[25]

RESULTS

Sonographic assessment

Ultrasound examination of the brain was taken since it is an excellent technique to determine if there is

any abnormality in the appearance, the result of rats related to control and moringa groups showed homogenous texture with normal echogenicity [Figure 1a and b].

On the other hand, the mobile data mode group showed irregular outlines, heterogeneous echogenicity multiple echogenic spots. Calling mode group showed irregular outline, heterogeneous echogenicity, change the shape of the specimen (shrunk), multiple echogenic foci and echo free spots [Figure 1c and d].

The results of mobile data mode with moringa group showed homogenous texture and with normal echogenicity. The results of calling mode with moringa group showed good improvement in the appearances to be normal in many areas in compared with rats in the control group, with less heterogeneity and less irregular outline edges of the brain [Figure 1e and f].

Histological assessment

The current study found that the normal architecture of the neurons, glial cells, and blood vessels was visible upon light microscopical examination of a section taken from the brains of control rats and moringa group at the end of week 10 of the experiment [Figure 2a and b].

However, a number of differences between the sections of control and mobile data-exposed rats (2h per day for 10 weeks) were found. The loss of the normal architecture exhibiting gliosis, vacuolization, congested blood vessels, vasogenic edema, and cytogenetic edema were some of these abnormalities [Figure 2c].

Additionally, a histological portion of a rat brain from the group that was exposed to the calling mode for two hours per day for ten weeks demonstrates severe vacuolization, liquefactive necrosis, vasogenic edema, and cytotoxic edema [Figure 2d].

In contrast, a histological portion of a rat's brain subjected to mobile data mode for 10 weeks (2h per day) while also receiving moringa leaf extract showed mildly congested blood vessels, minor vasogenic edema, and slight cytotoxic edema [Figure 2e].

Meanwhile, ethanolic moringa leaves extract group and calling mode-exposed rats (2h per day for 10 weeks) showed modest vacuolization, mild vasogenic edema, and mild cytotoxic edema in histological sections of the brain [Figure 2f].

DISCUSSION

Electromagnetic energy exposure can alter the electric current in the body, the histological abnormalities that have been observed may be caused by mobile phones disrupting the chemical composition of tissue. Organ functions are affected on both sides of the plasma

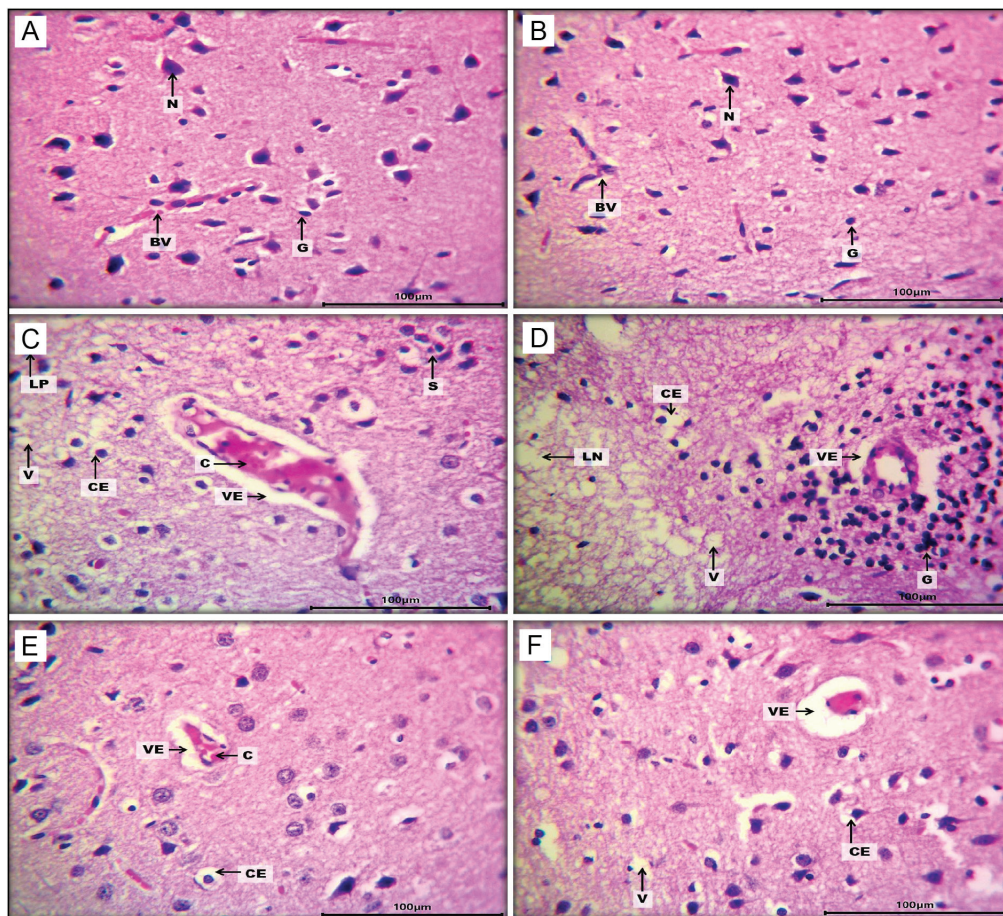


Figure 2: Histological section of rat brain of the (a) control group, (b) moringa group showing normal architecture of the neurons (N), glial cells (G), and blood vessels (BV). (c) mobile data mode group showing gliosis (G), vacuolization (V), congested blood vessels (C), vasogenic edema (VE), and cytotoxic edema (CE), (d) calling mode group showing severe gliosis (G), vacuolization (V), liquefactive necrosis (LN), vasogenic edema (VE), and cytotoxic edema (CE), (e) mobile data mode with moringa group showing mild congested blood vessels (C), mild vasogenic edema (VE), and mild cytotoxic edema (CE), (f) ethanolic Moringa extract and calling mode with moringa group showing mild vacuolization (V), mild vasogenic edema (VE), and mild cytotoxic edema (CE). (h and e) stain, 400×

membrane as a result of this exposure, and all free ions are forced to flow through it by the electric fields.^[26]

In this study, the neurons, glial cells, and blood vessels in control group and moringa group animals under light microscopy all displayed normal architecture. However, many changes were found in the rat groups exposed to mobile data mode and calling mode for 2h each day for 10 weeks. These alterations included the loss of the normal architecture, which was accompanied by gliosis, vacuolization, vasogenic edema, cytogenetic edema, congested blood vessels (in the case of mobile data), and liquid necrosis (in the case of calling mode).

In addition to biochemical changes to the membrane, these ion movements degrade the ion channels on the membrane, impairing all cellular activities. Numerous microscopical changes were discovered in the current research. These tissue damages may be caused by an increase in reactive oxygen species (ROS), excessive free radical generation, and insufficient endogenous defensive systems. Large biological molecules like lipids, proteins, and nucleic acid are all destroyed by free radicals.^[26]

In this study, gliosis is observed in (data mode and calling mode) this may be due to MP exposure of rats showed increased brain glial fibrillary acidic protein (GFAP) levels (GFAP is well known to have a role in homeostasis and brain injury control, jointly with the increased GFAP expression, reactive astrocytes are in parallel with cell hypertrophy and hyperplasia).^[27]

In this study, brain vacuolation is a frequent histopathologic finding that may be caused by a variety of spongiform encephalopathies, excitotoxins, and prion diseases-related possible pathways. The etiology of the vacuolation is yet unclear.^[28]

According to available data, oxidative stress may affect biological processes that result in apoptosis, necrosis, and autophagy. Additionally, it has been discovered that oxidative stress significantly alters biological processes that influence cellular integrity, leading to harmful variation in cellular proteins, lipids, and other macromolecules. Oxidative stress may cause DNA damage as a result of these fluctuations in the levels of protein and lipids.^[29] This study's vascular congestion may be caused by the EMF's ability to produce free radicals, which was also observed by Hanafy.^[30]

Even though the trial only lasted for a limited period, the negative impacts were still evident. The histopathological findings of our study make it clear that long-term use of cell phones might cause chronic inflammation in the brain, which can result in cerebral edema (cytotoxic or vasogenic). According to research by Sangi *et al.* (2020), there is a large inflammatory process in the various brain regions.^[31]

Vasogenic edema may develop as a result of BBB breakdown and extravasation of serum proteins like albumin.^[32]

Cells are lysed and necrotic tissue is transformed into a fluid phase in liquefactive necrosis that manifests in the calling mode group, which may be the result of hypoxia/ischemia brought on by a reduction in cerebral metabolism and BBF.^[33] Additionally, research has shown that radiation from mobile phones activate metabolic processes in the human brain.^[8]

It is unclear why liquefactive necrosis develops after ischemia injury to the brain, although it may be brought on by ROS-driven BBB opening, ROS-associated brain hypoxia brought on by an induced EMF drop in BBF, and/or EMF-induced hemoglobin deoxygenation. According to the study by Belpomme *et al.*,^[33] the ensuing hypoxia may cause metabolic neuronal malfunction as well as neuronal cell death by either apoptosis or necrosis.

However, compared to other cells, neurons are more vulnerable to oxidative stress due to the brain's high metabolic activity, high oxygen consumption needs, and extensive reliance on oxidative phosphorylation for energy. These results might shed light on the severe pathogenic alterations seen in the neurons of RF radiation-exposed animals. In high-lipid tissues like the brain, free radicals can disrupt lipids and cause lipid peroxidation, which results in necrosis and cell death.^[34]

There has long been proof that electromagnetic frequencies change a number of calcium-related functions. This is significant because calcium controls a wide range of cellular processes. RF-EMFs trigger calcium efflux. The initial event that causes changed protein structure, the production of ROS, and ultimately the activation of the molecular pathways to cancer may be calcium activation of proteins.^[35]

On the other hand, ethanolic moringa leaves extract group showed mildly congested blood vessels, moderate vasogenic edema, and minor cytotoxic edema in histological a view of rats brain subjected to mobile data mode (2h per day for 10 weeks), this due to the abundance of natural antioxidants in *Moringa oleifera*'s leaves, particularly the polyphenols, which have been demonstrated to be strong direct antioxidants in the oxygen radical absorbance capacity assay, appear to be the source of the plant's overall antioxidant activity, this agrees with González-Burgos *et al.*^[36] Flavonoids may also help to slow neurodegeneration, this also suggested by Stohs and Hartman.^[37]

There is early evidence that *Moringa oleifera* leaf powder has neuroprotective benefits that are mediated by antioxidants. In neuroblastoma cells, moringa has demonstrated a protective effect against hydrogen peroxide by lowering levels of ROS overproduction and lipid

peroxidation, raising GSH levels and antioxidant enzyme activity, and preventing mitochondrial malfunction.^[38]

Another study confirmed that *M. oleifera* extract lowers rat brain hypoxia.^[39] The malfunction and damage to the brain brought on by cerebral ischemia can be reduced by moringa leaves. Based on measurements of different antioxidant enzymes and declines in brain lipid peroxidation, it was thought that the protective effects were caused by lessened oxidative stress.^[40] Leaf extracts showed evidence of being neuroprotective by reducing acetylcholine esterase activity and increasing cholinergic function.^[41]

Neurosonography offers distinct advantages such as its cost-effectiveness, diagnostic utility and convenience.^[42] In this study, ultrasonography is used to evaluate the effect of mobile phone radiation on rats' brains (mobile data mode and calling mode), (mobile data mode with moringa and calling mode with moringa) and (moringa without radiation) in comparing to negative control sonogram.

Control and moringa groups showed homogenous texture with normal echogenicity. mobile data mode group showed irregular outline, heterogenous echogenicity multiple echogenic spots. Calling mode group showed irregular outline, heterogeneous echogenicity, change the shape of the specimen (shrunken), multiple echogenic foci and echo free spots. mobile data mode with moringa group showed homogenous texture and with normal echogenicity. Calling mode with moringa group shows less heterogeneity and less irregular outline edges of the brain.

By definition, tissue echogenicity describes its ability to create echo during ultrasound examination.^[43] There is no accepted and standardized method of tissue echogenicity assessment. The physics of ultrasound examination could be taken into account. Ultrasound echo is created due to reflection and scattering phenomena. As reflection occurs only on boundaries, it is scattering that actually produces tissue echogenicity. Magnitude of scattering produced echogenicity is proportional to the number of objects that actually interact with ultrasound wave.^[44] Therefore, the ratio between the object size and the ultrasound wave length is the most important factor, so the change in cell properties or arrangement seems to be significant.^[45]

Parenchymal increased echogenicity has been described as a sign of cerebral edema and/or hypoxic-ischemic injury, also cerebral hemorrhage may produce increased echogenicity.^[46] Echo free spots indicate liquefactive necrosis.^[47]

Meanwhile, moringa showed a partial protective role due to polyphenols and flavonoid antioxidants activity which is supported by histological findings in the current study.

CONCLUSION

Our findings show that the brain tissue exposed to mobile RF has histological alterations such as gliosis, vacuolization,

congested blood vessels, vasogenic edema, and cytogenetic edema. for example, exposure to cell phone RF caused severe morphological and structural abnormalities in brain tissues, which moringa can help to reverse. Future research is required to comprehend how these modifications may affect clinical outcomes. sonographic alterations such as irregular outline, heterogenous echogenicity multiple echogenic spots, change the shape of the specimen (shrunken) and echo free spots.

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Conflict of interest

There is no conflict of interest.

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