Original Article

Morphometric Study of Mobile Phone Induced Injury to Cerebellum with Preventive Effects of Ginger in Albino Rats

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ABSTRACT

Objective: To observe the effects of radio frequency (RF) radiation generated by third generation (3G) mobile phones on the rat cerebellum and possible protection provided by ginger in alleviating this injury in albino rats.

Study Design: Prospective Experimental Study

Place and Duration of Study: This study was conducted at the Basic Medical Sciences Institute (BMSI), Jinnah postgraduate Medical Centre (JPMC) for 6 months from January 2021 to July 2021.

Materials and Methods: Twenty four adult rats were procured from the animal unit of BMSI and arranged into four groups according to the assigned treatment. Group A: control; group B: 2100MHz RF radiation generated by 3G mobile phone; Group C: RF radiation and oral ginger 250mg/kg/day and Group D: ginger only. The rats were euthanized at termination of study. Brain of rats was processed for H & E stain to record thickness of granular layer as well as diameter of Purkinje fibers and Beilschowsky stain to observe microscopic anatomy of cerebellum.

Results: There was highly significant decrease in thickness of granular layer and decreased diameter of Purkinje neurons in RF radiation treated rats in group B as compared to controls. Distortion in the morphology of Purkinje cells and decreased number of axons were observed in tissue sections from group B animals as compared to control. These findings improved to a pronounced extent in group C animals who were given ginger concomitant to RF radiation by mobile phones.

Conclusion: The cerebellum of rat brain is sensitive to damage by radiofrequency radiations emitted by cell phones. However, ginger minimizes these damaging effects when used simultaneously.

Key Words: Radiofrequency radiation, Brain, Cerebellum, Ginger, Microscopic Anatomy

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INTRODUCTION

Brain is an indispensable organ of human body, comprising of nervous tissue enclosed in skull. It processes not only sensory input, but also controls vital functions such as regulation of blood pressure and respiration, motor activity and release of hormones. Thus damage to this organ results in disruption of all vital functions of human body.

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Damage to the brain can take place due to different conditions, diseases or injury, which includes poisoning, extended shortage of oxygen, physical trauma, infection, and oxidative stress[1].

There has been rapid growth in cell phone industry worldwide[2]. They operate at frequencies from 450 to 2700 MHz, which is included in radiofrequency field of non-ionizing radiation[3,4]**.** The radiofrequency radiation causes vibration of polar or charged molecules in the human body because of penetration of cells leading to thermal as well as non-thermal stress. This shows that exposure to radiofrequency radiation is hazardous for human health**,** especially brain[6]. Research has revealed that these injurious effects are caused by release of reactive oxygen species (ROS), which results in oxidative stress and lipid peroxidation, having a critical function in disease pathogenesis[1,7].

The use of medicinal plants as remedy for illnesses has increased over the past several years, especially because of their anti-oxidant effects[8]. Numerous studies have been carried out on Ginger as it defends against oxidative stress. Ginger (Z. officinale Roscoe) is an ancient herb[9]. It has different medicinal attributes like antioxidant, immunomodulatory, anti- hyperglycemic, anti-inflammatory, anti-apoptotic, anti- tumorigenic,

anti- lipidemic, hepatoprotective and neuroprotective activities[10].

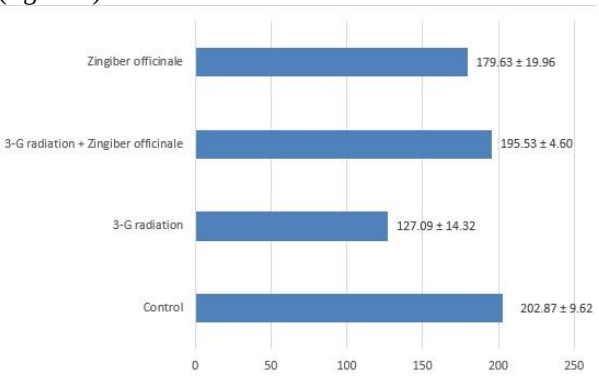
Many studies have shown that excessive use of mobile phones has harmful effects on brain, as the source emitting electromagnetic radiation is placed near to the user's head. These results may result in penetration of electromagnetic radiation into the human brain, causing damage[11]. With these facts in consideration, the present study was planned to analyze the damaging effects of electromagnetic radiation generated by 3G mobile phone on the cerebellum of rat, and prospective improvement provided by concurrent consumption of ginger by the albino rats.

MATERIALS AND METHODS

This study was undertaken at department of Anatomy, BMSI, JPMC, Karachi after securing ethical approval from the ethical review committee of JPMC. Twenty four adult albino rats of both sexes were acquired from the animal house. The rats were evaluated for one week before beginning of the study for their health by observing their diet intake and activity. The animals were sorted into groups based on treatment administered. Each group consisted of six animals. Group A: control, Group B: 2100MHz radiation generated by 3G mobile phone round the clock daily; Group C: dosage of radiation similar to group B with daily oral ginger 250mg/kg body weight; Group D: same dosage of ginger as administered to group C. The animals were allotted numbers and placed in plastic containers under laboratory conditions. Animals were irradiated with 2100MHz radiation generated by 3G mobile phones by hanging the phone 4-5cm from the floor of container with wire (Telenor Easy 3G, Model: W2). The animals were sacrificed at the end of study by administering ether anaesthesia in a glass jar. They were then fixed to an autopsy board. Parietal approach was used to remove brain, which was washed with normal saline and fixed by immersing in formal saline (10%) for a day. Then a small piece (about 2mm square) was sliced from the superior surface of cerebellar hemisphere and processed for making paraffin blocks for Haematoxylin and eosin and Bielschowsky stain[12]. Tissue block was fixed in a rotary microtome and 4 micron thick sections were made. The ribbons of tissue sections were soaked at 42° C in water bath and taken up on marked glass slides. Staining of cerebellar tissue was undertaken with Haematoxylin and eosin stain[12] for morphometry (measuring thickness of granular layer and diameter of Purkinje cells) and with Beilschowsky stain (Bancroft) to assess histological structure of axons and Purkinje cells at high magnification. Morphometry was done by calibrating ocular reticule with stage micrometer using 100 X objective. 50 small divisions of ocular micrometer coincided with 5 divisions of stage micrometer, which were equal to 50 µm, so one small division of ocular micrometer was equal to 1 µm at 100X objective. Quantitative data that is thickness of granular layer and diameter of Purkinje neurons were analyzed with SPSS software, version 21. The differences between the irradiated and control groups were analyzed by paired sample student "t" test. The difference between the groups was regarded significant statistically if p-value was equal to or less than 0.05.

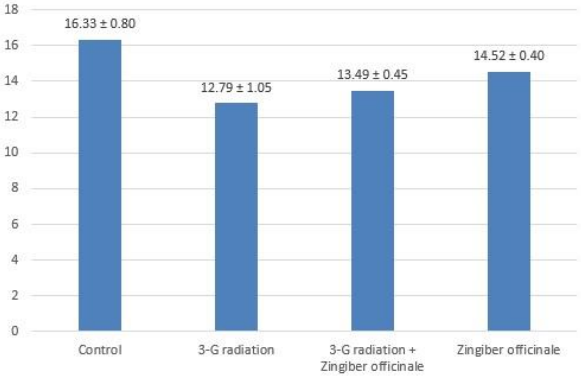
RESULTS

The mean values of thickness of granular layer in groups A, B, C and D are shown in figure-1. The results showed a highly significant decrease in the thickness of granular layer in 3G radiation treated group B as compared to control (p-value, 0.004). The data also revealed a highly significant restoration of granular layer thickness in radiation with ginger-treated group C (p-value, 0.009) when compared with group B. An insignificant decrease was also observed when ginger-treated group D was compared with control group A (figure-1).



Where n is the number of albino rats
Data is presented as Mean ± SEM (Standard Error of Mean)

Figure No.1: Thickness of Granular layer (µm) in different groups of Albino rats



Where n is the number of albino rats
Data is presented as Mean ± SEM (Standard Error of Mean)

Figure No.2: Mean Diameter of Purkinje Cell (µm) in different Groups of Albino Rats

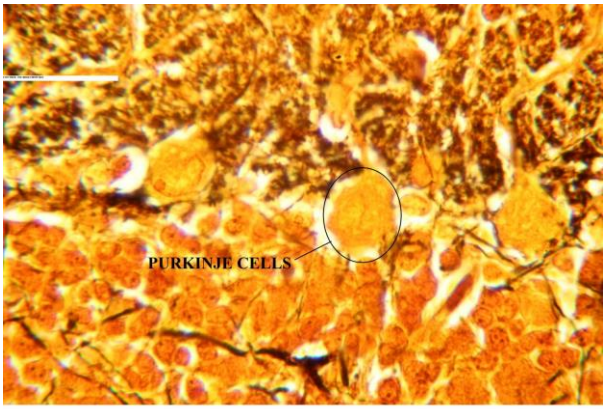


Figure No.3: Bielschowsky stained tissue section from cerebellum of control group A

Bielschowsky stained, 4µm thick section of control rat brain (cerebellum) showing Purkinje cells layer and pyramid-shaped Purkinje cells (Photomicrograph X 1000)

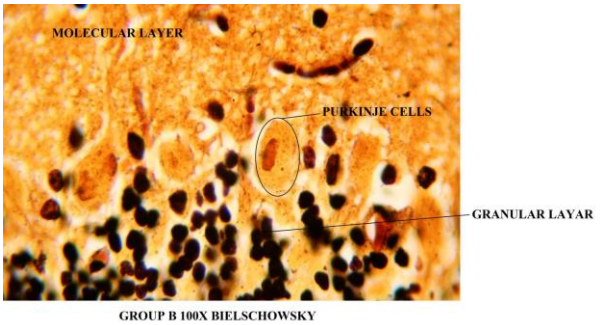


Figure No.4: Bielschowsky stained tissue section from cerebellum of mobile phone radiation treated group B

Bielschowsky stained, 4µm thick section of irradiated rat brain (cerebellum) showing decreased diameter of Purkinje cell with oedematous and degenerating nucleus. The Molecular & Granular Layers are also visible (Photomicrograph X 1000)

The mean values of diameter of Purkinje cell (µm) in group A, B, C and D are shown in figure-2. The size of Purkinje cell was significantly decreased in radiation-treated group B when compared to control group A (P value 0.012). However, insignificant restoration of Purkinje cell diameter was observed when radiation with ginger-treated group C was compared with radiation treated group B (p-value, 0.903). The mean value of Purkinje cell diameter in ginger-treated group D was near to control group A (figure-2).

On observation of Bielschowsky stained sections under oil immersion objective (100X), the Purkinje cells were seen as large pyramidal cells with apex directed towards the molecular layer and base towards the granular layer, lying in a regular pattern between molecular and granular layers (figure-3). The nerve fibers were seen as darkly stained fibers running in the molecular layer, parallel to Purkinje cell layer (figure-3).

Bielschowsky stained sections of cerebellar tissue from radiation treated group B animals under oil immersion lens showed dysmorphic changes in Purkinje cells represented by pyknosis of nuclei and reduction in their size, with shrinkage and degeneration of cell organelles. Granular layer also showed degeneration and nuclear accumulation with edematous spaces (figure-4).

Bielschowsky stained sections of group C animals (radiation with ginger) revealed restoration of cerebellar architecture similar to control (figure-3).

DISCUSSION

The most effective tool for communication these days are mobile phones. However, their use is associated with emission of radiofrequency radiation which has raised concerns about the safety related with their use[13]. Various effects of radiofrequency radiations have been reported in former studies, such as increased cellular excitability, lipid peroxidation, or enhanced stimulation of cellular reaction to stress etc[14]. Contrarily, literature search also reported absence of measurable biological effects of radiation on brain[15]. In view of these controversies, this study was conceived to detect microscopic anatomical changes in rat cerebellum in response to radiofrequency radiation emitted by third generation mobile phones.

The thickness of granular layer was highly significantly reduced in radiation exposed group B rats as compared to control. Marked improvement in thickness of granular layer was observed in histological sections taken from group C rats who were administered ginger in addition to radiation exposure. Hamzeh et al (2017)[16] observed significant improvement in level of anti-oxidants in rat brain in rats which were treated with ginger in addition to Diazinon as compared to Diazinon- treated group. This findings further show that histopathological effects leading to decreased thickness of granular layer are mediated by oxidative injury, which showed improvement in group C as rats assigned to this group consumed ginger along with RF radiation exposure, minimizing the effects of brain damage.

Degeneration of Purkinje cells was observed with decrease in the diameter of Purkinje cells in radiation treated group B tissue sections. Our results were similar to another study[17] who also observed reduced diameter of Purkinje cells in cerebellum of patients suffering from Alzheimer's disease as compared to senile controls. There was an insignificant restoration of the diameter of Purkinje cell diameter in group C receiving ginger in addition to 3G mobile phone emitted RF radiation. Ginger protects the brain by enhancing it's anti-oxidant defence system and down-regulation of MDA- levels to normal range as seen by a former study[18].

The Bielschowsky stained section from radiation treated group B showed disruption of architecture of cerebellar cortex, with dysmorphic Purkinje cells showing nuclear pyknosis and degeneration of other organelles. The molecular layer revealed decreased number of nerve fibers and granular layer showed disruption with degenerating neuron cell bodies. These findings were most likely due to the inflammatory process in the brain as a result of oxidative stress. Even though the duration of study was only 8 weeks, but these changes suggest that radiation emitted by third generation mobile phone produces histopathological changes which will be more enhanced longer the duration of exposure, and more advanced in the technology such as 4G mobile phones which are more common these days[19]. Numerous studies have determined excess production of reactive oxygen species (ROS) in experimental animals on exposure to radiofrequency radiation generated by mobile phones leading to oxidative damage to tissues, especially to brain because of close proximity of cell phone to it while in use.[20,21] Shahabi et al (2018)[22] found more vacuoles and of larger sized in brain tissue of male wistar rats after exposing them to 900 MHz RF radiation for 6 hour/day for a duration of one and two months as compared to the controls. Similar findings were ascertained by Zymantiene et al (2020)[23] who observed decrease in the number of Purkinje cells, presence of vacuoles in neurons as well as glial cells and interstitial oedema in cerebellum of BALB/c mice exposed to 1375 MHz radiofrequency radiation emitted by mobile phones continuously for 72 hours. Marked improvement in cerebellar architecture was observed in tissue slides of cerebellar cortex from radiation with ginger treated group C. These were most likely due to the anti-oxidant effects of ginger as it protects tissues from oxidative stress decreasing their inflammation. These results have been reported in previous studies as well[16,24]. These findings were in conformity to Sangi et al (2020)[19] who also observed significant improvement in microscopic architecture of cerebellar cortex in male Wistar albino rats exposed to mobile phones for 4 weeks with concomitant use of vitamin E in a dose of 50IU/Kg body weight. This shows that vitamin E, which is also a potent anti-oxidant like ginger, protected brain tissue from oxidative stress induced by cell phones, by removal of free radicals, stabilization of cell membranes and decreasing inflammatory changes in brain tissue.

Continuous exposure to radiofrequency radiation generated by 3G mobile phones for 8 weeks cause damage to the brain leading to histopathological changes in cerebellum, which improved markedly by simultaneous use of ginger in their diet. The results of the study should be disseminated to health care workers so they can advice masses about the daily use of ginger in their diet.

CONCLUSION

The cerebellum of rat brain is sensitive to damage by radiofrequency radiations emitted by cell phones. However, ginger minimizes these damaging effects when used simultaneously.

Author's Contribution:

Concept & Design of Study:	Syed Alamdar Raza Zaidi, Ashia Qamar
Drafting:	Syed Alamdar Raza Zaidi, Ashia Qamar
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Final Approval of version:	Syed Alamdar Raza Zaidi, Ashia Qamar

Conflict of Interest: The study has no conflict of interest to declare by any author.

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