Histological Study of Prolonged Exposure to Mobile Phone Radiations on Young Male Albino Rats' Cerebellar Cortex and the Role of Ginkgo Biloba Supplementation

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Abstract: Introduction: Mobile phone technology expansion has raised concerns regarding the effect of electromagnetic field exposure on the central nervous system. **Aim of work:** This work was done to demonstrate the possible histological changes that may occur in the cerebellar cortex of young male albino rats as a result of prolonged exposure to mobile phones radiations and the possible role of Ginkgo biloba supplementation. **Materials and methods:** Fifteen healthy young male albino rats were equally divided into three groups; control (I), mobile phone exposed (II) and mobile phone exposed concomitantly supplemented by Ginkgo biloba(III). Both groups; II and III were exposed daily to mobile phone radiations one hour/day for two continuous months. Group III were concomitantly supplemented with daily oral dose of Ginkgo biloba extract (100 mg/kg). Control one were housed away from irradiation for the same period. The cerebellum of all animals was dissected out carefully and processed for light and electron microscope examinations. **Results:** Most of Purkinje neurons in group (II) were shrunken, deeply stained, surrounded by perineuronal spaces and arranged in more than one row. They appeared distorted with different ultrastructural features. Some of granular neurons had deeply stained nuclei. Purkinje layer of group (III) showed disarrangement with few darkly stained shrunken Purkinje neurons were dispersed among numerous lightly stained ones. Few affected granular neurons were observed. Numerous GFAP positive cells were seen in the three layers of cerebellar cortex of group II in comparison with that observed in control group. GFAP positive cells in group III were less than that observed in group II. **Conclusion:** Prolonged exposure to mobile phone radiations provoked degenerative changes in cerebellar cortex where Purkinje neurons revealed several structural alterations with reactive gliosis. With Ginkgo biloba supplementation, these changes were minimal.
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1. Introduction

Electromagnetic radiations (electromagnetic fields; EMFs) are prevalent in our environmental modern society. EMFs can be classified into natural e.g. sunlight, earth's magnetic field and man-made. Man-made EMFs are formed of extremely low frequency (ELF), static and radiofrequency (RF). ELF-EMFs are mostly originating from the generation, transmission and consumption of electric power. Static EMFs are used in magnetic resonance imaging, some industrial processes and scientific research. RF-EMFs have different sources as mobile phone, radio, television, radar installations, wireless communications and microwave ovens **[1-3]**.

Mobile phones are widely used worldwide and considered as an integral part of modern telecommunications. It has become an important device in human daily life. Persons live near mobile phone base stations, welders, radar technicians, radio& telegraph operators and workers in dielectric heat sealing or in telecommunication manufacturing are more susceptible to mobile phone radiations**[4,5]**.

On using mobile phone, RF-EMFs are released and absorbed by various body tissues. The close proximity of mobile phones to users' head enhances the absorption of huge amount of radiations. Recent studies correlate between the wide use of mobile phones and various CNS diseases as Alzheimer's diseases, Amyotrophic lateral sclerosis, epilepsy and Parkinson's disease **[4,6,7]**.

Nowadays, the interest in traditional herbal medicines has grown rapidly as it is more safe and effective. Ginkgo biloba (Gb), an extract of maidenhair tree leaves, has gained great popularity over the last decades. The physiological effect of Gb extracts is based on their antioxidative activity depending not only on their flavonoid constituents but also on their content of Ginkgolides and Bilobalide. Physicians begin to prescribe Ginkgo biloba as a therapy for various medical conditions as cerebrovascular insufficiency, peripheral vascular insufficiency and for the cognitive impairment during aging and in neurodegenerative disorders **[8, 9,10]**.

So, this work was done to demonstrate the possible histological changes that may occur in the cerebellar cortex of young male albino rats as a result of prolonged exposure to mobile phones radiations

and the possible role of Ginkgo biloba supplementation.

2.Materials and Methods

Fifteen healthy young male albino rats (aged 3 weeks) weighing 40-60 g was used in this study. They were housed in stainless steel cages and maintained at room temperature. They were allowed water ad-libitum and were fed a standard diet. They were equally divided into three groups: control group (I), mobile phone exposed group (II) and mobile phone exposed group concomitantly supplemented by Ginkgo biloba(III). Rats of groups II and III were exposed daily to mobile phone radiations one hour/day for two continuous months. Mobile phones were put in a direct contact to the top of the exposure cages. At this position, rats' heads were the nearest part of the body to the source of radiations **[11]**. Rats of the group III were concomitantly supplemented with daily oral dose of Ginkgo biloba extract (100 mg/kg) by using gastric tube**[9]**. Rats of the control group were housed in similar cages away from irradiation for the same period.

At the end of the experiment, rats of all groups were anesthetized with 50 mg sodium pentobarbital per kilogram body weight intraperitoneally and then intracardiac perfusion was done by 2.5% glutaraldhyde buffered with 0.1 M phosphate buffer at pH 7.4 for partial fixation of the cerebellum. The cerebellum of all animals was dissected out carefully and was processed for light and electron microscopic examinations. Specimens for light microscope examination were fixed in Bouin's solution for 24 hours and were processed to prepare 5 µm thick paraffin sections for H&E stains and immunohistochemical reaction for glial fibrillary acidic protein (GFAP) in astrocytes **[12]**.

Immunostaining was performed using avidin-biotin peroxidase technique for GFAP to demonstrate the astrocytes. Sections underwent deparaffinization and hydration. They were treated with 0.01M citrate buffer (pH 6.0) for 10 minutes to unmask antigen. Then, they were incubated in 0.3% H_2O_2 for 30 minutes to abolish endogenous peroxidase activity before blocking with 5% horse serum for 1-2 hrs. Slides were incubated with the primary antibody (Thermo scientific company) (1:100 monoclonal mouse anti GFAP) at 4°C for 18-20 hrs, then washed and incubated with biotinylated secondary antibodies followed with avidin–biotin complex. Finally, sections were developed with 0.05% diaminobenzidine slides, were counter stained with hematoxylin, dehydrated, cleared and mounted. GFAP positive cells exhibited brown color in astrocytes cytoplasm and their processes. Nuclei appeared blue **[12]**.

Specimens for electron microscopic examination were immediately fixed in the same perfusion fixative (2.5% glutaraldehyde) for 2 hrs and postfixed in 1% osmium tetroxide buffered with 0.1 M phosphate buffer at pH 7.4 for 1h. Then, they were dehydrated in ascending grades of ethyl alcohol and were embedded in resin to prepare semithin and ultrathin sections using a Leica ultracut (Glienicker, Berlin, Germany). Semithin sections (1µm thick) were stained with 1% toluidine blue for light microscopic examination **[12]**. Ultrathin sections were stained with uranyl acetate and lead citrate **[13]**. They were examined using a JEOL JEM 1010 electron microscope (Japan) at the Electron Microscope Research Laboratory of the Histology and Cell Biology Department, Faculty of Medicine, Zagazig University (Egypt).

3.Results

Light microscope examination of the sections of the cerebellum of the control young male albino rats showed that the cerebellar cortex was formed of three layers; outer molecular, middle Purkinje and inner granular. The molecular layer contained scattered small neurons, nerve fibers and blood vessels. Purkinje layer was formed of a single row of large pyriform shaped cells **(Fig. 1)**. Purkinje neurons had rounded pale stained nuclei surrounded by deeply stained cytoplasm. Among these neurons, few astrocytes with pale stained nuclei were noticed. The granular layer was packed with closely related small neurons with pale stained nuclei**(Fig. 2).**Few GFAP positive cells were scattered in molecular, Purkinje and granular layers**(Figs. 3)**.

Electron microscope examination of the ultrathin sections of the cerebellar cortex of the same group showed that Purkinje cells had large indented euchromatic nuclei with prominent nucleoli. Their cytoplasm contained numerous free ribosomes, scattered cisternae of rough endoplasmic reticulum and few mitochondria **(Fig. 4)**. Bergmann astrocytes had regular euchromatic nuclei with electron lucent cytoplasm containing few ribosomes and mitochondria. Granular neurons had nuclei with clumps of heterochromatin and thin rim of cytoplasm containing few free ribosomes **(Fig. 5)**.

Light microscope examination of the cerebellum of the mobile phone exposed rats showed that most of Purkinje neurons appeared shrunken and deeply stained. They were surrounded by perineuronal spaces **(Fig. 6)**. Purkinje neurons had corrugated cell boundaries with indistinct nuclear profile. They were arranged in more than one layer rather than the one row in the control group. Numerous Bergmann astrocytes with pale stained nuclei and clear cytoplasm were observed among these Purkinje cells.

Granular layer contained closely related neurons. Some of them had deeply stained nuclei**(Fig. 7)**. Numerous GFAP positive cells were seen in the three layers of cerebellar cortex **(Fig. 8)** in comparison with that observed in the control group.

Electron microscope examination of the ultrathin sections of the cerebellar cortex of mobile phone exposed rats showed numerous distorted Purkinje neurons. This distortion had different ultrastructural features. Some of them had electron dense cytoplasm containing fragmented Golgi complex and rough endoplasmic reticulum. Ill-defined nuclear membranes leaving nuclear ghosts were observed **(Fig. 9)**. Others had irregular shrunken heterochromatic nuclei. Their cytoplasm contained dilated cisternae of rough endoplasmic reticulum, distorted mitochondria; abnormal shapes, variable sizes, ruptured cristae, swollen ones**(Fig. 10)** and secondary lysosomes **(Fig. 11)**. Some of the distorted Purkinje cells with highly corrugated nuclear envelope were shifted among the granular neurons(**Fig. 12)**. Bergmann astrocytes preserved their ultrastructural features **(Fig. 13)**. Many granular neurons had shrunken heterochromatic nuclei with indistinct cell membranes **(Fig. 14)**.

Light microscope examination of the cerebellum of the Ginkgo biloba supplemented rats showed disarrangement of Purkinje layer. Few darkly stained shrunken Purkinje neurons were dispersed among numerous slightly stained ones **(Fig. 15).** Few astrocytes were observed in between Purkinje neurons. The granular layer contained closely related small neurons with pale stained nuclei**(Fig. 16)**. GFAP positive cells were less than that observed in group II**(Fig. 17)**.

Electron microscope examination of the ultrathin sections of the cerebellar cortex of the same group showed that numerous Purkinje neurons had large euchromatic nuclei. Their cytoplasm contained mitochondria, rough endoplasmic reticulum and free ribosomes **(Fig. 18)**. Few Purkinje neurons had shrunken electron dense nuclei and electron dense cytoplasm containing small vacuoles **(Fig. 19)**. The affected granular neurons were few in comparison with that observed in group II **(Fig. 20)**.

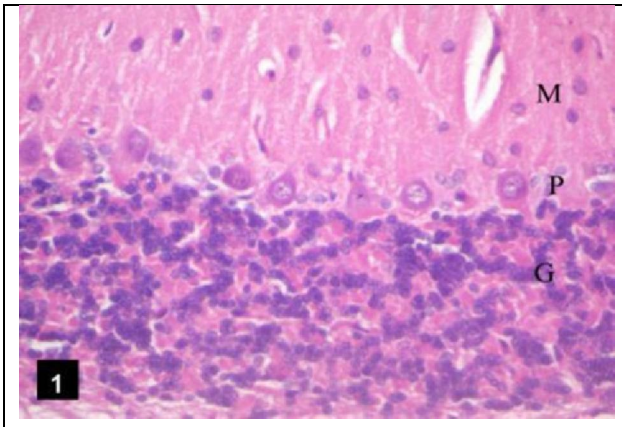

Figure (1): A photomicrograph of a section in the cerebellum of the control rats showing the outer molecular (M), middle Purkinje (P) and inner granular (G) layers**.** The molecular layer contains scattered small neurons, nerve fibers and blood vessels. Purkinje layer is formed of a single row of large pyriform shaped cells (H&E X 400).

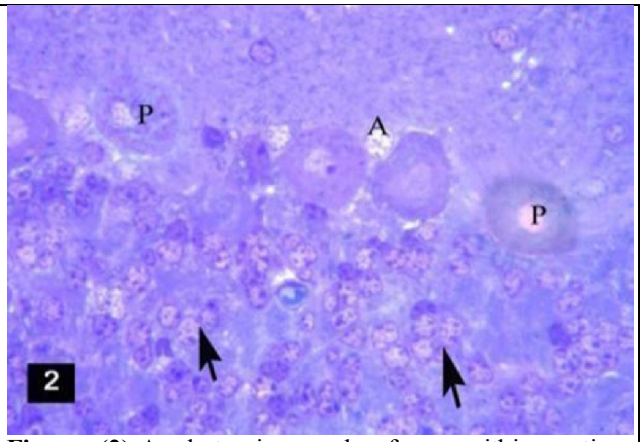

Figure (2):A photomicrograph of a semithin section (1µm thick) in the cerebellum of the control rats showing Purkinje neurons (P) with large rounded pale stained nuclei surrounded by deeply stained cytoplasm. Among Purkinje neurons, few astrocytes (A) with pale stained nuclei are noticed. The granular layer is packed with closely related small neurons (arrows) with pale stained nuclei (Toluidine blue X 1000).

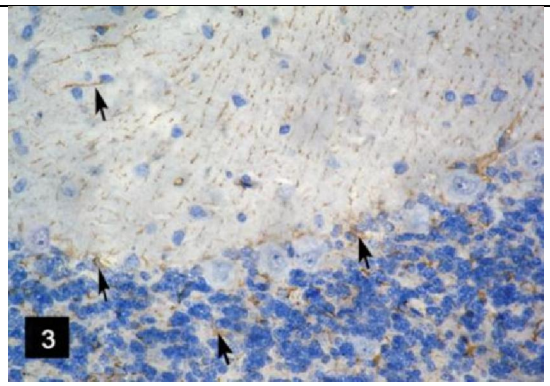

Figure (3): A photomicrograph of a section in the cerebellum of the control rats showing few GFAP positive cells (arrows)are scattered in molecular, Purkinje and granular layers (Avidin biotin peroxidase system X 400).

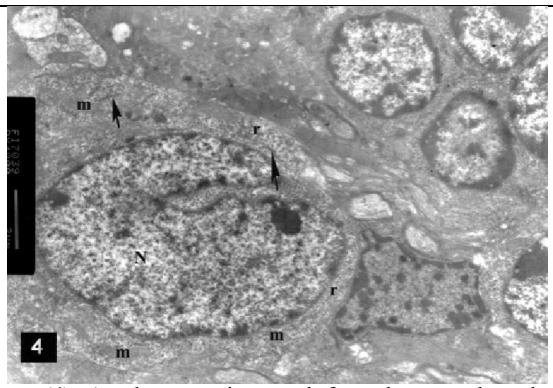

Figure (4): An electron micrograph from the control cerebellar cortex showing Purkinje cell with large indented euchromatic nucleus (N) and prominent nucleolus. Its cytoplasm contains numerous free ribosomes (r), scattered cisternae of rough endoplasmic reticulum (arrows) and few mitochondria (m) (Mic. Mag.X4000).

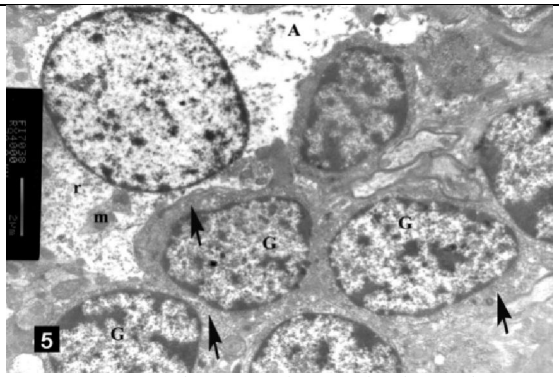

Figure (5): An electron micrograph from the control cerebellar cortex showing astrocyte (A) with regular euchromatic nucleus and electron lucent cytoplasm containing few free ribosomes(r) and mitochondria (m). Granular neurons (G) have nuclei with clumps of heterochromatin and thin rim of cytoplasm containing free ribosomes(arrows) (Mic. Mag.X 4000).

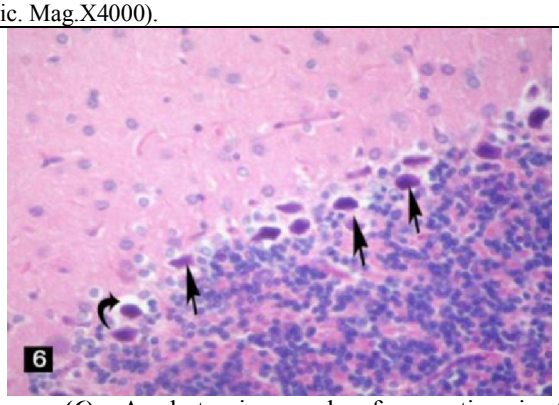

Figure (6): A photomicrograph of a section in the cerebellum of the mobile phone exposed rats showing that most of Purkinje neurons appear shrunken and deeply stained (arrows). They are surrounded by perineuronal spaces (curved arrow) (H&E X 400)

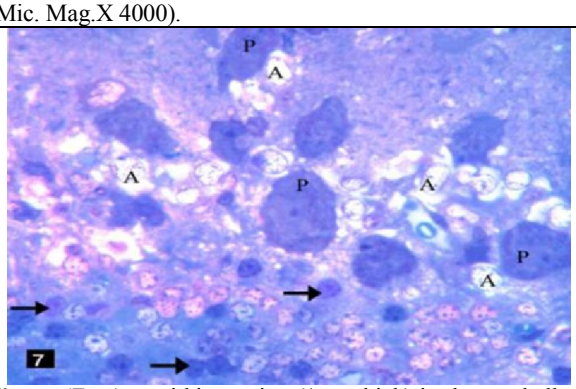

Figure (7): A semithin section (1µm thick) in the cerebellum of the mobile phone exposed rats showing that Purkinje cells (P) have corrugated cell boundaries with indistinct nuclear profile. They are arranged in more than one layer. Numerous astrocytes (A) with pale stained nuclei and clear cytoplasm are observed among these Purkinje neurons. Granular layer contains closely related neurons. Some of them have deeply stained nuclei (arrows) (Toluidine blue X 1000).

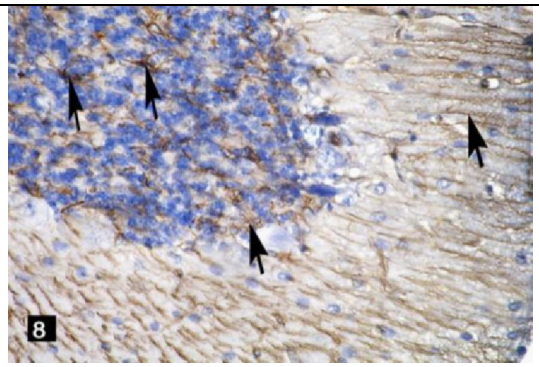

Figure (8): A photomicrograph of a section in the cerebellum of the mobile phone exposed rats showing that numerous GFAP positive cells (arrows) are seen in the three layers of cerebellar cortex in comparison with that observed in fig. 3 (Avidin biotin peroxidase system X 400).

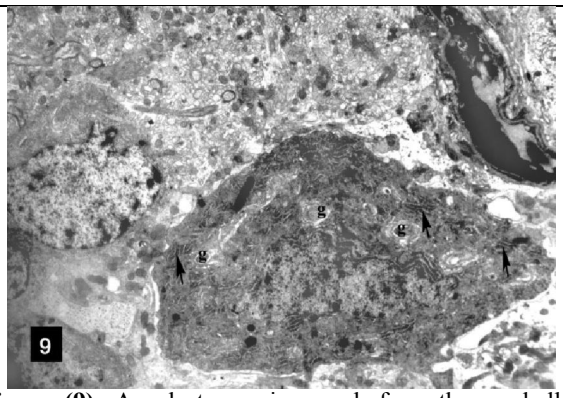

Figure (9): An electron micrograph from the cerebellar cortex of the mobile phone exposed rats showing a Purkinje neuron with electron dense cytoplasm containing fragmented Golgi complex (g) and rough endoplasmic reticulum (arrows). Ill-defined nuclear membrane leaving nuclear ghost is observed (Mic. Mag.X4000).

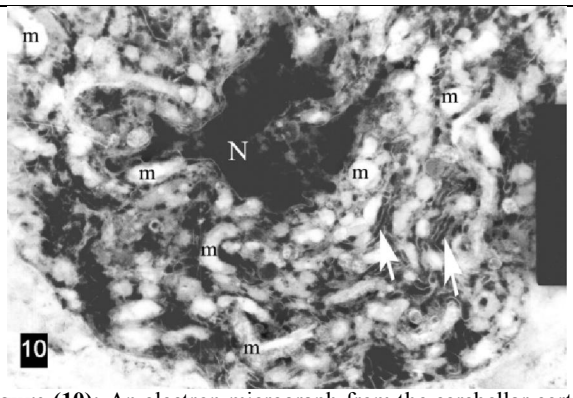

Figure (10): An electron micrograph from the cerebellar cortex of the mobile phone exposed rats showing a Purkinje neuron with irregular shrunken heterochromatic nucleus (N). Its cytoplasm contains dilated cisternae of rough endoplasmic reticulum (arrows) and distorted mitochondria (m); abnormal shapes, variable sizes, ruptured cristae and swollen ones (Mic. Mag.X4000).

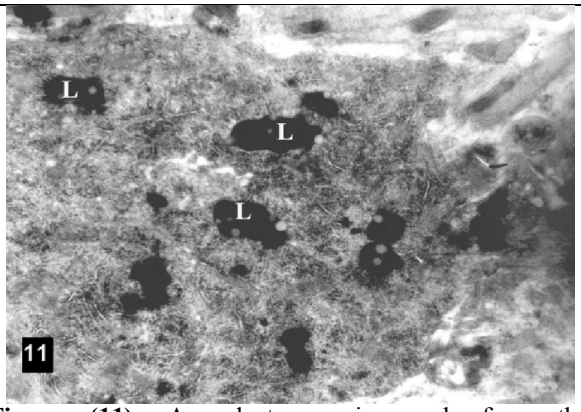

Figure (11): An electron micrograph from the cerebellar cortex of the mobile phone exposed rats showing a Purkinje neuron with secondary lysosomes (L) in its cytoplasm (Mic. Mag.X4000).

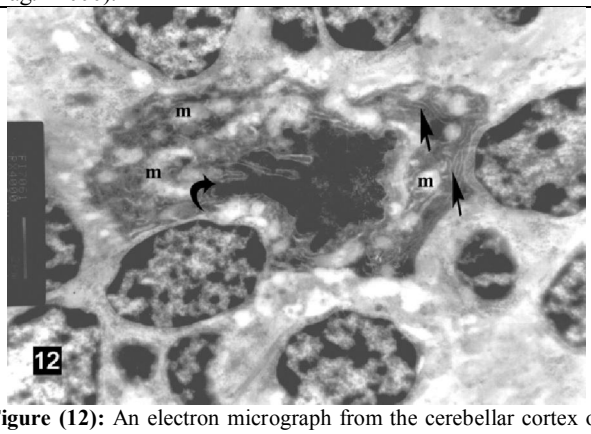

Figure (12): An electron micrograph from the cerebellar cortex of the mobile phone exposed rats showing that a distorted Purkinje neuron with highly corrugated nuclear envelope (curved arrow) is shifted among the granular neurons. Dilated rough endoplasmic reticulum (arrows) and distorted mitochondria (m) are also noticed (Mic. Mag. X 4000).

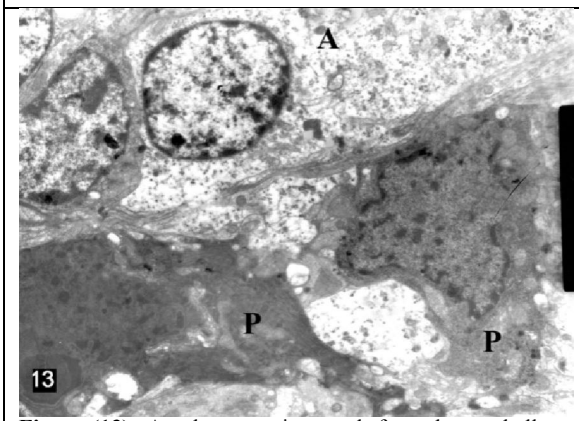

Figure (13): An electron micrograph from the cerebellar cortex of the mobile phone exposed rats showing that astrocyte (A) preserves its ultrastructural feature. It has regular euchromatic nucleus and electron lucent cytoplasm containing ribosomes and mitochondria. Electron dense Purkinje neurons (P) are also noticed (Mic. Mag. X 4000).

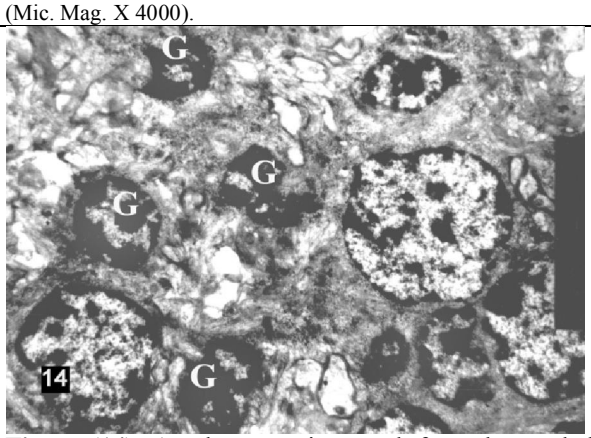

Figure (14): An electron micrograph from the cerebellar cortex of the mobile phone exposed rats showing many granular neurons (G) with shrunken heterochromatic nuclei and indistinct cell membranes (Mic. Mag.X4000).

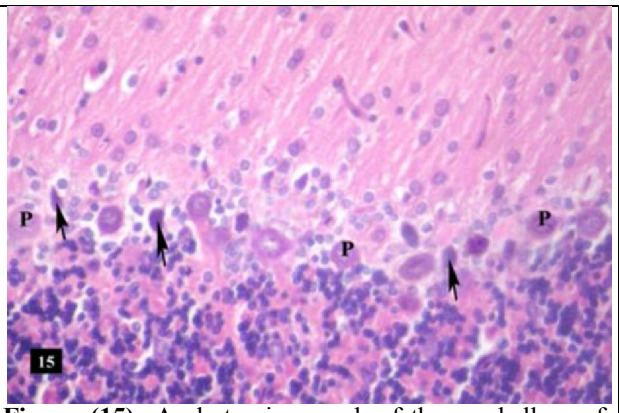

Figure (15): A photomicrograph of the cerebellum of the Ginkgo biloba supplemented rats showing disarrangement of Purkinje layer. Few darkly stained shrunken Purkinje neurons (arrows) are dispersed among numerous lightly stained ones (P) (H&E X400).

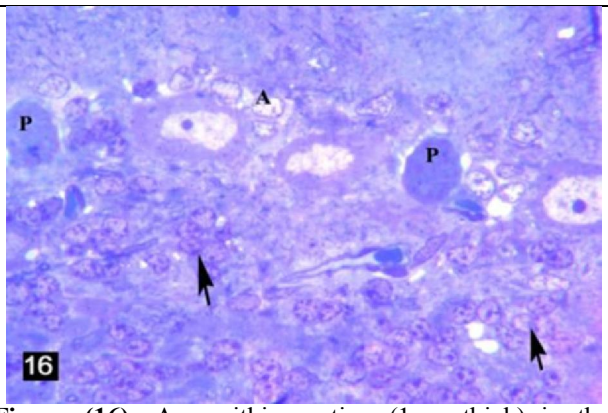

Figure (16): A semithin section (1µm thick) in the cerebellum of the Ginkgo biloba supplemented rats showing that few astrocytes (A) are observed in between Purkinje neurons. The granular layer contains closely related small neurons (arrows) with pale stained nuclei. Few deeply stained Purkinje neurons (P) are also noticed (Toluidine blue X 1000).

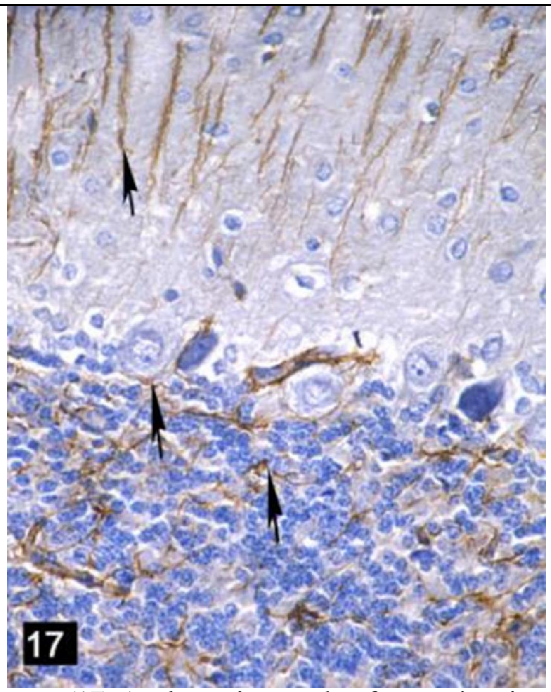

Figure (17):A photomicrograph of a section in the cerebellum of the Ginkgo biloba supplemented rats showing that GFAP positive cells (arrows)are less than observed in fig. 8 (Avidin biotin peroxidase system X 400).

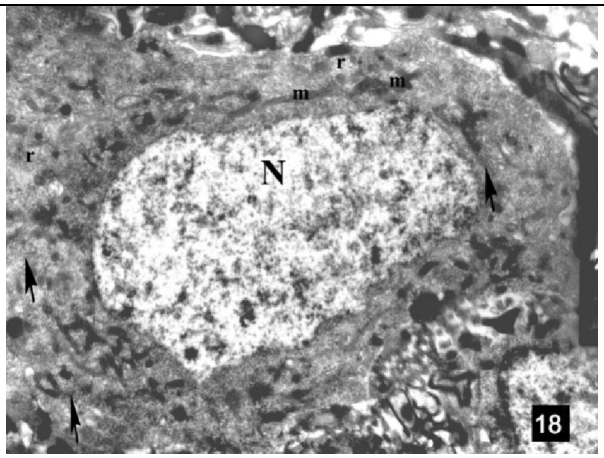

Figure (18): An electron micrograph from the cerebellar cortex of the Ginkgo biloba supplemented rats showing a Purkinje neuron with large euchromatic nucleus (N). Its cytoplasm contains mitochondria(m), rough endoplasmic reticulum (arrows) and free ribosomes (r) (Mic. Mag. X4000).

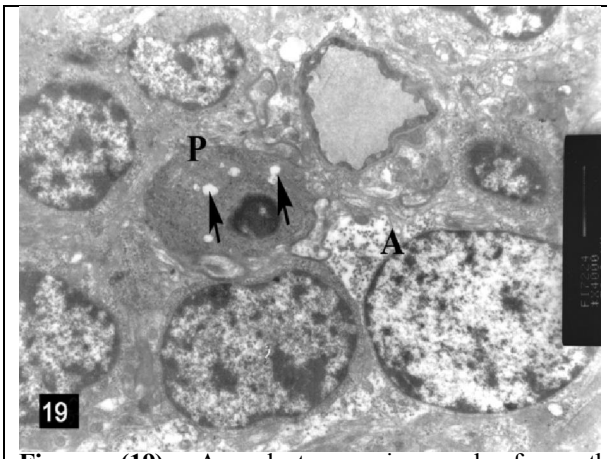

Figure (19): An electron micrograph from the cerebellar cortex of the Ginkgo biloba supplemented rats showing that a Purkinje neuron (P) has shrunken electron dense nucleus and electron dense cytoplasm containing small vacuoles (arrows). Astrocyte (A) with euchromatic nucleus and electron lucent cytoplasm containing free ribosomes is also noticed (Mic. Mag. X4000).

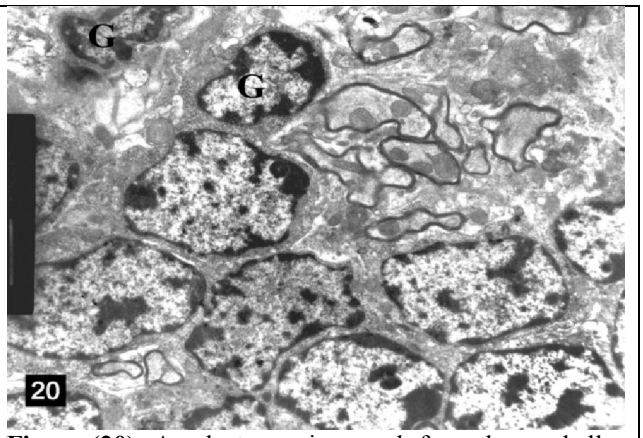

Figure (20): An electron micrograph from the cerebellar cortex of the Ginkgo biloba supplemented rats showing that the affected granular neurons (G) are few in comparison with that observed in fig 14. (Mic. Mag. X4000).

4.Discussion

The increasing number of available telecommunication devices and length of time spent using mobile telephones has aroused interest of possible interactions between human and radiofrequency radiations. This extensive mobile phones use in our life increased public, governmental and scientific attention to the issue of whether or not adverse effects are induced after exposure to radiofrequency electromagnetic fields (RF-EMF). Mobile phones transmit RF-EMF in all directions, especially brain regions. Human head absorbs up to 80% of these radiations **[14,15,16]**.

In this work, the cerebellum of mobile phone exposed group revealed that most of Purkinje neurons appeared shrunken and deeply stained. They were surrounded by perineuronal spaces. Purkinje neurons had corrugated cell boundaries with indistinct nuclear profile. Ultrastructurally, ill-defined nuclear membranes leaving nuclear ghosts were observed. It had been described**[17]** that apoptosis is a cellular suicide mechanism which occurs in mammalian cells during normal development and also as a response to injury or disease. In RF-EMF exposed rats**[18],** scattered and grouped dark neurons were detected. The apoptotic cell death is mainly characterized by nuclear condensation and DNA fragmentation without major ultrastructural changes of cytoplasmic organelles**[19]**. These dark neurons were explained by several investigators and attributed them to different causes. Some investigators**[6,20]** claimed that RF-EMF increases the permeability of the blood–brain barrier (BBB) which significantly increased leakage of the albumin through the BBB into the nervous tissue and subsequently leading to neuronal damage. Others**[21]** stated that cells contain high levels of iron as well as metabolically active cells would be more susceptible to EMF, because more hydrogen peroxide is generated by their mitochondria and subsequently DNA damage. Cumulative DNA damage in nerve cells can accelerate neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. Other investigators**[22]** reported that the CNS consumes the highest amount of oxygen and although most oxygen is converted into CO_2 and water, a small amount of O_2 forms reactive oxygen species (ROS). Nervous tissue is particularly vulnerable to ROS due to its high metabolic rate, its deficient oxidant defense mechanisms, high concentration of polyunsaturated fatty acids and its diminished cellular turn over. RF-EMFs radiation produced an excess of ROS via inhibition of the mitochondrial respiratory chain, prolongation of the life-span of free radicals and impairment of the antioxidant defense system**[23,24]**. High level of ROS results in oxidative damage of cellular macromolecules such as lipids, proteins and nucleic acids**[24,25]**. It was also found**[4,26]** that this high level ROS increase leakage through the cell membrane by acting on polyunsaturated fatty acids; subsequently the influx of calcium and sodium ions increases. Physiologic calcium entry into neurons regulates normal neuronal development, metabolism, ageing and is involved in

the control of synaptic transmission and long-term modulation. Elevated calcium levels can lead to neuronal degeneration.

At the level of electron microscope examination, Purkinje neurons in group II revealed several cellular alterations. Some of them had electron dense cytoplasm containing fragmented Golgi complex and rough endoplasmic reticulum. Others had irregular shrunken heterochromatic nuclei. Their cytoplasm contained dilated cisternae of rough endoplasmic reticulum, distorted mitochondria; abnormal shapes, variable sizes, ruptured cristae, swollen ones and secondary lysosomes. It had been reported**[27]** that mitochondria, endoplasmic reticulum, Golgi-complex and lysosomes considered to be direct intracellular targets of radiations. Alterations in mitochondrial function and structure (swelling and disappearance of cristae) occur in early stages of apoptosis and may precede and/or accompany nuclear changes. ROS are continually produced at a relatively high amount in mitochondria during physiological conditions. However, their concentration greatly increases following radiations and the products of lipid peroxidation accumulate. Elongation and branching of the mitochondria and a reversible increase of their size and the development of giant forms are the most frequently reported changes in radiated mitochondria. Additionally**[28],** increased free radical reactions trigger oxidative damage of mitochondrial membranes and mitochondrial DNA. Previously**[29],** the detected dilated cisternae of rough endoplasmic reticulum with observed nuclear changes signify a reduction in the ability of protein synthesis by affected neurons which can ultimately influence the overall functions. Moreover**[27],** fragmentation and rearrangement of the Golgi cisternae are commonly observed upon radiations. Alteration of the Golgi complex may be connected with the radiation-induced destruction of the cytoskeletal system. Also, the expansion of the lysosomal/autophagic compartment may be due to a decreased rate of digestion of the segregated material, which may cause the overload and expansion of the lysosomal compartment. Furthermore**[18]**, it was found that the neuronal changes may be mediated through organelles damage with release of not only hydrolytic lysosomal enzymes but also sequestered harmful material as heavy metals, stored away in cytoplasmic organelles (lysosomes).

Also, Purkinje neurons of mobile phone exposed group were arranged in more than one layer rather than the one row in the control group. Some of the distorted Purkinje cells with highly corrugated nuclear envelope were shifted among the granular neurons. It was reported**[30]** that prolonged exposure to neuronal insult could lead to 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.

The granular layer of mobile phone exposed group contained closely related neurons. Some of them had deeply stained nuclei. Ultrastructurally, many granular neurons had shrunken heterochromatic nuclei with indistinct cell membranes. Some researchers focused in exposure of cerebellar granule cells to oxidative stress that triggers apoptosis**[31]**.It was thought **[32]** that the gathered darkly stained nuclei of the granular layer in a clumping manner, were secondary to the changes occurred in the Purkinje neurons. As the degenerated Purkinje cells failed to establish contact with the granule cells, this will lead to lack of normal synchronism between both that might minimize the regulatory role on them. Additionally**[21],** EMFs exposure enhances free radical production which damages various macromolecules such as DNA, protein and membrane lipids leading to cell death, aging or cancer.

Numerous Bergmann astrocytes with pale stained nuclei and clear cytoplasm were observed among the Purkinje cells of mobile phone exposed group. They preserved their ultrastructural features. Also, numerous GFAP positive cells were seen in the three layers of cerebellar cortex in comparison with that observed in the control group. It was reported**[33]**that glial cells perform structural and nutritional functions. They have a role in neurotransmission and in the control of the blood-central nervous system interface. In addition, they contribute to the synthesis and secretion of neurotrophic factors. It was stated **[34]**also**,** that glial fibrillary acidic protein (GFAP) is an intermediate-filament (IF) protein that is highly specific for astroglial cells lineage. An estimation of neuronal damages can be carried out by measuring the astrocyte proliferation and reactivity. So**[26,35],** astrogliosis represents a remarkable response of astrocytes to all CNS injuries and GFAP expression is an early hallmark of reactive gliosis during these injuries.

In this work, examination of the cerebellum of the Ginkgo biloba supplemented rats showed disarrangement of Purkinje layer. Few darkly stained shrunken Purkinje neurons were dispersed among numerous lightly stained ones. These numerous neurons had large euchromatic nuclei. Their cytoplasm contained mitochondria, rough endoplasmic reticulum and free ribosomes. Few Purkinje neurons had shrunken electron dense nuclei and electron dense cytoplasm containing small vacuoles. The granular layer contained closely related small neurons with pale stained nuclei. The affected

granular neurons were few in comparison with that observed in group II. Some authors**[36]**claimed that Gb could inhibit intracellular Ca2+ increase that has the ability to restore the neuronal physiology. However, others**[9]**stated that mobile phones cause oxidative damage by increasing the levels of reactive oxygen species (ROS) activities in brain tissue and Gb administration significantly reduce these ROS leading to greater protection to neuronal cell population and prevented apoptosis. This antioxidant mechanism of Gb is based upon scavenging free radicals directly or indirectly by activation of antioxidant enzymes and inhibiting the formation of free radicals**[37,38]**. Another study**[39]** reported that Gb can up-regulate brain-derived neurotrophic factor expression that plays a key role in the survival, maintenance and growth of neurons. Others demonstrated that Gb can inhibit the activation of caspases 9 and 3; a member of caspase family of cysteine proteases that have been implicated in apoptosis **[40]** or induce over expression of Bcl-2 protecting the cells from apoptotic process**[41]**.

Few astrocytes were observed in between Purkinje neurons of group III. Also, GFAP positive cells were less than that observed in group II. It was known **[42- 44]** that neurons and astrocytes are two major types of cells in the central nervous system and astrocytes account for one-third of the total cortical volume in the brain. The bi-directional communication between neurons and astrocytes is essential for normal functions of nervous system. Additionally, it was reported**[45-47]** that astrocytes secrete some of trophic factors as nerve growth factor, basic fibroblast growth factor, brain-derived neurotrophic factor, glial cell line derived neurotrophic factor, erythropoietin and neuregulins. These factors promote neuronal survival and regeneration after injury. Gb could induce astrocytes to produce excess erythropoietin, enhancing indirectly neuronal viability.

In conclusion, prolonged exposure to mobile phone radiations provoked degenerative changes in cerebellar cortex where Purkinje neurons revealed several structural alterations with reactive gliosis. With Ginkgo biloba supplementation, these changes were minimal.

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