Effects of Non-Ionizing Radiation on the Ultrastructure of the Retina of Albino Mice

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Abstract: Due to the extensive use of non-ionizing radiation (NIR) in everyday life, more information is required for the detection of their possible side effects on the different tissues of the organism. Therefore, in this paper, the possible adverse effects of 900- 1800 MHz pulsed radiation emitted from a mobile phone on the retina of developing albino mice *in vivo* were investigated by electron microscopy (EM). Twenty male albino mice of 4 months old and 35-50gm body weight were assigned to receive microwave irradiation. Microwaves were delivered to the whole body of the animals by a mobile phone (SAR 0.78 w/ kg) 1h/ day for about 140 days including all the prenatal and a period (4 months) of postnatal life. After exposure for the scheduled duration the retinae of the control and irradiated animals were dissected out and processed for ultrastructural examination. It was found that the NIR of the mobile phone has a marked degenerative effect on the retinae of exposed animals at the ultrastructural level. All the retinal layers exhibited an obvious reduction in their height and cell population. The retinal pigment epithelia (RPE) were the most affected cells, where they are completely degenerated and disappeared with their melanin granules. The photoreceptor cells underwent shortening, shrinking, disorganization, nuclear pyknosis, disruption, interruption of their membrane lamallae, death and loss. The outer limiting membrane (OLM) appeared interrupted as affected by microwave irradiation. The outer nuclear layer (ONL) appeared with shrunken chromatin and karyolysed nuclei. Others with lobulated and fragmented nuclei with mild vacuolization. The outer plexiform layer (OPL) appeared with vacuolated and degenerated elements depleted from their cytoplasmic components. The inner nuclear layer (INL) exhibited apoptotic and necrotic cells with extensive vacuolization. The inner plexiform layer (IPL) illustrated atrophic degeneration and degenerating synaptic buttons. The ganglionic cell layer (GCL) underwent extensive vacuolization developed to vacuolar degeneration with degenerating synaptic areas. Others appeared with crenated nuclear envelops and clumped heterochromatin. Other GCs were apoptotic nuclei and vacuolated cytoplasm. The inner limiting memebrane (ILM) lost its double nature. In conclusion, the results of the present investigation support and confirm the findings of previously published studies that the NIR of mobile phone induce different ultrastructural lesions in the retina of the exposed animals particularly the growing ones.
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1. Introduction

The neural retina is the most complex structure in the eye, processing light signals from the environment into patterns in the visual cortex through the optic nerve **(Dillon, 1991).** The mobile phones are being used more frequently in present days than in the past. These handsets operate in close proximity to the human head which has raised concerns about the biological interactions between electromagnetic radiation (EMR) and eye **(Knave, 2001 and Repacholi, 2001)**. The eye is a critical organ that can be injured by these non-ionizing radiation (NIR). **Kues *et al.* (1985)** used histological techniques to confirm damage to both cornea and retina of primates after microwave exposure at 2.45GHz. Microwave induced-retinal injury was noted by **Kues and Monahan (1992)** as submacula detachment, degenerative changes in photoreceptor outer segments, vacuolization of the outer retinal layers, focal retinal detachments, karyolysis of photoreceptors and pyknotic changes of the pigmented epithelium in the monkey. Broadband blue light (400-520nm) has been found to disrupt the blood-retinal barrier properties of the retinal pigment epithelium (RPE) of the rabbit eye **(Liu *et al.*, 1995)** at a dose 18 J/ cm^2. The threshold dose for funduscopic evidence of damage **(Gorgels *and* Van Noreen, 1995)** after exposure to short-wavelength UVA radiation (320 nm) has been observed to lie at 0.35 J/ cm^2. On histological examination, the damaged tissue was shown to consist of cells with pyknotic photoreceptor nuclei. Other studies that examined the lethal effect of near UV radiation peaking at 365 nm showed that killing of RPE cells was dependent on the total energy dose of irradiation

and the state of confluence of the culture **(Van Best *et al.*, 1997)**.

The study of **Patton *et al.* (1999)** confirmed that DNA single-strand breaks occurring either directly or from alkali-labile lesions are present in the RPE DNA after exposure to UV radiation (which forms part of the electromagnetic spectrum lies between visible light and ionizing radiation). **Yao *et al.* (2008)** recorded a DNA damage by 1.8GHz radiofrequency (RE) field for 2h, which was mainly single strand breaks (SSBs), and may be associated with the increased reactive oxygen species (ROS) production in human lens epithelial cells. Electromagnetic noise could block RF-induced ROS formation and DNA damage. In contrast to the above mentioned studies, there are also authors who contest the adverse effects of electromagnetic fields (EMF). Some researchers are convinced that there are not measurable EMF associated changes **(Kizilay *et al.*, 2003).**

Since, no sufficient information is available concerning the effects of these NIR on retinal cells at the ultrastructural level and keeping in view the rising trend of mobile telephony and insufficient knowledge regarding its health effects. Therefore, the purpose of the present study was to investigate the effects of the long term exposure of NIR emitted by a mobile phone on the ultrastructure of the nervous tissue of the developing mice, selecting the retina of as the study organ of this paper.

2. Materials and Methods:

Animals:

Twenty male albino mice were collected from the first generation of sixteen pregnant females. The dams and their pups were kept in an environment of controlled temperature (24-26°C) and controlled photoperiod (12 h of light and 12 h of dark) for one week before the start and during the time of experiment. A commercially balanced diet (standard pellets) and tap water were provided *ad libitum*. The twenty animals were categorized into 2 groups, each one comprised 10 mice. The first 10 coming from microwave- exposed dams and served as exposed or treated group. The second 10 coming from control (unexposed) dams and served as control group.

Irradiation:

Exposure of animals was carried out by a mobile phone (Nokia, model 3220, SAR 0.78 w / kg) microwaves (900- 1800 MHz). The pregnant dams were exposed (irradiated) from the beginning of gestational period (day 0) until day 21 (the end of the gestational period). Then the resulting pups continue to receive microwave irradiation for the following 120 days (4months) of the postnatal life. Irradiation was performed as a series of repeated (1h / day) exposures for up to 140 h during about 140 days. During exposure, animals were caged in groups of three in perforated plastic containers suitable for their size to permit good ventilation and free motion. A ringing mobile phone set on silent tone was placed in a direct contact to the bottom of the exposure cage during the exposure of pregnant dams and on the top of cage during the exposure of their pups. This allowed a whole body microwave exposure of 1h / day for 140 days. The control animals were housed in a similar container away from irradiation for the same period of time every day. Each animal aging 4 months and weighting 35-50 gm immediately before sacrifice.

Electron microscopy technique:

The posterior eye cups (containing retinae) were dissected and removed from the control and microwave-exposed animals and immediately fixed in cold 4% glutaraldehyde in 0.2% phosphate buffer (pH = 7.4) then embedded in plastic resin **(Robenson et al., 1987)**. Semithin sections were cut (1µm thick) by an ultratome and stained with toluidine blue to select suitable areas for ultrastructural studies. Ultrathin sections were cut, mounted on copper grids, stained with uranyle acetate and lead citrate **(Echlin, 1964)** for electron microscopic examination (JEOL-JEM 1010) at the electron microscopy unit, Al-Azhar University. Hamamatsu C4742-95 digital camera was used for photo.

3. Results:

The present study is focused upon the ultrastructure of the neural retina which is the posterior photosensitive portion of the retina lining the choroids from the papilla of the optic nerve posteriorly to the ora serrata anteriorly.

Results from semithin sections:

By investigating the semithin sections of the normal and microwave exposed retina it was found that there is a great reduction in the height and cell population of most layers of retina of exposed animals as compared to controls. Also, it was noted that there is a complete absence of RPE and melanin pigments of choroid (Figs. 1&2). Conversely there is an abnormal thickening of choroid in exposed retina.

Results from ultrathin sections:-

i. Retina of control animals:

The electron microscopic (EM) examination of the retina of control mice revealed the normal histological criteria of this important nervous tissue. The electron micrograph (Fig. 3) demonstrated part of the choroid (CH), part of the retinal pigmented epithelia (RPE) and part of the outer segment (OS) of the photoreceptor cells (rods, cones) of the eye.

The RPE appeared as simple cuboidal epithelium resting upon the Bruch's membrane (BM)

and their apical processes having many long microvilli (Mv) which project between the OS of rods and cones. The Mv containing the characteristic melanin pigments of the retina which responsible for absorption of light. Choroid (the middle vascular layer of the eye) appeared with its characteristic melanocytes (Mc), fibroblasts (Fb) Bruch's membrane (BM). The BM is considered as a fusion of the basal lamina of both RPE and the endothelia of blood capillaries of choroid. In a magnified part of the last EM, the collagen fibers and melanocytes of choroid with its characteristic abundance of melanin granules are well demonstrated (Fig. 4)

The layer of photoreceptors (first order neurons) appeared differentiated into outer segments (OS) and inner segments (IS) with a normal cell population and adequate height (Fig. 5). The outer limiting membrane (OLM) and a part of the outer nuclear layer (ONL) are well demonstrated in the same field. The OLM is not a true membrane but a series of junctional complexes between photoreceptors and glial cells of the retina (Müller's cells). The IS of the photoreceptors is a part of this layer containing many mitochondria, polysomes, smooth and rough endoplasmic reticulum, Golgi apparatus and glycogen granules. The magnified picture of the OS of photoreceptors which appeared as cylindrical structures containing stacks of disc membranes illustrated in Fig (6). The membrane lamellae of these discs are packaged in a regular manner inside the stacks of these outer segments. The photopigments rhodopsin and iodopsin of rods and cones respectively are localized upon the membrane lamellae of these discs.

The outer nuclear layer (ONL) is well illustrated in Fig. (7) with a normal thickness and dense cell population. It contains the nuclei of cones and rods. The magnified picture of the ONL demonstrated the integrity and homogenity of the nuclear components of the constituent elements of this layer (Fig. 8). The outer plexiform layer (OPL) appeared in (Fig. 9) containing synapses between the axons of rods and cones (first order neuron) and dendrites of other neuronal cells (second order neuron) and associated horizontal cells. The characteristic ribbon synapse of this layer could be also demonstrated.

The inner nuclear layer (INL) contained nuclei of the bipolar cell bodies (second order neuron), amacrine, horizontal and Müller's cells with normal height and cell population (Fig. 10). In a magnified EM the normal architecture of the inner plexiform layer (IPL) could be demonstrated (Fig. 11), where it comprised the axons of the second order neuron in synapses with the branched dendrites of the ganglion cells. Also, short amacrine cells synapse in this layer.

Semithin sections of retina of control and exposed animals (Figs. 1 & 2)

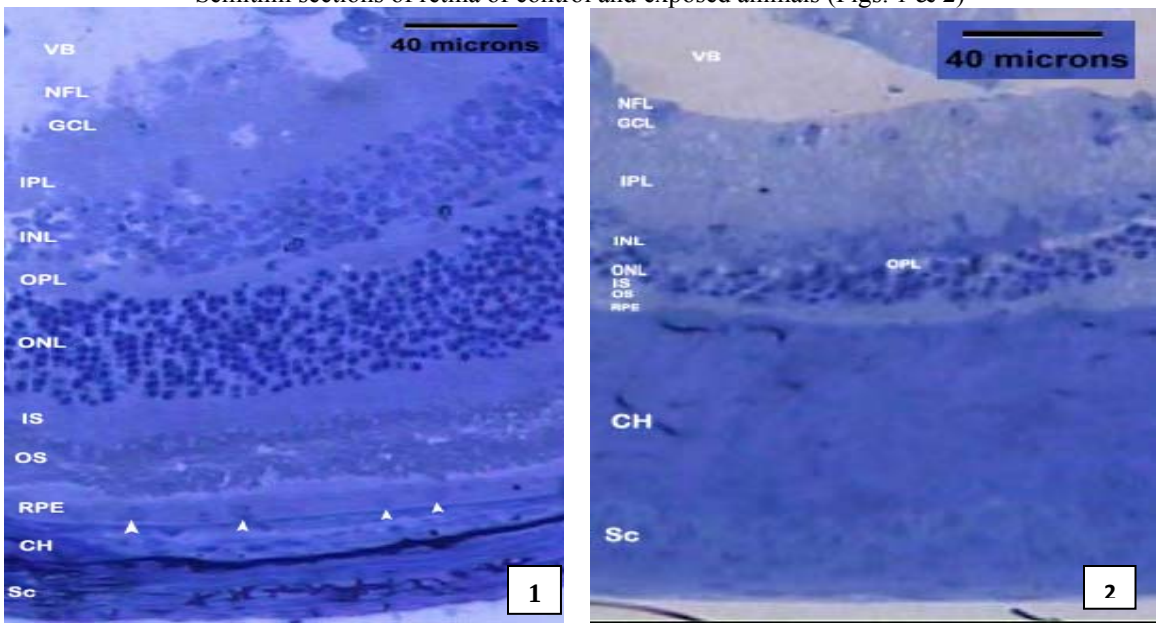
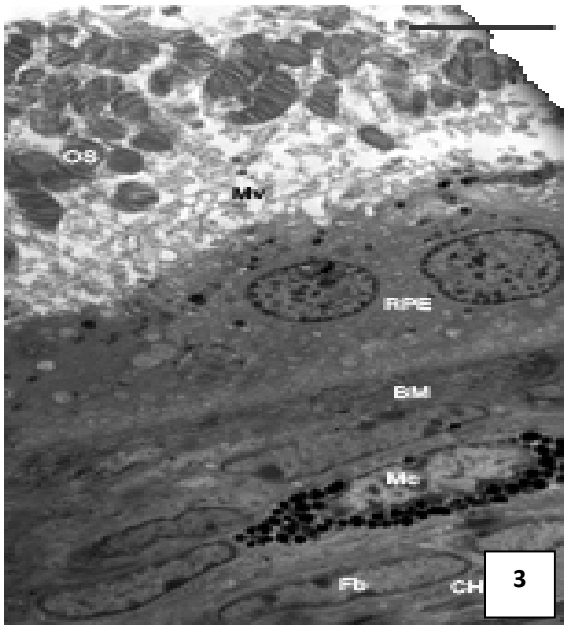
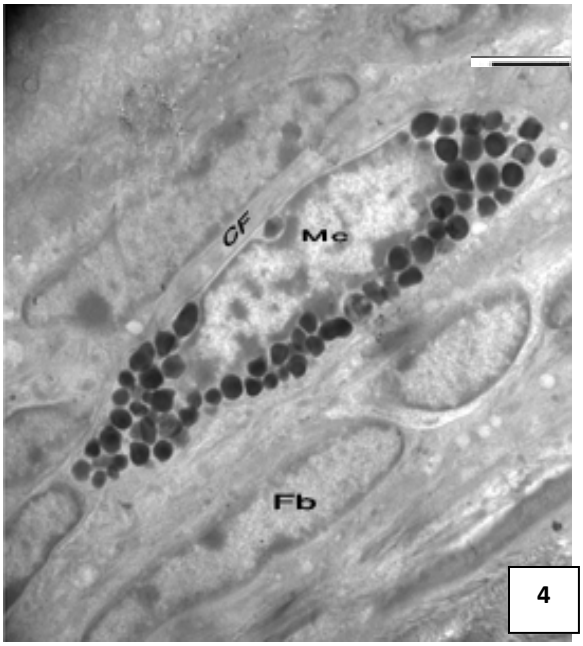
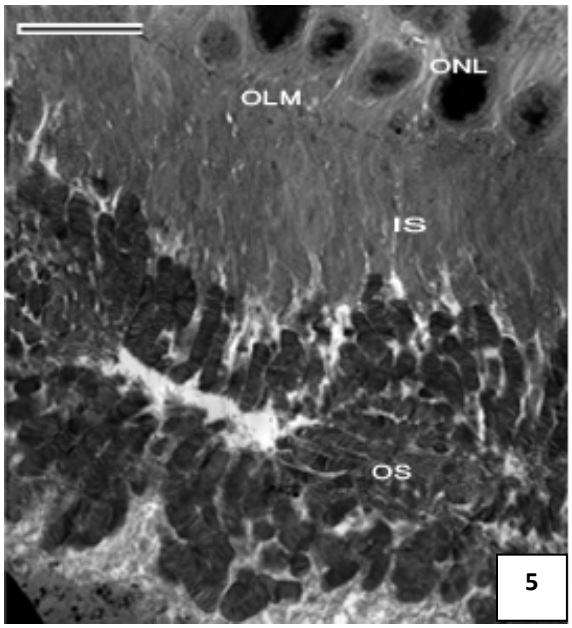


Fig. (1): illustrating the different layers of retina of control animals: sclera (Sc), choroid (CH), retinal pigmented epithelia (RPE), outer segment (OS) & inner segment (IS) of photoreceptors, outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglionic cell layer (GCL) and nerve fiber layer (NFL). Compare the height or thickness of different layers. Fig (2) Retina of exposed animals showing reduced cell population in different layers absence of RPE and melanin pigments of choroid and abnormal thickening of choroid.

Electron micrographs (EM) of retina of control albino mice (Figs: 3-11)







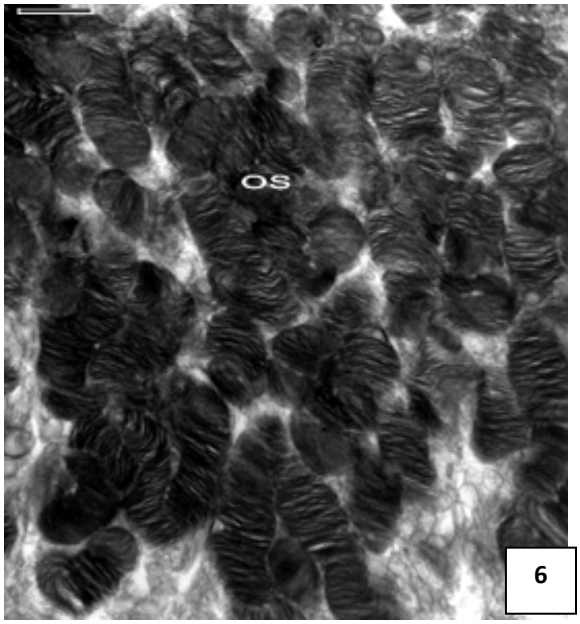


Fig. (3): showing CH, melanocyte (Mc) fibroblast (Fb), Bruch's membrane (BM), RPE, microvilli (Mv), OS of rods & cones (photoreceptor cells). Scale bar = 10µm.
Fig. (4): a magnified part of the last EM demonstrating Mc with melanin granules, Fb and collagen fibres (CF) of choroid. Scale bar = 2µm.
Fig. (5): showing OS, IS, OLM and a part of the ONL. Notice, the height of photoreceptor layer. Scale bar = 10µm.
Fig. (6): showing OS of photoreceptors. Notice the regularity of the membrane lamellae of the discs. Scale bar = 2µm.

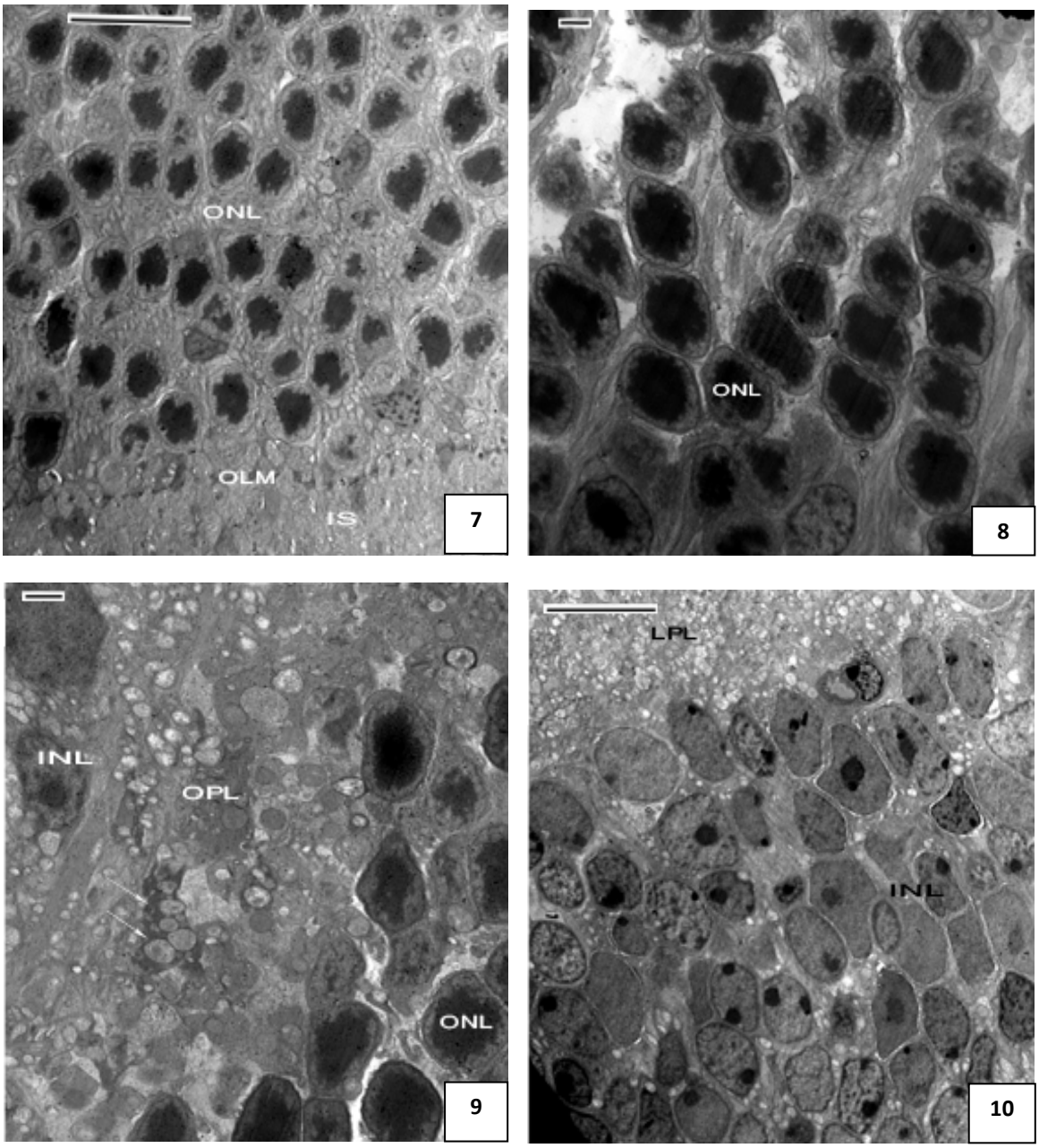


Fig. (7): Showing the IS, OLM and the outer nuclear layer (ONL). Notice, the height & cell population of this layer. Scale bar = 2µm.
Fig. (8): Showing the ONL. Notice, the width and integrity of the nuclear components of these elements. Scale bar = 2µm.
Fig. (9): Showing part of the ONL, the OPL and part of INL. Notice, the characteristic ribbon synapse(arrows) of the OPL. Scale bar = 2µm.
Fig. (10): Showing the INL and part of the IPL. Notice, the height and cell population of the INL. It contains the nuclei of bipolar, amacrine, horizontal and Müller cells. Scale bar = 10µm.

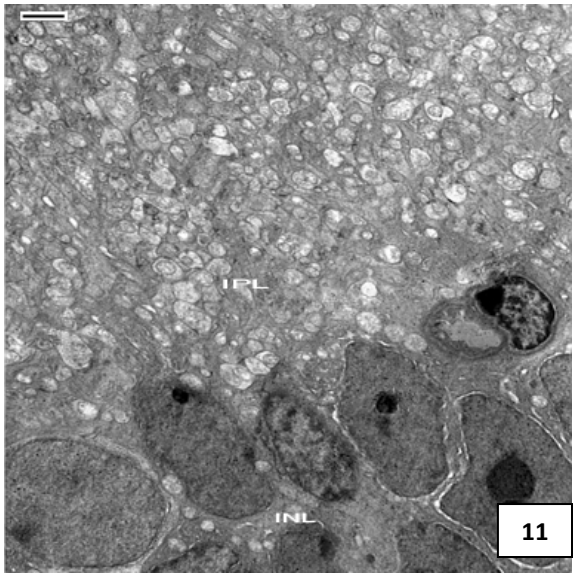


Fig. (11): Showing a magnified part of the last EM to illustrate the histological architecture of the IPL and INL. Scale bar = 2µm.

ii. Retina of irradiated animals:

The electron microscopic investigation of retina revealed many degenerative changes allover the different layers due to the long term microwave exposure of the developing animals in the prenatal and postnatal life. The striking histological feature is the reduction of the cellular population in these layers where they appeared decreased in their height or thickness. (Fig. 12). The layer of RPE is unremarkable. The photoreceptor layer (OS & IS) appeared thin, short and undergo disorganization and degeneration leading to death and loss of these elements. The ONL is represented in not more than 3 rows of degenerated cells, while the OPL is represented by one row of vacuolated neurons appeared depleted of their cytoplasmic constituents.

The most prominent ultrastructural event is the severe degeneration and complete absence of the RPE due to radiation exposure (Fig. 13). The RPE are completely destroyed and disappeared together with their melanin granules and the underlying Bruch's membrane. The EM examination of more than one field of exposed retina in all exposed animal confirmed this observation. Also, the field in Fig. (13) demonstrated severe destruction and disorganization of the photoreceptor elements (OS & IS) with atrophic nuclear degeneration inside the inner segment. Moreover, the melanocytes of choroid appeared with depleted melanin granules, disrupted mitochondria and crenated nuclear envelops.

The ultrastructural lesions in retinal cells produced by microwave exposure become to be more evident in magnified electron micrographs (Fig. 14). The continuity of the disc membranes was interrupted in some elements of OS and desintigrated completely in others. In this field one could demonstrate a focal interruption of the outer limiting membrane (OLM) between the photoreceptors and Müller cell processes. Moreover, the IS of photoreceptor cells undergo a marked process of shrinking and vacuolization.

Further EM investigation of other specimens of radiation-exposed retina disclosed one of the reactional behavior of the RPE where they appeared in a process of phagocytosis to the lower destructed portions of OS (Fig. 15) in spite of their severe degeneration. Other patterns of disorganization and degeneration due to radiation exposure in the IS and OS of photoreceptors could be detected. A devastating effect for the NIR on the organization of most OS_s was recorded, where the disc stacking was severely distorted and vesiculated (Fig. 16_a). Isolated portions of damaged OS within the layer of IS In addition to scattered pyknotic nuclei with densified and granular inner segments also appeared in Fig (16_b) together with greatly vacuolated and disrupted portions of IS.

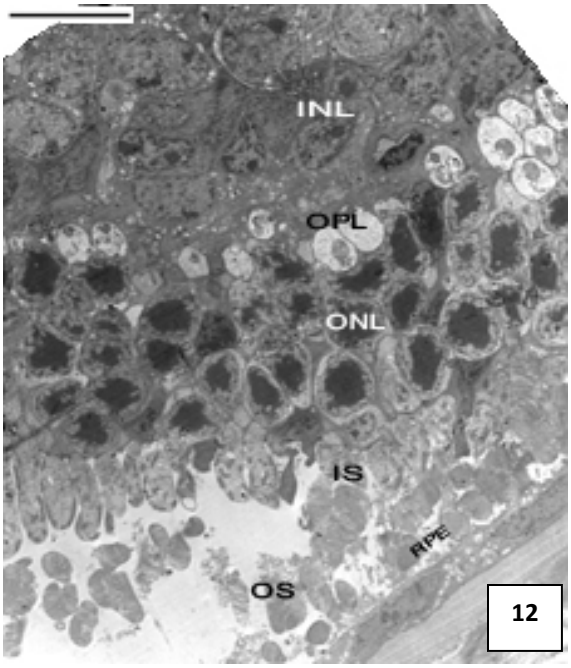
Concerning the impact of microwave irradiation on the ONL and OPL of retina, the EM exhibited marked ultrastructural lesions in these layers. The ONL appeared containing nuclei with lobulated and fragmented hetero-chromatin with mild vacuolization (Fig. 17), in other field appeared with shrunken chromatin leaving large vacuoles or having completely karyolysed nuclei (Fig. 18). At the same time the OPL of the same field appeared in reduced population of one layer with vacuolated and

degenerated elements depleted from their cytopasmic constituents (Fig. 17).

As regards the ultrastructure of the INL and IPL after microwave irradiation, the INL disclosed a highly reduced cellular population with apoptotic and necrotic cells together with extensive vacuolization in most of the constituent cells (Fig. 19). The IPL in its turn exhibited evident atrophic degenerations in the form of empty spaces containing cellular debris which may be originated from the damaged cells and being surrounded by degenerating synaptic buttons (Fig. 20).

By the EM examination of another fields for the IPL and ganglionic cell layer GCL, it was found that, the two layers contained extensive vacuolization developed to vacuolar degeneration with pronounced degenerating synaptic areas in the IPL. Whereas, the ganglionic cells (GC) appeared with crenated nuclear envelope, clumped heterochromatin and disrupted mitochondria (Fig. 21). The GCL exhibited more drastic ultrastructural changes in response to the microwave exposure of retina (Fig. $22_{a,b,c}$), where necrotic and completely degenerated GCs could be demonstrated near the inner limiting membrane (ILM) with concomitant intensive vacuolar changes. The double nature of the ILM was lost (Fig. 22_a). Moreover, Fig. (22_b) revealed swollen and vacuolated Müller's fibres (Müller cell processes) passing inbetween two GCs having disrupted mitochondria. The field also recorded the presence of a completely degenerated GC with a concomitant large vacuole. However the 3rd field (Fig. 22_c) demonstrated two affected GCs, one was apoptotic and the other with fragmented nucleus and vacuolated cytoplasm. There is also sub-ILM vacuoles.

Electron micrographs of the retina of microwave-exposed animals (Figs: 12-22)



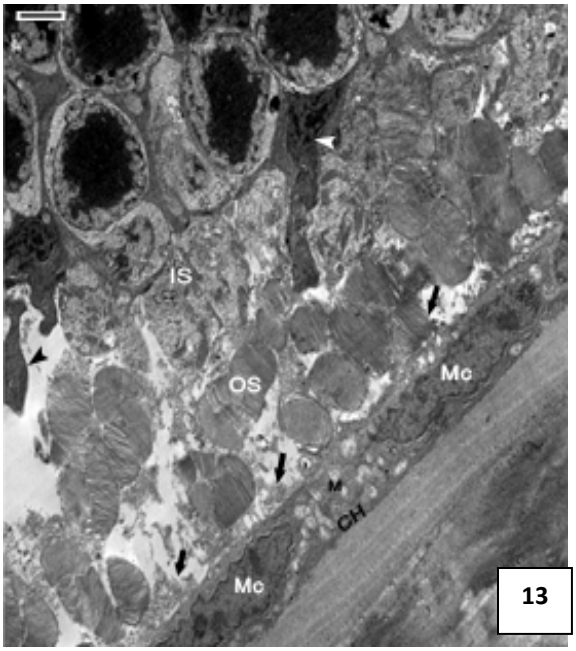
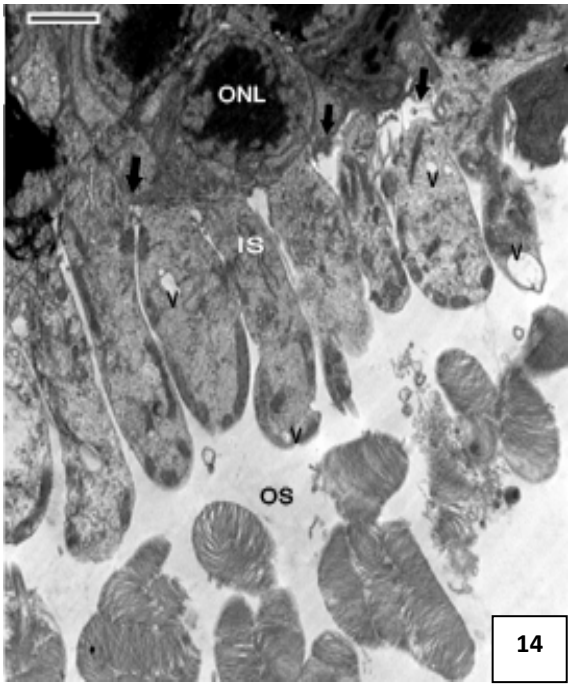


Fig. (12): Showing unremarked RPE, and several layers of the retina (OS, IS, ONL, OPL and INL) with reduced width and population. The OS and IS elements undergo shortening, degeneration, disorganization and final loss.
Fig. (13): A magnified part of Fig. (10) showing complete absence of RPE (arrows) obvious destruction of OS and IS. Atrophic nuclear degeneration (arrow heads), melanocytes (Mc) of CH with depleted melanin granules, disrupted mitochondria (M) and crenated nuclear envelops. Scale bar = 2µm.



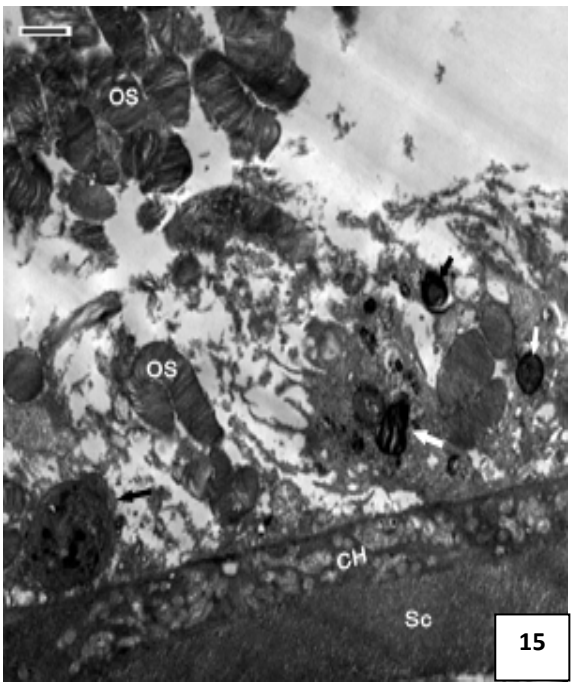
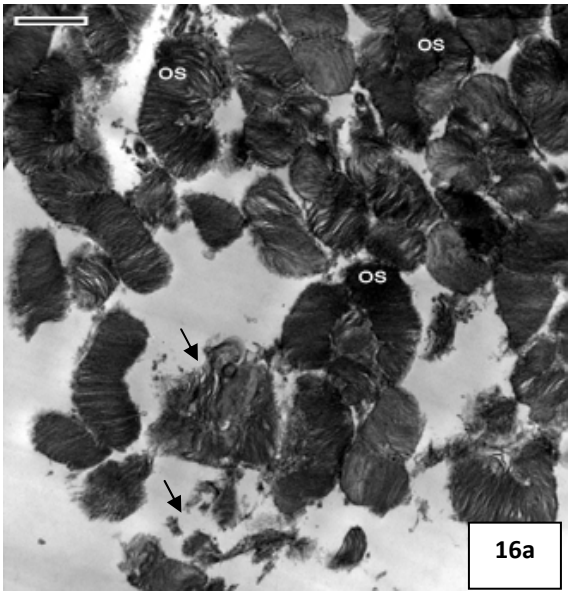
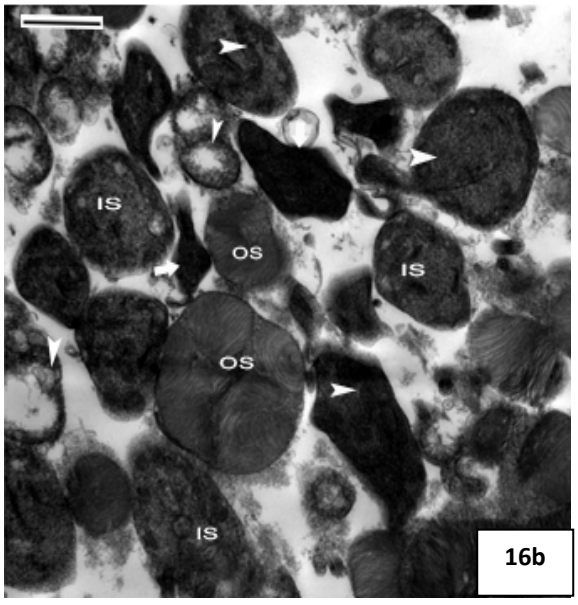
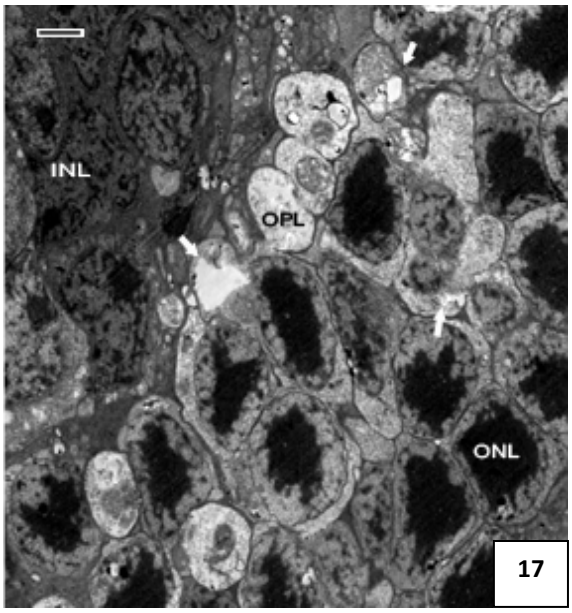


Fig. (14): Another magnified part of Fig. (10) to illustrate interruption and desintigration of disc membranes inside OS – shrinking and vacuolization (V) inside IS- focal interruption of OLM (arrows) – ONL. Scale bar = 2µm.
Fig. (15): showing degeneration and complete absence of RPE- phagocytosed portions of OS by RPE (arrows) – severe destruction of OS elements – vacuolated choroid (CH) – collagen fibres of sclera (Sc). Scale bar = 2µm.





Figa. (16_a & 16_b): Showing other patterns of OS disorganization and degeneration where in Fig (16_a) the disc stacking was severely distorted and vesiculated (arrows). While in Fig (16_b) there are isolated portions of OS inside the layer of IS together with scattered pyknotic nuclei (arrows) densified, granular and vacuolated disrupted portions of IS (arrow heads). Scale bars = 2µm for a & b.



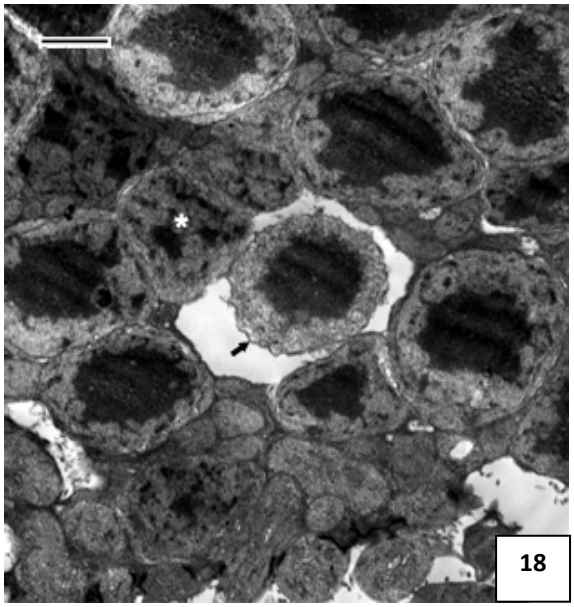
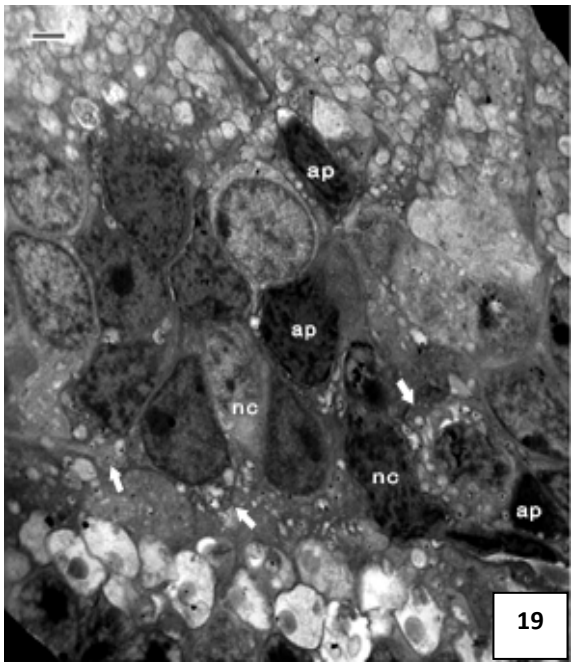


Fig. (17): showing the ONL, OPL and a part of the INL. ONL comprises nuclei with lobulated and fragmented heterochromatin and vacuolization (arrows), vacuolated and degenerated elements of OPL with depleted cytoplasmic constituents. Scale bar = 2µm.

Fig. (18): Another field of ONL containing nuclei with shrunken chromatin (arrow) and others underwent karryolysis (*). Scale bar = 2µm.



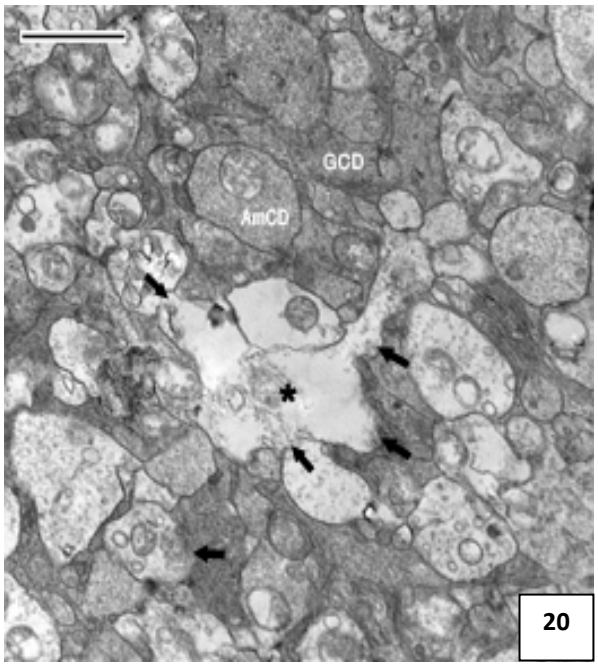
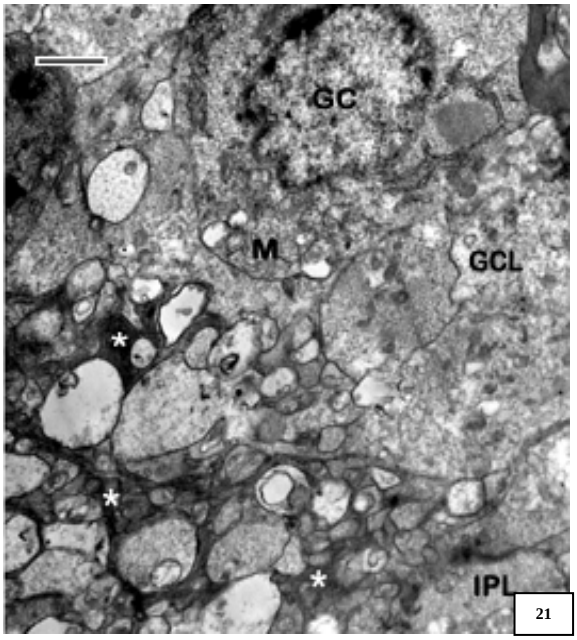


Fig. (19): showing INL with reduced cell population, apoptotic (ap) and necrotic (nc) cells and extensive vacuolization (arrows) – scale bar = 2µm.

Fig. (20): showing IPL with evident atrophy (*) and many degenerating synaptic buttons (arrows). Notice, a ganglion cell dendrite (GCD), amacrine cell dendrite Am CD. Scale bar = 2µm.



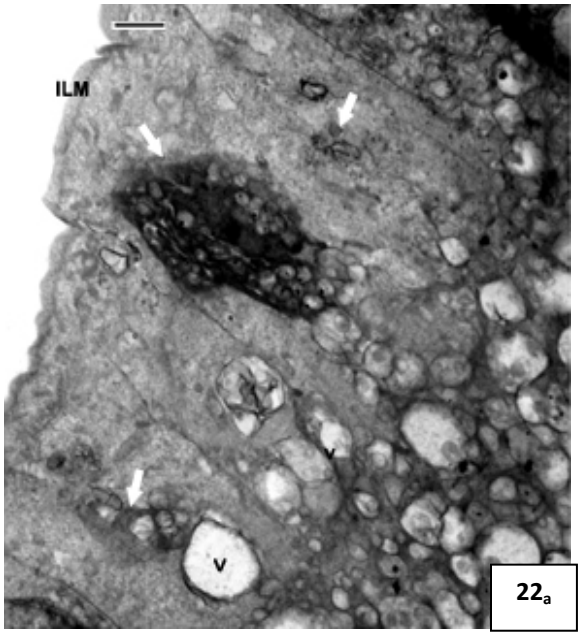
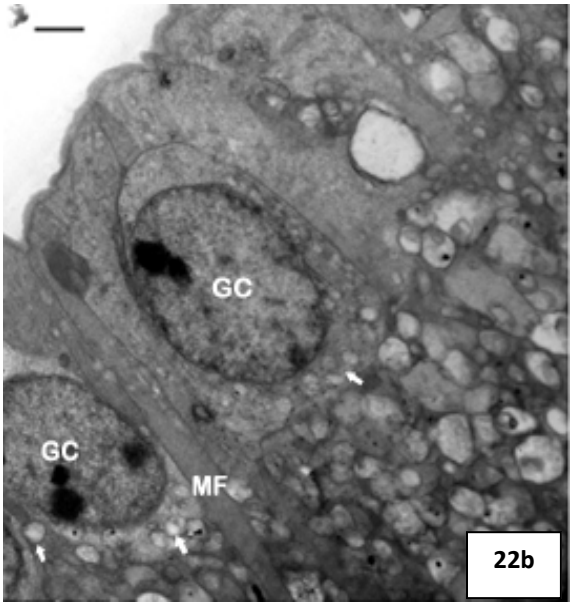


Fig. (21): showing part of IPL and part of the GCL illustrating extensive vacuolization in both of them (arrows). GC with crenated nuclear envelope, clumped heterochromatin and disrupted mitochondria (M). Degenerating synaptic areas (*) were evident inside IPL. Scale bar = 2µm.

Fig. (22_a): showing necrosis and complete degeneration of GCs (arrows) with intensive vacuolization (v), ILM = internal limiting membrane.



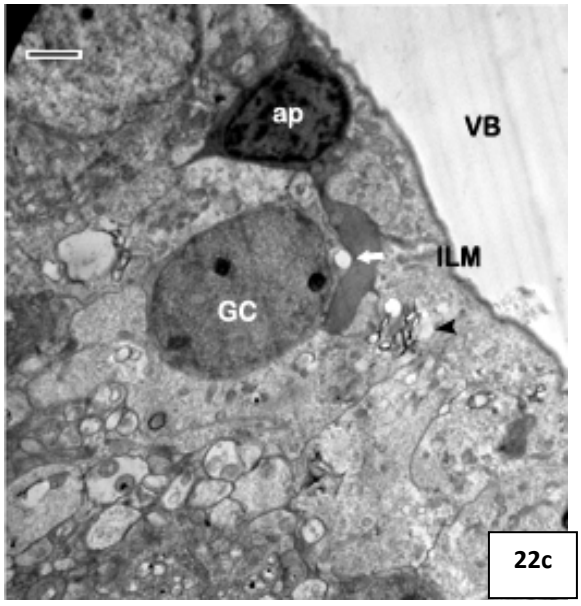


Fig. (22_b): exhibited a swollen and vacuolated Müller's fibres (MF) passing between two GCs with disrupted mitochondria (arrows)

Fig. (22_c): showing 2GCs, one is apoptotic (ap) & the other with fragmented nucleus and vacuolated cytoplasm (arrow). There is also sub- ILM vacuoles (arrow head). VB: Vitreous body. Scale bar = 2µm.

4. Discussion:

The use of mobile phones in last years has raised many questions about their safety. Moreover, it has been reported that mobile phones induce free radical formation in many tissues **(Knave, 2001 and Bortkiewicz, 2001).** The retina is expected to be very sensitive to microwave irradiation due to the polar character of the photoreceptor cells as well as to its high water content **(Kovacs et al., 1995).**

In the present investigation, the long term exposure of animals to the microwaves (900-1800 MHz) of a mobile phone exhibited different ultrastructural alterations in the retina of the developing mice. The application of microwave exposure to animals started at a critical point of development (embryonal life) and extended for a vast range of developmental time (4 months) postnataly. Development is a process characterized by a highly ordered sequence of cell proliferation, differentiation, migration and programmed cell death (particularly in nervous tissues). These events are guided by endogenous ionic currents and electric fields and disruption of these fields through microwave exposure can potentially affect these processes.

Concerning the RPE (first layer of retina), one of the most prominent observations recorded in the current study was the degenerative effect of the NIR on the RPE of the developing animals. These RPE underwent complete degeneration and disappearance together with their melanin granules in most of the studied specimens. But in other exposed retinae they appeared highly degenerated with captured phagolysosomal structures for the damaged OS of cones and rods. These results support and confirm the previous findings of **(Paulsson et al., 1979 and Zareen et al., 2009). Paulsson *et al.* (1979)** recorded degenerative changes in the retinal neurons of rabbit due to microwave exposure. The authors recorded ultrastructural changes in the glial cells and the retinal neurons (RPE) appeared containing phagolysosomal structures with myelin bodies. While **Zareen *et al.* (2009)** reported that microwave-exposure of mobile phone at first retarded the normal steps of retinal differentiation. This retarded process also included a hypopigmentation of the RPE, which further contributed to the depressed neuronal growth of the retina. The RPE plays an extremely important role in maintaining the functional activity of the photoreceptors, including degradation of photoreceptor outer segments **(Grierson *et al.*, 1994)**. Thus, agents that damage the RPE may have adverse effects on the photoreceptors and ultimately, on vision **(Remé *et al.*, 1996).** Therefore, in the present experiment the recorded degenerative effect of microwaves on the RPE and the depletion of their melanin granules is an important histological event as reported by several investigators. **Ilia and Jeffery (2000)** reported that vertebrate retinal pigment epithelium (RPE) cells are derived from the multipotent optic neuroepithelium develop in close proximity to the retina, and are indispensable for eye organogenesis and vision. Retinal cell mitosis is regulated by dopa, a melanin precursor present in the developing retinal pigment epithelium. Its absence results in retinal deficits. These granules have also been reported to be involved in many important functions, such as protection from oxidative stress, detoxification of peroxides, and binding of zinc and drugs, and therefore, serve as a versatile partner of the retinal pigment epithelial cell. There is an evidence that melanin granules are connected to lysosomal degradation pathway and deficit of melanin pigment is associated with age-related macula degeneration, the leading cause of blindness **(Schraemeyer and Heimann, 1999)**. Studies have also suggested that melanin granules in retinal pigment epithelial cells have a role in preventing the cytotoxicity **(Akeo *et al.*, 2000).** The pigmentation abnormalities reflect a possible deranged developmental process and damage vulnerability of the embryonal retina. Ocular melanin synthesis modulates rod photoreceptor production **(Ilia and Jeffery 2000)**. In addition, regional abnormalities in retinal development are associated with local ocular hypopigmentation **(Giménez *et al.*, 2005).**

The reduced retinal height (or thickness), due to radiation exposure and the depletion of the cellular population of the different layers particularly the OS & IS of photoreceptors, the ONL, the OPL and the INL noticed in this study was a striking histological feature. This may be explained as a lag of melanin maturation of the epithelium. Because melanin pigment and products associated with it regulate the maturation of the neural retina. It has been reported that in hypopigmented mammals the central retina has failed to develop fully **(Jeffery *et al.*, 1994).** Also, since melanin pigment guards against cytotoxicity, the hypopigmented retinae of exposed animals might be more vulnearable to cytotoxic insult **(Zareen *et al.*, 2009).** It may be also attributed to the degeneration of melanocytes of choroid and depletion of their melanin granules recorded in the current study. The onset which may impair the choroidal function as a middle vascular layer of the eye and finally affect the retinal tissue. This come in agreement with those of **Tanito *et al.* (2007)** who reported that the collapsed choroidal layer and circulation is involved in the loss of retinal tissue 1 and 3 months after severe light exposure. On the other hand the recorded abnormal thickening of choroid in semithin sections of exposed retina is explained as a sign of retinopathy **(Roy *et al.*, 2011)**

The layer of photoreceptor cells (rods and cones) was highly affected by mobile phone irradiation in the present study. They underwent destruction, disorganization, disruption, shrinking and shortening of IS, interrupted contiuity of their disc membranes, distorted and vesiculated disc stacking of OS_s, nuclear pyknosis and atrophic nuclear degeneration leading to complete

degeneration and photoreceptor cell death evidenced by the depletion of this layer. All these criteria are typical signs of radiation damage. A possible explanation of these changes in the structure of disc membranes could be the fatty acids composition of the membranes and radiation which render the ROS susceptible to lipid peroxidation. Lipid peroxidation has damaging effects on membrane integrity of the photoreceptors leading to retinal damage **(Anderson *et al.*, 1984).** This degenerative effect of NIR on the photoreceptor cells of retina may be also attributed to the free radicals generated in the living tissues due to the long term exposure to these NIR. These observations agree with other earlier studies showing that the low power microwaves (2.45 GHz) affect the membrane structure of the rod photoreceptor cells of retina and attributed it to a modification in the membrane fluidity of these cells which being strongly dependent on the power density of incident radiation **(Pologea-Moraru *et al.*, 2002). Ozguner *et al.*, (2006)** recorded protective effects of melatonin and caffeic acid phenethyl ester against oxidative stress in rat retina after long-term exposure to 900 MHz emitting mobile phone. The investigators attributed this oxidative damage to the reactive oxygen species (ROS) generated under the experimental conditions employed. Two mechanisms have been proposed that might lead from rod damage to cone damage. One is that rods produce a factor essential for cone survival **(Chalmel *et al.*, 2007)** and that rod loss reduces the expression of this factor below the levels required for cone integrity. In 1999 **Stone *et al.*,** suggested a more general mechanism involving oxidative damage. The authors proposed that depletion of the photoreceptor population (rod and cone) by any cause would reduce consumption of oxygen flowing from the choroidal circulation. Because this flow of oxygen is unregulated, photoreceptor depletion will cause a chronic increase in oxygen tension in the outer retina **(Yu *et al.*, 2000 and Yu *et al.*, 2004)**, and this increase in oxygen tension is toxic to surviving photoreceptors. The vulnearability of photoreceptors to hyperoxia has also been confirmed **(Walsh *et al.*, 2004 and Wellard *et al.*, 2005)** and evidence has been reported that rod loss results in oxidative damage to cones **(Shen *et al.*, 2005).**

The increased incidence of apoptotic and necrotic neural cells in the INL and GCL recorded in the present work come in agreement with those of **Sasaki *et al.*, (1999).** In the nervous system, neurons were excessively generated during the early stages of development and were all eliminated by apoptosis, except for those with appropriate functions in the course of development. It is believed that the selection by apoptosis is genetically programmed. But here, the present experiment, the chronic exposure of mice to mobile phone microwaves triggers the inducation of apoptosis in the mouse retina during the postnatal period. This suggests some type of apoptosis depends upon outside stimuli.

The vascular degeneration and pronounced degenerating synaptic areas recorded by the present investigation in the IPL due to radiation exposure come in agreement with those of **Paulsson *et al.* (1979).** The authors recorded many degenerating synapses in the same layer by EM after repeatedly microwave-exposed retina (1h exposure for up to 53h during about 100 days).

The results of the current experiment suggest that the ultrastructural changes recorded in the retinal neurons of irradiated (900-1800 MHz) mice may be regarded as an indicator of increased ROS production due to pathological processes driven by mobile phone exposure. Numerous previous studies demonstrated that ROS are directly involved in oxidative damage of cellular macromolecules (such as lipids, proteins and nucleic acids) after exposure to electromagnetic radiation from cellular phones **(Irmak *et al.*, 2002** and **Ilhan *et al.*, 2004).** Oxygen ROS free radicals may play a role in mechanisms of the biologic effects induced by electromagnetic radiation **(Mostafa *et al.*, 2001** and **Stopczyk *et al.*, 2005)**. In aerobic cells, reactive oxygen species (ROS) are generated as by-product of normal mitochondrial activity. If not properly controlled, ROS can cause severe damage to cellular macromolecules, especially DNA **(Barzilai and Yamamato, 2004)**. There may be some association between the overproduction of ROS and DNA damage induced by electromagnetic radiation.

In summary, the results of the present study confirm the retina's susceptibility to microwave injury particularly in the developing animals. This is because ten out of 10 albino mice exposed to microwaves (900- 1800 MHz, SAR 0.78 w/ kg) for 140 days (including the embryonal life) exhibited different ultrastructural changes in their retina.

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