The Egyptian Journal of Hospital Medicine (2009) Vol., 36: 585 - 607

Effect of Nonionizing Radiation onThe Cerebellum of Neonatal Mice

• Morphological, Histochemical And Ultrastructural Study

Samir A. Nassar
Zoology Department, Faculty of Science, Zagazig University, Egypt

(Received 13 December 2008 ; accepted 3 January 2009)

Abstract:

Introduction: Although the use of mobile telephones is common, increasing and beneficial, it is still considered as an environmental pollutant nowaday. This is because these devices require to be held close to the head and the exposure effects on the brain remain controversial. Being so, we designed this study.
Aim: The present study was done in an attempt to investigate the morphological, histochemical and ultrastructural changes produced in the cerebellum of neonatal mice as a result of exposure to the nonionizing radiation of the mobile phone.
Material and Methods: Eleven neonatal mice were used in this study. Five of them were exposed (as experimental group) to mobile phone microwaves (900- 1800 MHz, SAR: 0.92 w/kg) during their late prenatal and early postnatal life (1 hour/day for 30 consecutive days). While the other six served as control animals. Comparable parts of cerebella were removed from all animals and processed for the examination by the light and the transmission electron microscopes.
Results: The whole body exposure of the neonatal mice to this type of nonionizing radiation resulted in several morphological, histochemical and ultrastructural changes. These changes included a statistically significant decrease in the mean cell distribution, DNA content and total protein content of Purkinje cells and other cerebellar elements of exposed animals. On the other hand an increase in the Purkinje cell volume was recorded. In addition, the ultrastructural observations were corrugated plasma and nuclear membranes, ruptured mitochondria, destruction of Golgi apparatus , dilatation and disintegration of RER, scarcity of ribosomes and Nissl bodies in Purkinje cells. Damage in the cell membranes, chromatin clumping and increase in electron density of the cells of granular layer also observed. In the molecular layer; degeneration of axons and dendrites, increased electron density and damage of neurons occurred.
Conclusion: The whole-body exposure of neonatal mice to the nonionizing radiation produced many pathological lesions in their cerebella at the cellular and subcellular levels.

Introduction:

Radiofrequency fields of cellular phones may affect biological systems by increasing free radicals, which appear mainly to enhance lipid peroxidation and by changing the antioxidase activities of the subject blood leading to oxidative stress. Although the use of mobile telephones is common and increasing and requires these devices to be held close to the head, exposure effects on the brain remain controversial and prevent regulatory agencies giving an unqualified assurance of public safty *(Repacholi, 1998).* Furthermore, experimental findings have often been contradictory due to variable exposure conditions in diverse animal species and inadequate dosimetry. The brain, particularly cerebellum, is very metabolically active organ. The cerebellum has the propensity to be affected by environmental stress. It was proved that the cerebellum is the most sensitive part of the brain because it recorded the highest value of specific absorption rate "SAR" *(Chou et al., 1999).* Also, Purkinje cells can be easily identified for quantitative and

qualitative analysis. Therefore, the cerebellum and its Purkinje cells were chosen in this study since they may provide a good experimental model for the effects of the non-ionizing radiation on the mice brain. No DNA damage in brain cells was detected following exposure of rats to 2450 MHz microwaves pulsed wave at a specific absorption rate of 1.2 w/kg regardless of whether or not proteinase K was included in the assay. Thus, the results supported the conclusion that low- level 2450 MHz pulsed wave microwave exposure did not induce DNA damage detectable by the alkaline comet assay *(Lagroye et al., 2004). Monteiro et al. (1994) and Mohamed (2003)* recorded ultrastructural alterations in the rough endoplasmic reticulum (RER) cisternae in Purkinje cells and granular neurons of cerebellum of microwave exposed rats. They also recorded nuclear alterations to conclude a reduction in the ability of protein synthesis by these neurons which can influence the overall neuronal functions. The exposure of aged animals (6- 25 months of age) to the high powered microwaves (2.06 GHz, 2.2 w/cm^2) resulted in an accumulation of heat shock protein (HP70) in the cerebellum of male rats. This was recorded by *Walters et al. (2001)* during the investigation of the effect of aging on the capacity of the brain to produce HSP70 in response to heat stress using these non-ionizing radiations. A detectable histochemical reduction in the total protein content in all the cellular elements of cerebellum of ♂ and ♀ rabbits was recorded by *Shkorffo (2007)* after the exposure to mobile phone microwaves (900 MHz, 30 min. per day for 90 days, SAR rating 0.62 w/kg). *Ammari et al. (2008)* indicated that chronic exposure to GSM 900 MHz microwaves (SAR= 6 w/kg) may produce persistent activation of the acidic proteins in the astroglia of the rat brain. Therefore, the current study was designed to investigate the possible effects of mobile phone microwaves on the cerebellum of neonatal mice from the morphological, histochemical and ultrastructural points of view with special emphasis on Purkinje cells. Another purpose of this study is to shed some additional light on the so-called dark Purkinje (DP) and clear Purkinje (CP) cells.

Material and methods

i- Animals:

Two pregnant mothers of mice were used to obtain the mice pups required for this study. One of them was separated and subjected to the whole body exposure of mobile phone microwaves 6 days before delivery. It gave 5 pups served as exposed (experimental animals). Then the pups were exposed postnataly for 24 consecutive days to complete their exposure course into a total of 30 days. The second mother was chosen from the pregnant mothers of the animal house that underwent delivery at the same day with the first one. It gave 6 pups served as control (unexposed) animals. They were housed in plastic cages. Animals also were kept under normal room conditions of temperature, humidity and normal day and night cycle and freely supplied with food and water.

ii- Experimental design:

Mice pups were categorized into 2 main groups:

Group (1): Comprising the 6 control (unexposed) mice pups.

Group (2): Comprising the 5 experimental (exposed) mice pups. Irradiation of these newly- born mice was performed for 30 consecutive days (6 prenataly and 24 postnataly) using 900-1800 MHz microwave of mobile phone (model Nokia 6210, SAR 0.92 w/kg). During exposure animals were housed in a special circular plastic container of 9 cm diameter and 3 cm height. The top, sides and bottom of this container were perforated to permit proper ventilation. Irradiation was performed by putting the used mobile phone in a direct contact to the top of the exposure cage 0.5-1 cm distant from their bodies (Fig. 1).

Animals received whole- body microwave irradiation 1 hour per day for 30 consecutive days. The nearest parts of the animal bodies to the source of emission were their heads. This is due to continuous movement as a result of irradiation perturbations which made their heads permanently arrayed in a circle near the source of emission. The control (un-exposed) animals were housed in a similar container away from irradiation for the same period every day.

iii- Histological and histochemical techniques:

Animals were euthanized and fresh small pieces from identical areas of cerebella of control and experimental animals were fixed in 10% neutral buffered formalin & carnoy's fluid for histological and histochemical studies. Paraffin sections were prepared 3µm thickness and stained with Harris haemotoxylin and eosin *(Bancroft and Gamble, 2002)*. DNA content was detected by the Feulgen reaction *(Feulgen and Rossenbeck, 1924)* and counter stained with light green. Proteins were detected by mercuric bromophenol blue (MBB) method (Mazia et al., 1953). Nissl substances were stained by 1% methylene blue followed by a mild differentiation in equal parts of absolute alcohol and dioxane until the slides become colourless against the stained Nissl granules.

iv- Morphological studies:

a) The Purkinje cell distribution in the stained histological sections of cerebella of control and exposed animals was performed as follows: we draw a line parallel to the Purkinje cell line in the section and we got
the length of this line. Then we find out the number of cells along this line and then
divide the number of cells by the length of that line to obtain the cell distribution. This was performed on different slides and different localities in each slide to obtain an average mean for cell distribution.

b) Mean Purkinje cell volume was performed for both groups of animals using a large number of Purkinje cells that having conspicuous nuclei and nucleoli.

v- Image analysis*:*

a) Measurements of the optical density of protein material were performed using IP win 32 software. Data were statistically analyzed using Microsoft Excel 2007 software.

b) Measurements of the optical density of Nissl substances and DNA content were

performed using IP win 4 image proplus version 4S 0.29. Data were statistically analyzed using Microsoft Excel 2003.

vi- Electron microscopy technique:

Ultrastructural studies have been conducted on specimens prepared as follows: fresh cerebellar specimens were removed from the animals of both groups, cut into small pieces (0.5- 1mm) and immediately fixed in cold 4% glutaraldehyde in 0.2 M cacodylate buffer (pH= 7.2) and kept for 24 hours at 4°C *(Sabatini et al., 1963)*. After washing in the same buffer, specimens were post fixed in 1% O_SO_4 in cacodylate buffer (pH= 7.2) then embedded in plastic resin *(Robenson et al., 1987)*. Semithin sections (1µm thickness) were cut using the ultratome, mounted on glass slides and stained with toluidine blue for light microscopic examination and to select areas for ultrastructural studies. Ultrathin sections were also cut, mounted on coated copper grids, stained with uranyl acetate and lead citrate *(Echlin, 1964)* for transmission electron microscopic examination (Jeol- JEM) at the electron microscopy unit, Ein-Shams University. Negative Kodak film (black & white) were used.

Results:

The exposure of mice pups to the mobile phone microwaves (900/1800 MHz and SAR rating 0.92 w/kg) resulted into many morphological, histochemical and ultrastructural changes in cerebella of these animals:

* ***Qualitative and quantitative observ-ations:***

i: Purkinje cell distribution and volume:

Examination of the cerebellar sections, stained with H & E, from irradiated animals revealed an obvious degeneration and paucity of Purkinje cells (PC) as compared to those of control animals (Figs. 2, 3). The statistical analysis of the mean cell distribution of Purkinje cells in control and exposed animals confirmed this observation where the probability was $P \leq 0.05$ i.e. approaching a significant value (Table 1, Fig. 6). At the same time a statistical analysis for the mean Purkinje cell volume was performed to reflect the activity and the metabolic status of these cells. This study recorded an increased difference in the PC volumes in exposed animals than control ones. But the increase was statistically non- significant (Table 2, Fig.7).

ii- DNA content*:*

The histochemical detection of the DNA material in the cerebellar tissue of both groups of animals provided a detectable change. In controls, the granular cells gave a strong (+++) positive reaction while the Purkinje cells and molecular cells gave a weak (+) and moderate (++) reactions respectively. But, the intensity of Feulgen reaction in cerebellar cells of exposed animals was reduced as compared to that of controls (Figs. 4, 5). These findings were confirmed by the image analysis of the stained sections. The mean optical density (MOD) values of the DNA content in the three cortical layers were analyzed. A statistically significant decrease was recorded for the three cortical layers where the P values were 0.0001, 0.0142 and 0.0374 for the granular, Purkinje and molecular layers respectively (Table 3, Fig. 8).

iii- Nissl substances in Purkinje cells*:*

The histochemical assessment of the Nissl bodies was performed on 1% methylene blue- stained slides. The light microscopic examination revealed an intense positive reaction for these tigroid substances in the nucleoli and cytoplasm of Purkinje cells of controls. The reaction was in the form of compactly- packed rods characteristic for Purkinje cells (Fig. 9). There was a change in the pattern of Nissl bodies of many Purkinje cells in the exposed animals as compared to controls. The Nissl substances appeared in the form of finely-dispersed granules instead of the compact rod-shaped appearance generally demonstrated under the light microscope in the normal Purkinje cells (Fig. 10). The image analysis for the MOD values of Nissl positive material in control and exposed animals confirmed the qualitative observations. The analysis recorded a statistically significant (P= 0.03) decrease in exposed as compared to control animals (Table 4, Fig. 13).

iv- Total proteins:

Examination of cerebella of irradiated mice pups with the light microscope revealed a marked decrease in the total protein content

of all constituent cells as compared to those of controls (Figs. 11,12). This histochemical reduction in the protein material was confirmed quantitatively by the image analysis. The recorded MOD values of the protein content of the three cortical layers revealed a highly significant decrease (Table 5, Fig. 14) where the P value was ≤ 0.000001.

v- Results obtained from semithin sections: Examination of semithin sections stained with toluidine blue under the light microscope revealed many cytological features in both groups of animals. In the control mice pups, the cerebellar cortex showed the two types of Purkinje cells; DP and CP. The cytoplasm of CP appeared voluminous and diffusely stained with small but more intensely-stained areas. These areas representing sites of Nissl granules. The granular layer exhibited multiple closely packed cells. Their nuclei contain small clumps of chromatin (Fig. 15). In irradiated (exposed) mice pups, the cerebellar cortex showed several Purkinje cells, (CP and DP). They were in different stages of degeneration. Some of them degenerated leaving empty spaces. Many granular cells were deeply stained and clumped together with glial cells in some sort of gliosis. The degeneration of granular cells resulted in a pericellular oedema in the granular layer (Fig. 16).

vi- Ultrastructural observations:

control animals:

Examination of cerebella of unexposed animals with the EM revealed many histological criteria. The molecular layer showed a basket cell with large nucleus, little granular cytoplasm and aggregated mitochondria at the axon hillock. It contained also dendrites of Purkinje cells and unmyelinated axons from the granular layer (Fig. 17). The EM examination of the Purkinje layer revealed large cells containing large nuclei. They are of two types; dark (DP) and clear (CP) Purkinje cells. The DP in Fig. (18) appeared with large nucleus and voluminous cytoplasm. Both of them are electron dense. The nucleolus and Golgi apparatus are well-developed. The elements of RER and ribosomes are present at the vicinity of the nuclear membrane forming a nuclear cap. The nuclear membrane is slightly invaginated at different sites. In a magnified part of the last EM, the Golgi apparatus units appeared mostly in the form of elliptical stacks taking a straight aspect associated with a number of vesicles. Many mitochondria of variable sizes were also present (Fig. 19) together with orderly arrays of rough endoplasmic reticulum. In another EM of CP of control animal (Fig. 20), the cellular details appeared more clearly where the nucleus is pale with fine dispersed chromatin (euchromatic). Its karyoplasm having many coarse granules. The cytoplasm is also pale, voluminous and filled with dispersed ribosomes most of them aggregated with the RER as a nuclear cap. A large number of mitochondria of variable shapes and sizes is found throughout the cytoplasm. The nuclear membrane is slightly invaginated and visible but not dilated. Fig. (21) illustrated an EM of another CP from a control animal revealing the abundance of the free and membrane-bounded ribosomes which represent the Nissl bodies upon which organized the elements of the RER. In that field one Golgi apparatus unit is present in the form of spherical shape together with some vesicles. The nuclear membrane is double-layered but not dilated. The EM examination of the granular layer (Fig. 22) showed closely packed cells. They having well-defined nuclei and thin shell of scanty cytoplasm with few apparently normal mitochondria .Most of their bodies were rounded or polygonal. Interstitial microgliacytes having a hyperchromophilia appeared in the field. The RER is scanty in their cytoplasm.

Exposed animals:

The ultrastructural examination of cerebellar cortex in the molecular layer (Fig. 23) illustrated different signs of degeneration including vacuolated and degenerated foci inbetween the dendrites of Purkinje cells and the non-myelinated axons of the granular cells. Marked shrinkage and distortion of cell bodies. The cells appeared with ill-defined nuclear membrane. Some nuclei and cytoplasm of the molecular cells appeared more electron dense. Mitochondria were few and disrupted outside the cell membrane. Examination of the DP cell by EM (Fig.24) revealed also several signs of degeneration. It exhibited corrugated plasma and nuclear

membranes. The nucleus and cytoplasm are very electron dense with nuclear shrinkage. This type of Purkinje cells called DP-2 cells which having no nucleolus. It is completely absent. Another field of EM (Fig. 25) exhibiting 2 Purkinje cells (CP and DP)from exposed animal showing high grade of affection and degeneration. The two cells having abnormal polymorphous nuclei. The CP showed a more electron dense nucleus and cytoplasm as compared to those of controls. The nucleolus appeared fragmented. The cell boundary is distorted and giving several perisomatic processes having large accumulation of mitochondria. Some of these processes making synapses with the neighbouring cells and axons. Also, there is a scarcity of Nissl bodies (ribosomes) and disintegration of RER in the cytoplasm Some degenerated granular cells with ruptured cell membranes appeared in the field. the cytological features of the second Purkinje cell (DP) will be discussed on the next electronmicrograph (Fig. 26). This part of the cell is magnified (× 12000) to illustrate clearly the cytological lesions occurring to the DP cells as a result of microwave irradiation. The cell appeared as a bizarre looking immature Purkinje cell. It showed a lobulated part of the nucleus. As a rule this surface of the nucleus is pointing upward toward the surface of the cortex. The RER in the form of disorderly arrayed stacks. They appeared dilated with clear contents. The cytoplasm having distorted mitochondria being swollen with ruptured cristae. In another EM from an exposed animal (Fig. 27) illustrating part of CP exhibiting a group of lesions to confirm the radiation hazards at the subcellular level. The most prominent feature is the disruption of mitochondria which appeared swollen with ruptured cristae. The cell also showed a shrunken nucleus and disintegration of RER. The cytoplasm appeared more electron dense than that of the control animal. Examination of another field of cerebellar cortex of exposed animals (Fig. 28) illustrating degenerated elements of the granular layer. Most of the granular cells appeared with ruptured cell membranes and nuclei with clumped chromatin material. Two Purkinje cells appeared in the field each having disrupted mitochondria.

Explanation of Figures :

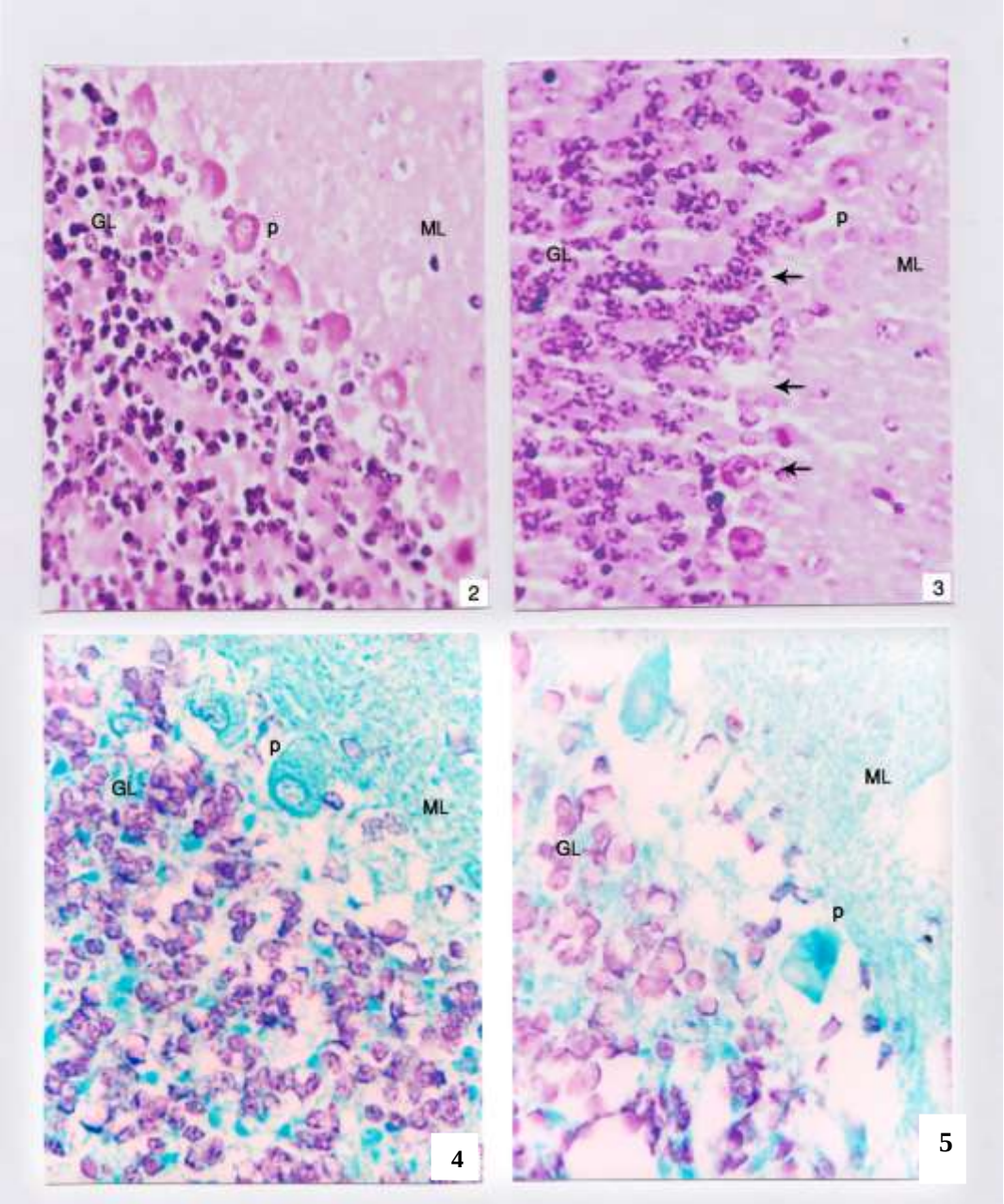


Fig. (2): Representative micrograph of cerebellum of control animal stained with H & E (× 400) showing Purkinje cells (P) in a linear arrangement at the interface between molecular (ML) and granular (GL) layers.

Fig. (3): Representative micrograph of cerebellum of exposed (treated) animal stained with H & E (× 400). Arrows point to the scarcity and destruction of Purkinje cells (P). GL= granular layer. ML= Molecular layer.

Fig. (4): Section in cerebellum of control animal stained with Feulgen's reaction for DNA and counter stained with light green (× 400) showing intense reaction in granular layer (GL), a weak reaction in Purkinje cells (P) and moderate reaction in molecular layer (L).

Fig. (5): Section in cerebellum of exposed animal stained with Feulgen's reaction for DNA and counter stained with light green (× 400) showing a marked decrease in the DNA content of the three cortical layers (GL, P, ML).

Table 1: Mean cell distribution of Purkinje cells in control and exposed (treated) animals.

	Control	Treated
Mean cell distribution	12.77221±3.233	10.05264±2.372*

(t-test) p≤ .05

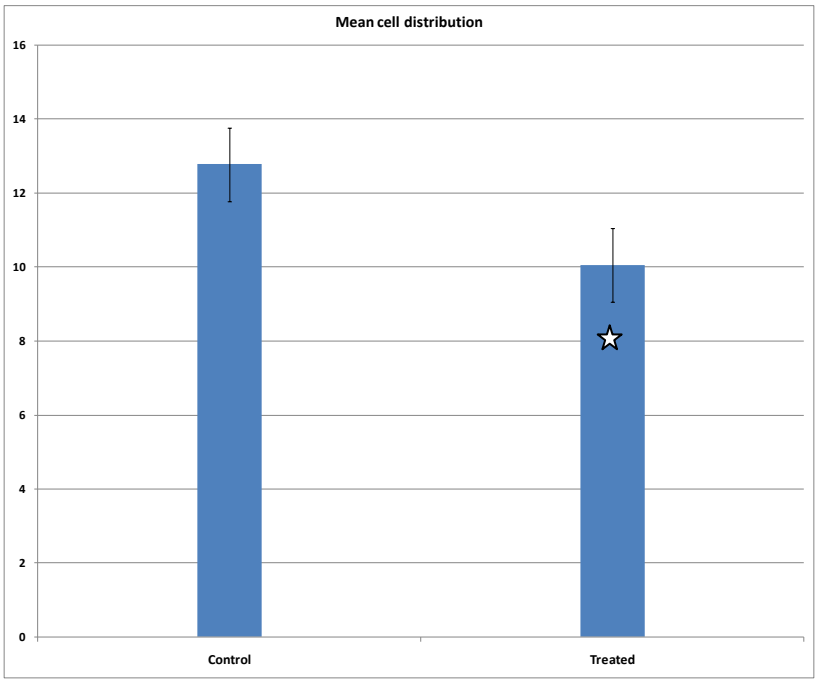


Fig. (6): Histogram showing the mean cell distribution of Purkinje cells in both groups of animals.

Table 2: Showing mean Purkinje cell volume:

	Control	Treated
Mean	85645.57	92228.14
SE	2232.452	5256.686
p (t-test)		0.173146

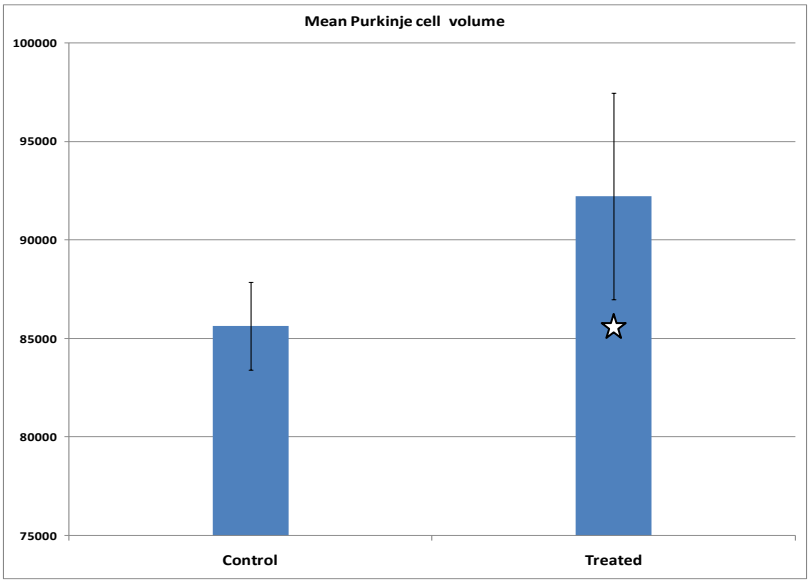


Fig. (7): Histogram showing mean Purkinje cell volume in both groups of animals. Error bars are standard error of the mean.

Table (3): Showing the mean optical density values (MOD) of the DNA content of the three cortical layers in both groups of animals.

	Control			Treated		
	Molecular layer (M)	**Purkinje's cell layer (P)**	**Granular layer (G)**	**Molecular layer (M)**	**Purkinje's cell layer (P)**	**Granular layer (G)**
Average	0.1208	0.0788	0.2074	0.0969	0.0672	0.149
S.D.	0.029431	0.0073454	0.022804	0.014449	0.016732	0.045031
t test				0.037406	0.014214	0.000181
Probability				Sig.	Highly Sig.	Very Highly Sig
% of change				-19.7848	-14.7208	-28.1581

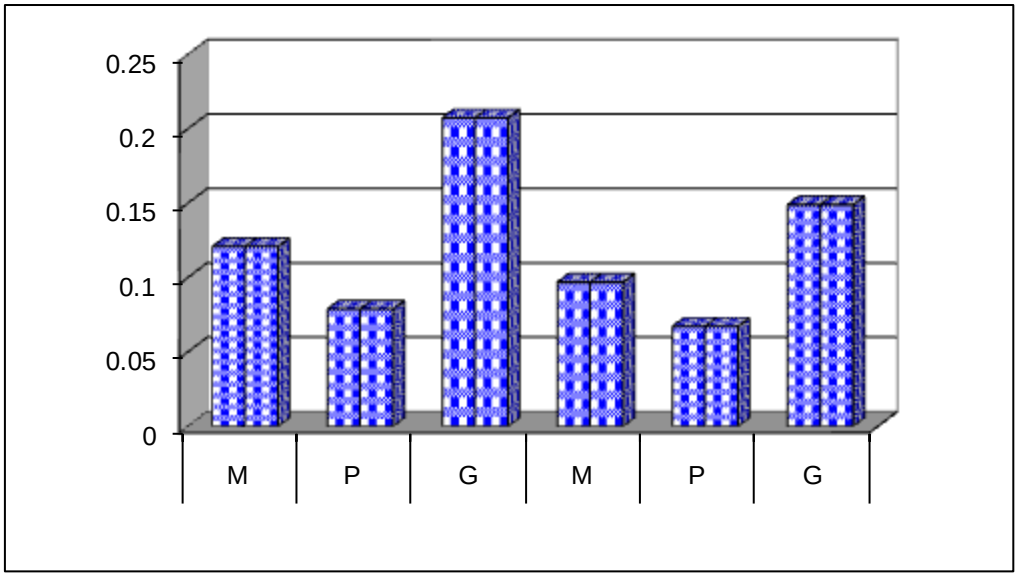


Fig. (8): Histogram representing the relation between the MOD values of DNA positive material in the cortical cerebellar layers of the two different groups

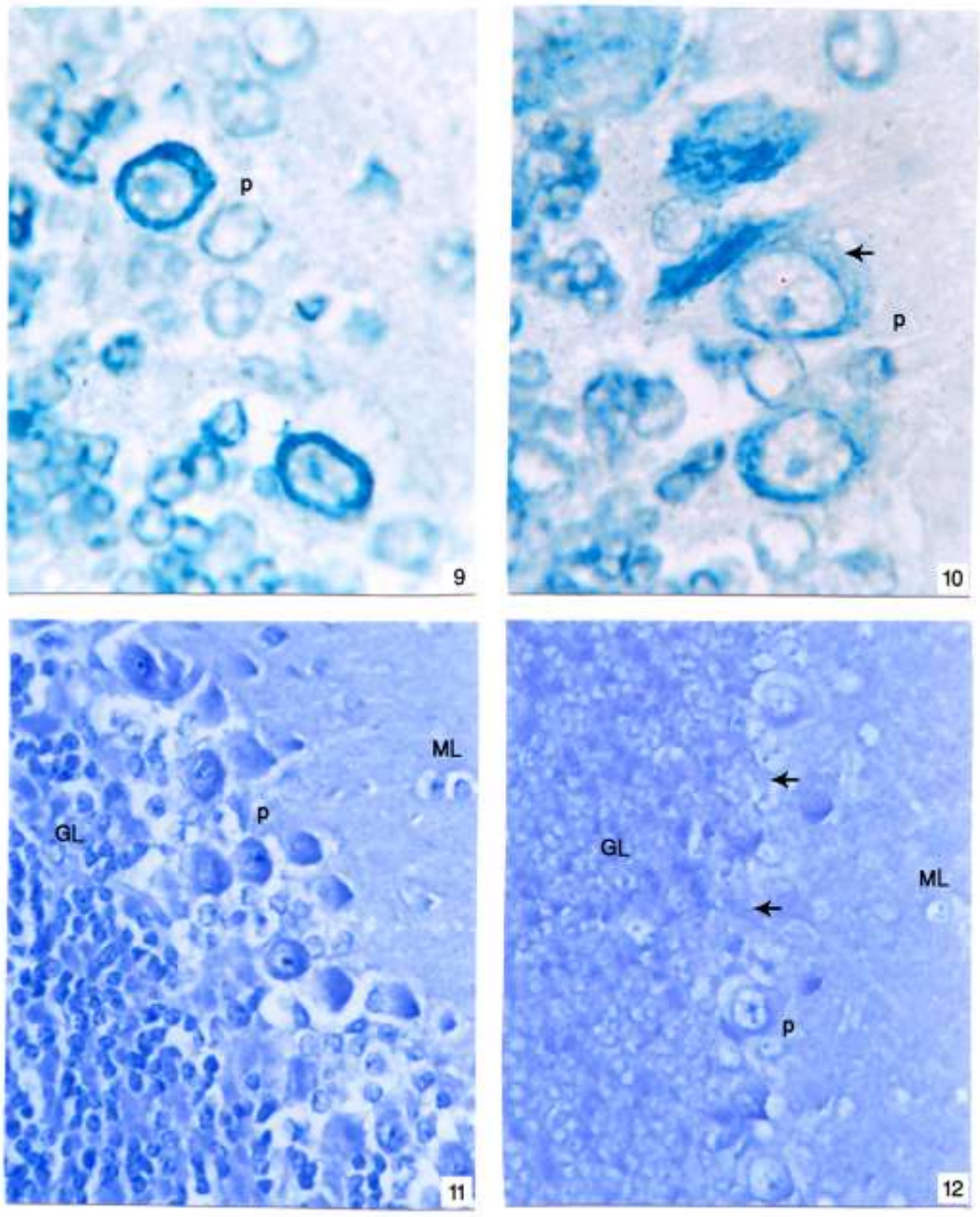


Fig. (9): Section in cerebellum of control animal stained with 1% methylene blue for Nissl substance (× 400) showing intense reaction of this material in the nucleoli and cytoplasm of Purkinje cells (P), in the form of compactly packed rods characteristic of Purkinje cells.
Fig. (10): Section in cerebellum of exposed animal stained with 1% methylene blue for Nissl (× 400) showing a weak reaction in Purkinje cells (P) in the form of fine granules (arrow).
Fig. (11): Section in cerebellum of control animal stained with MBB for protein (× 400) showing a high positive reaction inside the cells of the three cortical layers (GL, P, ML) particularly those of the granular layer.
Fig. (12): Section in cerebellum of exposed animal stained with MBB for protein (× 400) showing a marked decrease in the protein positive material in the cells of the three cortical layers (GL, P, ML). Notice the absence and destruction of Purkinje cells (↑).

Table (4): Showing the MOD values of Nissl positive material in control and treated (exposed) animals

	Control	Treated
Average	0.133	0.113
S.D.	0.010625	0.025166
t test		0.030477
Probability		Sig.
% of change		-15.0376

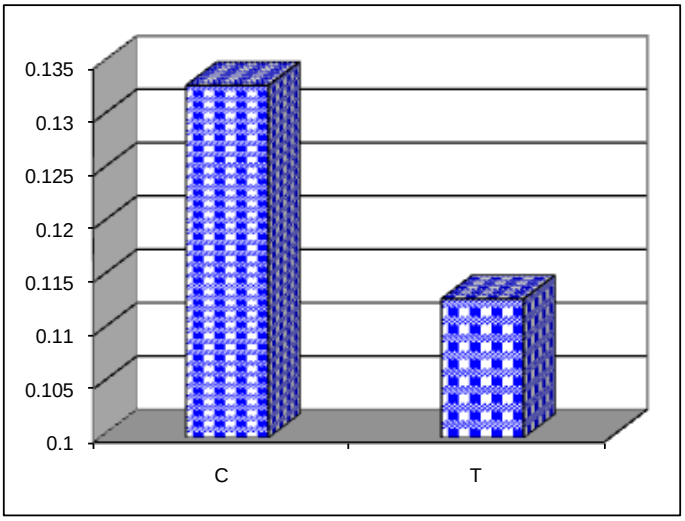


Fig. (13): Histogram illustrating the relation between MOD values of Nissl positive material in Purkinje cells of control and treated (exposed) animals.

Table 5: Showing the MOD values of protein content of molecular layer (ML), Purkinje cell layer (PL) and granular layer (GL).

	Molecular layer	Purkinje cell layer	Granular layer
Control	0.172662 ±0.021066	0.188297 ±0.035528	0.189117±0.027938
Treated	0.139636 ±0.017362*	0.140173 ±0.016014*	0.133091±0.016285*

- T-test :$p \leq .000001$

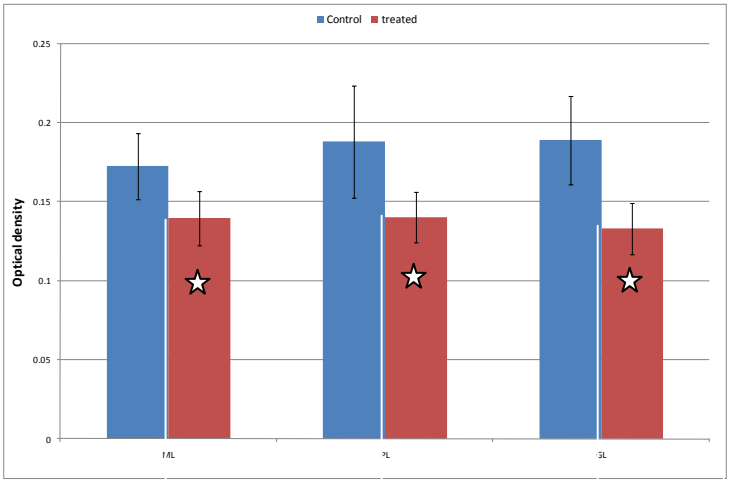


Fig. (14): Histogram revealing the relation between MOD values of protein material in the three layers ML, PL and GL of control and treated (exposed) animals.

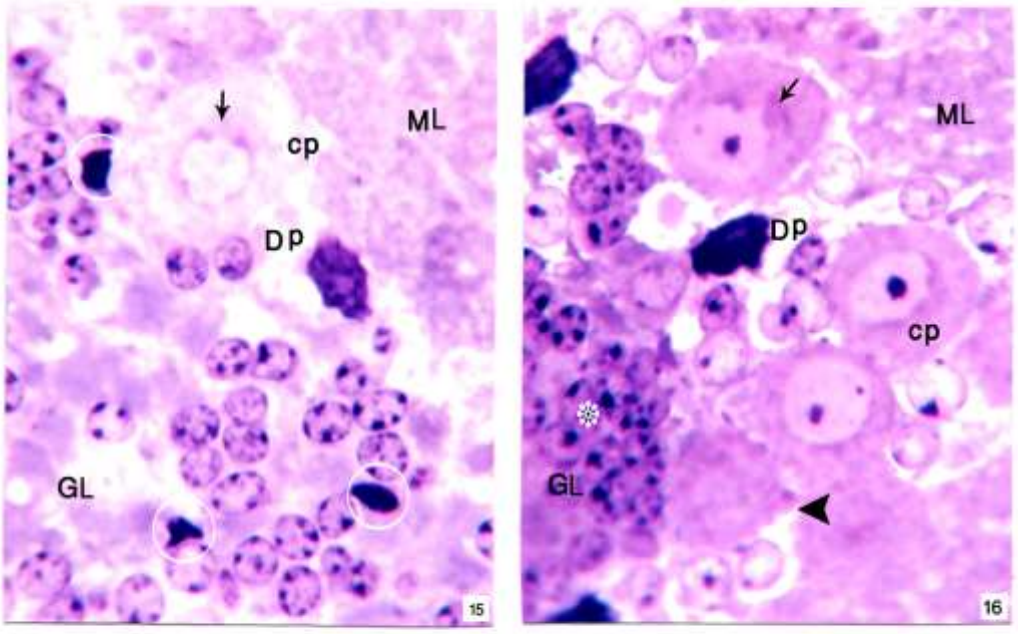
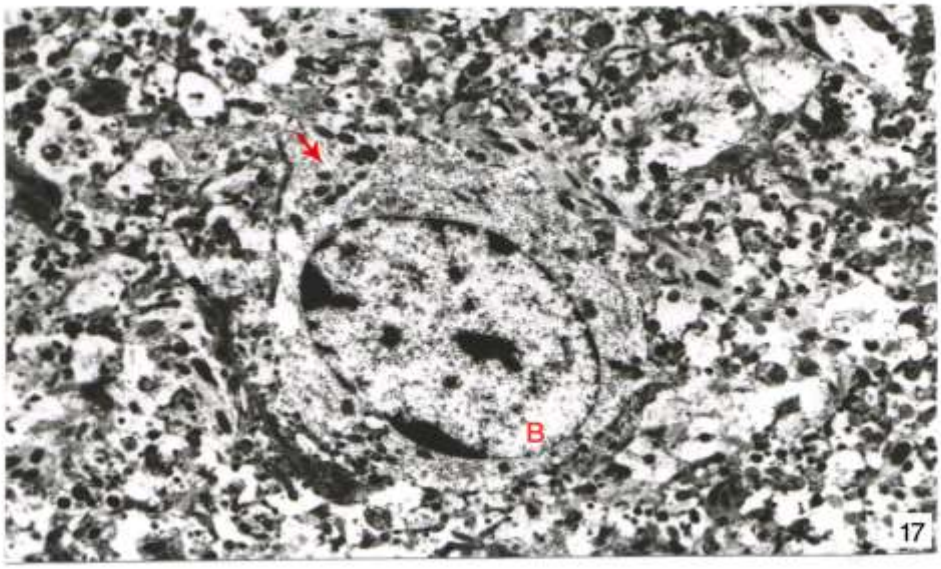


Fig. (15) : A semithin section of cerebellar cortex of a control mice pup revealing part of the molecular layer (ML), tow Purkinje cells one dark DP and one clear (CP) at the interface between molecular layer (ML) and granular layer (GL). The DP showing the most distinct chromophilia from that of the adjacent CP. The CP having lightly stained cytoplasm except at the site of Nissl bodies which appeared intensely stained (↑). The granular layer showed multiple closely packed cells. In the field 3 interstitial microgliacyte (circles) having hyperchromophilia appeared.

Fig. (16): A semithin section of cerebellar cortex of irradiated (exposed) mice pup illustrating deeply stained Purkinje cells (DP & CP) in different stages of degeneration. Notice the site of Nissl bodies in DP (↑), gliosis (*) and pericellular oedema (arrow head).



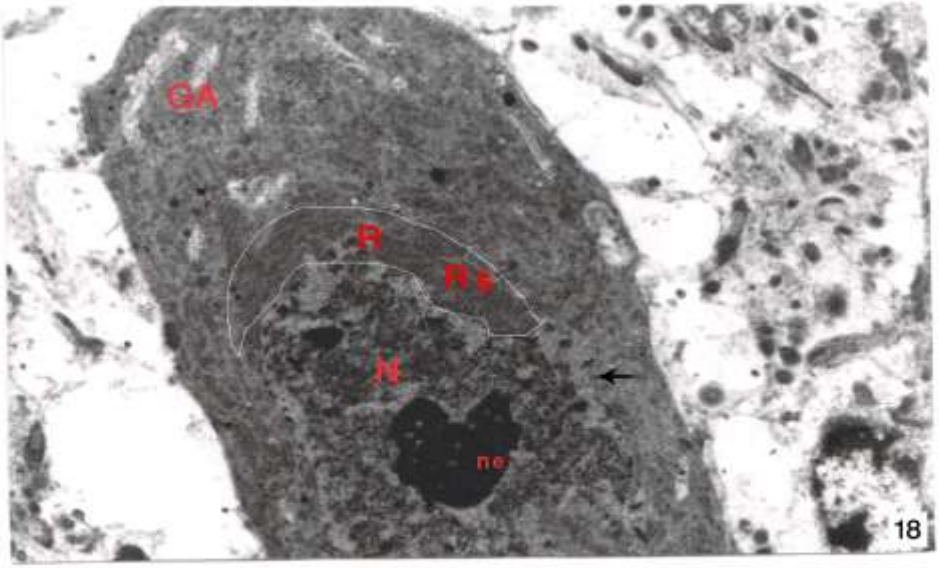
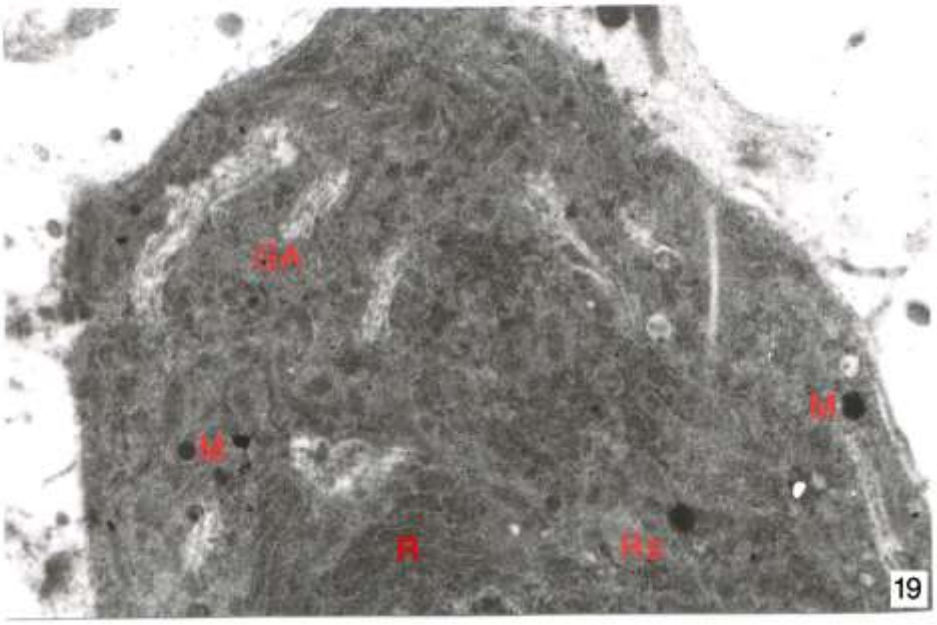


Fig. (17): Electron micrograph (EM) of cerebellar cortex (× 3000) of control mice pup showing: a basket cell (B) with large nucleus and little granular cytoplasm. Notice, an aggregation of mitochondria at the axon hillock (↑). It is surrounded by many dentrites of Purkinje cells and unmyelinated axons of the granular cells.

Fig. (18): EM of cerebellar cortex of control animal (× 4000) showing dark Purkinje cell having normal appearance, nucleus (N) with irregular outline and well-developed nucleolus (ne) and Golgi apparatus (GA). Cytoplasm is electron dense. RER (R) and ribosomes (Rs) forming a nuclear cap (limited). Invaginated nuclear membrane (↑).



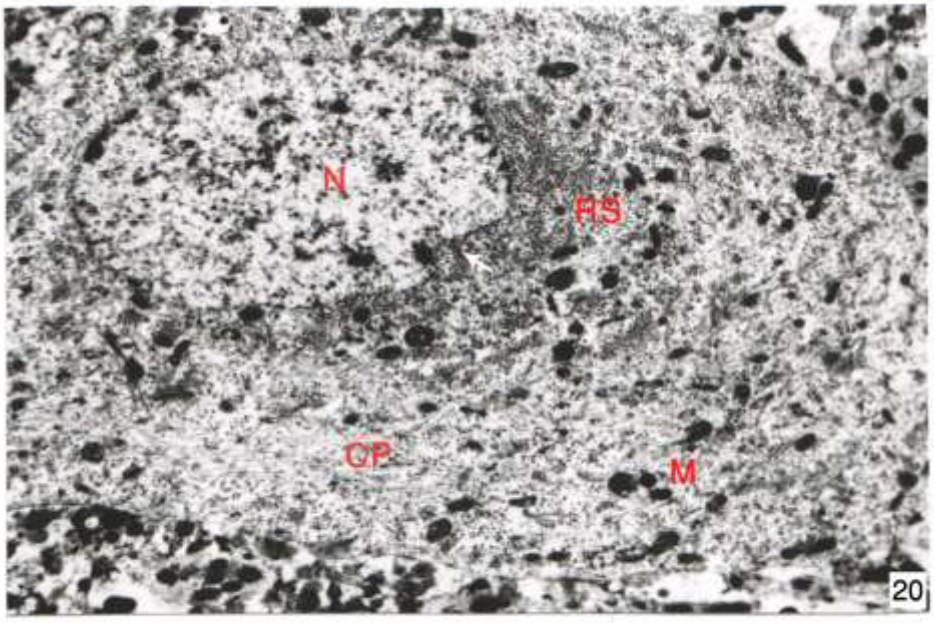


Fig. (19): Magnified part of the last EM (× 15000) illustrating characteristically well-developed Golgi apparatus (GA) units appeared either spherical or elliptical, abundant ribosomes (RS), aggregated between the cisternae of RER, orderly arrays of RER (R), mitochondria (M) of variable sizes.

Fig. (20): EM of CP of control mice pup (× 4000) showing: its outstanding size, normal and numerous mitochondria (M), euchromatic nucleus (N) with karyoplasm having many coarse granules, invaginated, visible but not dilated nuclear envelope (↑) and numerous free ribosomes (Rs).

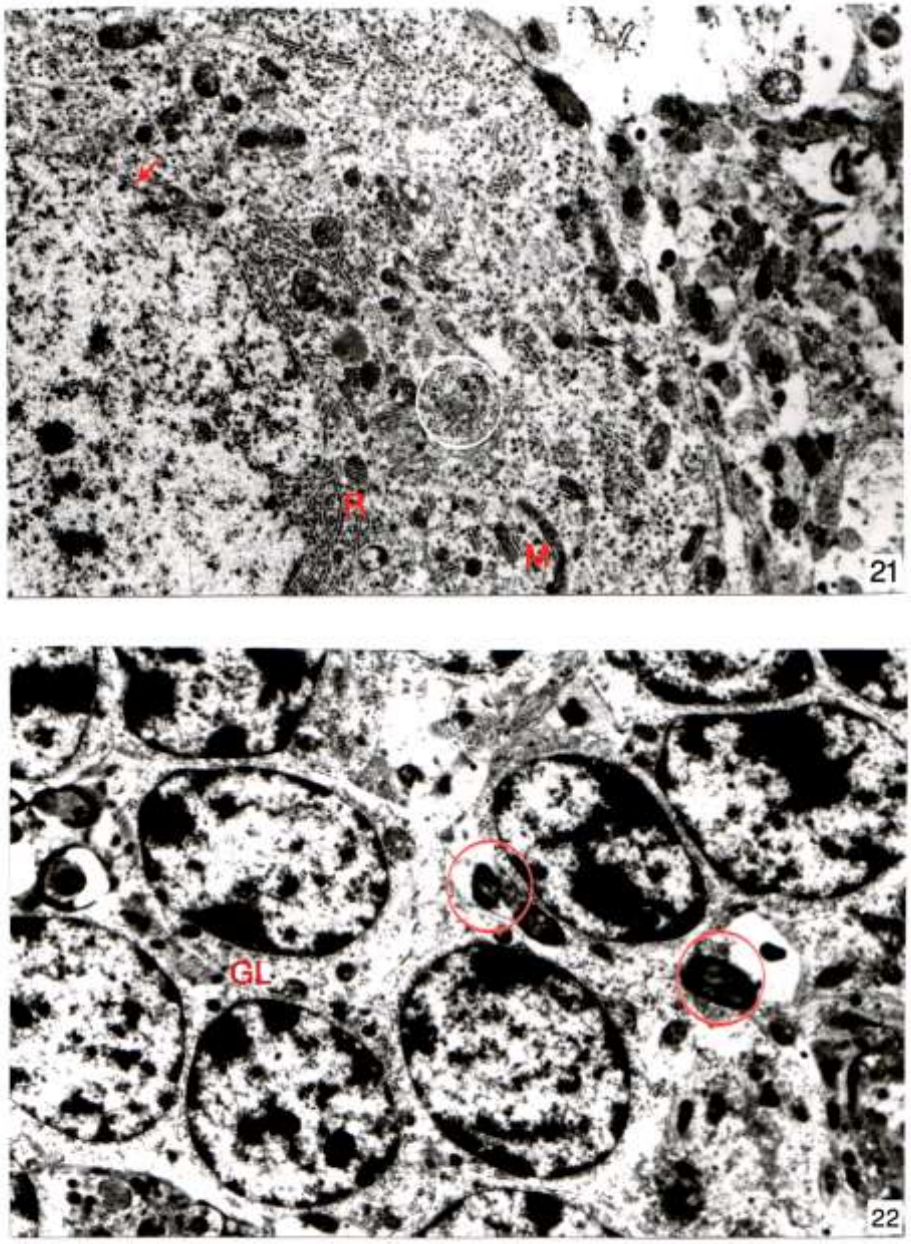


Fig. (21): EM showing a magnified part (× 6000) of another CP of control animal showing normal abundant mitochondria (M), slightly indented double layered nuclear membrane (↑) but not dilated, Golgi apparatus unit (circle) and prominent and numerous RER (R) organized on Nissle bodies. RER appear in orderly stackes.

Fig. (22): EM of cerebellar cortex of control animal (× 4000) showing the cells of the granular layer (GL). Each having a large and well-defined nucleus and thin shell of scanty cytoplasm and few apparently normal mitochondria. Interstitial microgliacytes (circles) having a hyperchromophilia appeared in the field.

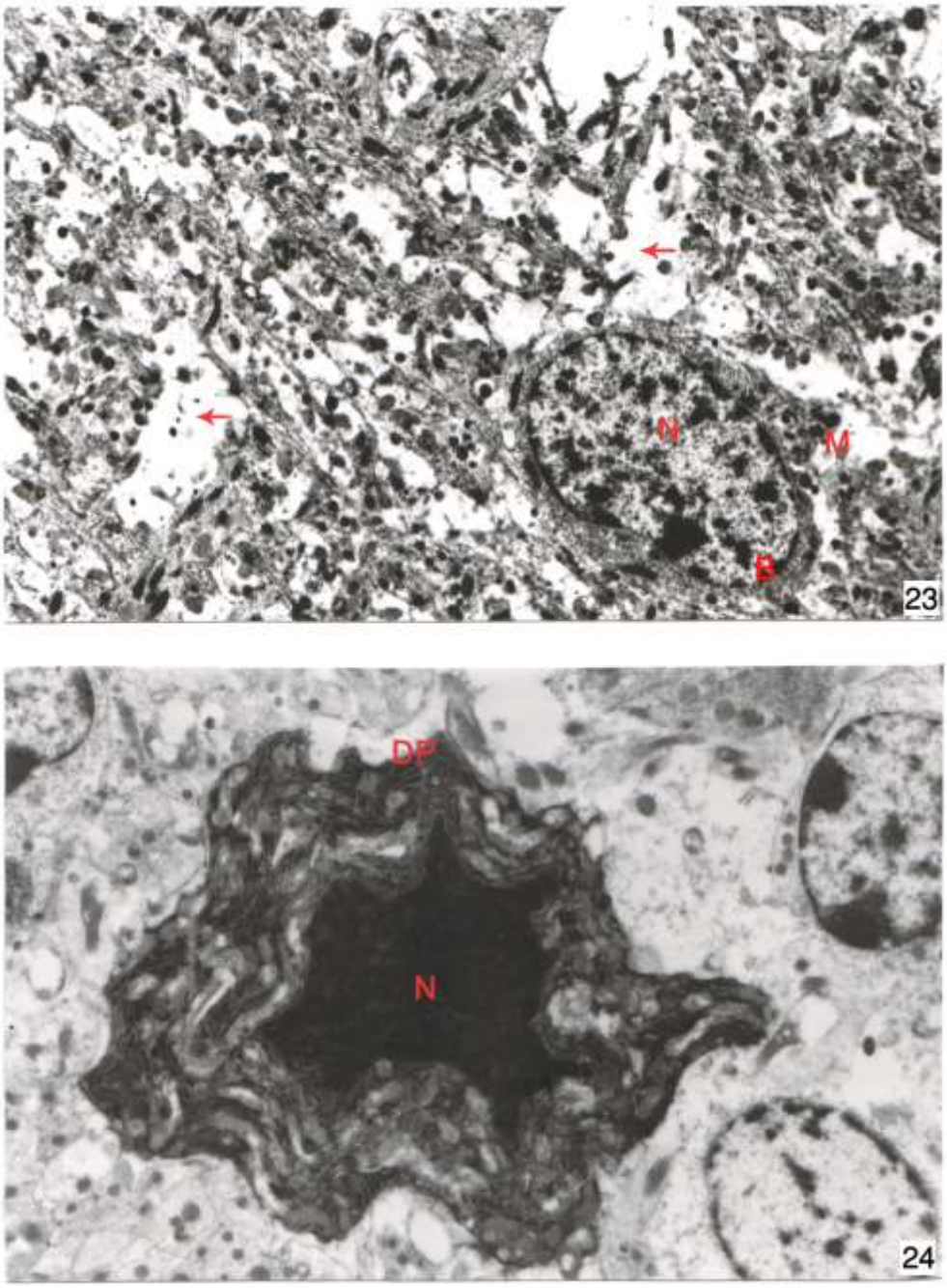
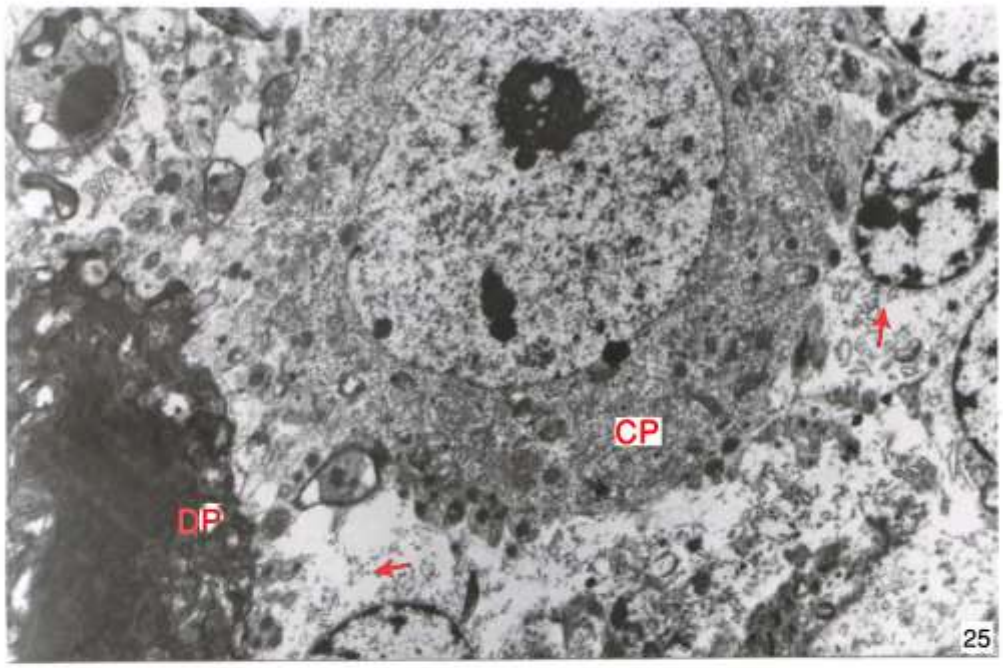


Fig. (23): EM of cerebellar cortex of irradiated (exposed) animal (× 3000) showing the molecular layer with vacuolated and degenerated foci (↑), a basket cell (B) with large nucleus (N) and narrow rim of cytoplasm. Notice, the increased electron density in the nucleus and cytoplasm and loss of the cell membrane integrity of these cells. Few mitochondria (M) are present .

Fig. (24): EM of cerebellar cortex of exposed animal (× 4000) showing DP with corrugated plasma and nuclear membranes, electron dense cytoplasm, shrunken nucleus (N), very electron dense karyoplasm. Notice the complete absence of the nucleolus.



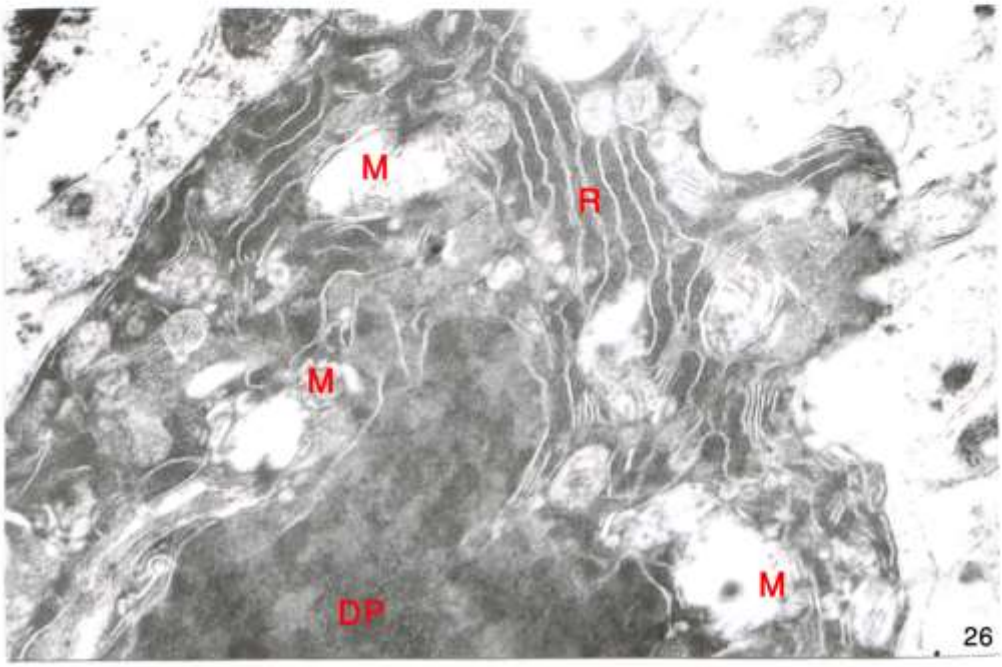


Fig. (25): EM of cerebellar cortex of exposed animal (× 3000) showing 2 affected Purkinje cells CP and DP with abnormal polymorphous nuclei and several perisomatic processes having large accumulation of mitochondria. Notice also degenerated cells of the granular layer (arrows).

Fig. (26): A magnified part of the last EM (× 12000) disclosing the DP with highly irregular plasma and nuclear membranes, electron-dense cytoplasm, disorderly- arrayed stacks of RER (R) which appeared dilated with clear contents, destroyed Golgi apparatus units, and severly destructed mitochondria (M).

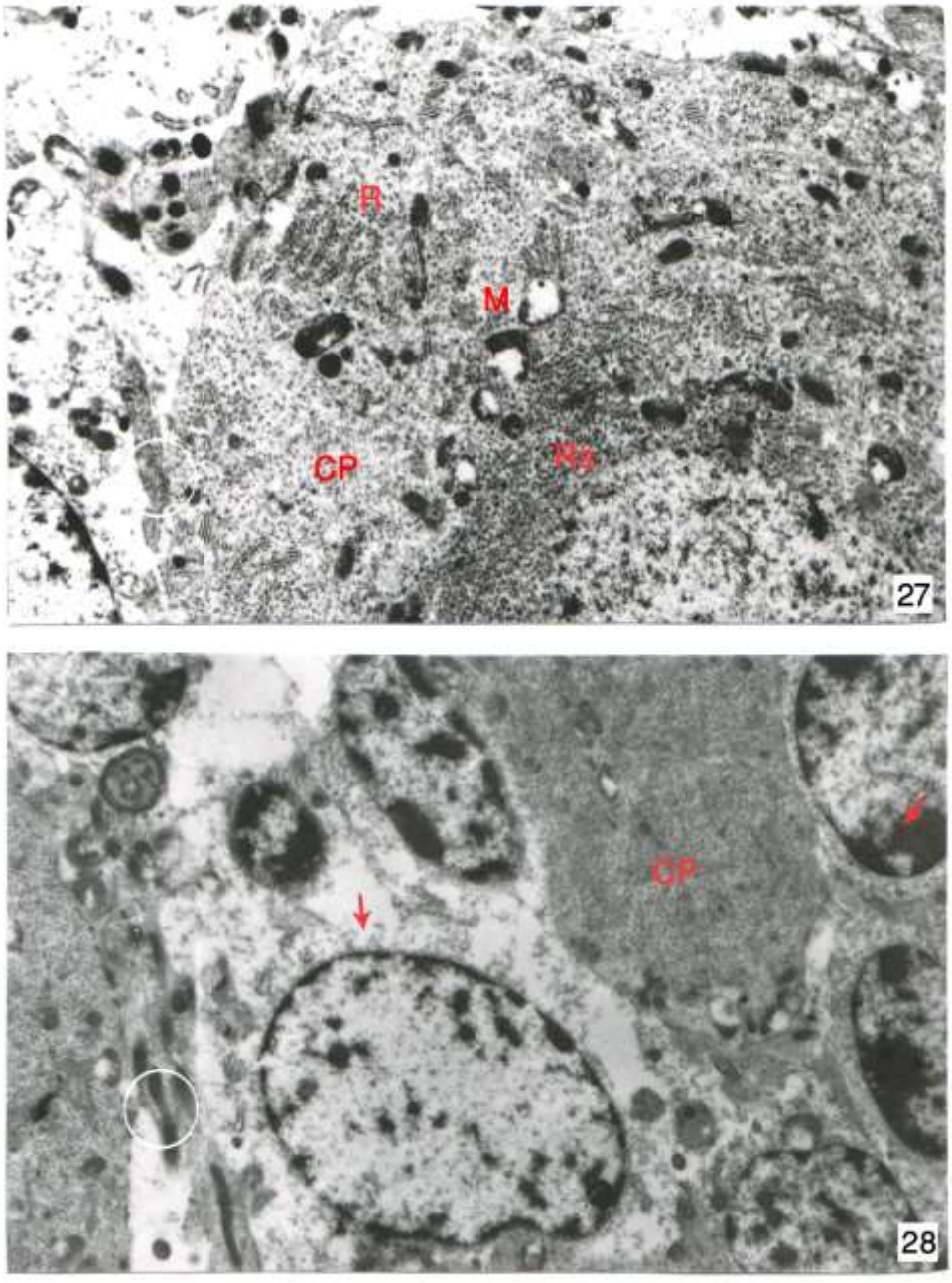


Fig. (27): EM of cerebellar cortex of exposed animal (× 6000) showing another part of CP revealing several destructed mitochondria (M), disintegrated RER cisternae (R) organized on ribosomes (Rs). The cell appears in synapse with one climbing fiber (circle).
Fig. (28): An EM of cerebellar cortex (× 4000) from an exposed animal showing degenerated elements of the granular layer. The granular cells with ruptured cell membrane and clumped chromatin in their nuclei (↑). CP with disrupted mitochondria

Discussion:

Because the nervous system is particularly vulnerable to reactive oxygen species (ROS) due to its high metabolic activities. The morphological, histochemical and ultrastructural alterations in cerebellum of mice pups are investigated to reflect the oxidative stress produced by the exposure to these non-ionizing microwave radiation (NMR). This oxidative stress is considered as a contributor to the initiation or progression of neurodegenerative events ***(Albers and Beal, 2000)***. The Purkinje and granular cells are the main neuronal cell populations in the cerebellar cortex, so these cells are the highly sensitive parameters to evaluate the histological changes in the cerebellum. Exposure of mice pups to the non-ionizing microwave radiation (NMR) during their prenatal and early postnatal life (for 30 days one hour per day) produced several changes in their cerebella at the cellular and subcellular level. The principal changes involved a statistically significant decrease in the mean cell distribution of Purkinje cells in cerebella of exposed animals. The observed significant decrease in the mean relative number of Purkinje cells of irradiated animals can be explained by suggesting that the radiation may affect the migration pattern of Purkinje cells and prevented them from reaching their final destination at the interface between molecular and granular areas and forming the normal synaptic contacts with the parallel fibers. Similar observations were obtained by *Albert and Sherif (1988).* Also, the current study investigated the mean Purkinje cell volume in irradiated animals in relation to that of controls in an attempt to detect the metabolic status of these cells during irradiation. Observations recorded a slight but not statistically significant increase in the mean PC volume of exposed animals. The subject which may suggest increased catabolic processes inside these cells due to radiation damage. *Gona et al. (1993)* studied the effects of extremely low frequency (ELF) electromagnetic fields (EMF) on the maturation of rat cerebellum. They exposed newborn rats to 60 Hz electric and magnetic fields and reported a small but statistically significant increase in cerebellar mass of the exposed rats.

Investigating the DNA content of the cerebellar cortex of irradiated animals in this study led to a decrease in that content. The decrease in the DNA content was detected histochemically and confirmed by image analysis to obtain a statistically significant value. This observation is in accord with those of *Lai and Singh (1995, 1996)* who reported that the acute exposure of rats to continuous and pulsed microwaves at whole body averaged SAR of 0.6 or 1.2 w/ kg for 2 h produced a dose dependant increase in single and double strand DNA breaks in the brain. On the contrary other investigations *(Malayapa et al., 1998)* did not confirm the previous results of *Lai and Singh.*

Examination of the cytology of Purkinje cells in the cerebella of exposed animals at the light microscopy level, revealed a change in the pattern of their Nissl substance. These tigroid substance appeared finely dispersed in the cytoplasm as if it had been disintegrated. This was confirmed by the quantitative histological analysis using the image proplus program. Also, at the electron microscopy level; these data were confirmed where the RER appeared in the form of a few small disordered arrays. On the contrary, the elements of RER of Purkinje cells of control animals were in the form of orderly stacks of parallel arrays characteristic of normal Purkinje cells *(Palay and Chan- Palay 1974& Albert and Sherif, 1988).* These observations are in accord with those of *Hansson (1988)* who recorded alteration of the RER and disintegration of Nissl bodies of Purkinje cells in rabbits exposed to electromagnetic field of 14 kv/m (undisturbed field, 50 Hz AC). The observed alteration in the pattern of Nissl granules and RER is indicative of a change in the metabolic status of the cell, especially the common protein metabolic pool. This could be considered as an early sign of degeneration.

In the present study, the observations recorded a reduction in the protein content of all cerebellar elements of irradiated animals. This reduction exhibited a highly

significant value by image analysis of the studied material. This effect may be due to a reaction between electromagnetic field induced free radicals and animal proteins which may produce biproducts that delay the initial protein synthesis. The present observations confirm and document those of *Monteiro et al. (1994), Kula et al. (1999) and EI-Abiad and Marzook (2005). Monteiro et al. (1994)* recorded some histological criteria at the level of E.M such as dilatation of RER cisternae in the neurons of cerebellar cortex of the experimental animals in association with observed nuclear alterations. These ultrastructural findings signifies a reduction in the ability of protein synthesis by those neurons which can ultimately influence the overall functions. *Kula et al. (1999)* observed a decrease in the levels of total proteins β- and γ globulins. They explained that it may have resulted from disturbed protein synthesis in the liver, which is controlled by steroid hormones . The availability of tissue proteins , release of amino acids and their metabolism in the liver are triggered by catabolic action of glucocorticoids, but the changes observed cannot be attributed to glucocorticoids only The action of glucocorticoids is also determined by function of the liver and nutritional status. *EI-Abiad and Marzook (2005)* recorded alterations in the protein fractions in both young and senile rats which exposed to radiofrequency radiation (RFR). They attributed these alterations to the interactions among the electric charges on ions, molecules and membranes of protein fractions. Another explanation was provided by another investigator *(Valberg et a1.,1997)* who suggested that , the electromagnetic radiations (EMR) may also exert some forces on the fixed and moving charges on these membranes. In contrast to our observations, some investigators described an increased heat-shock protein levels after RF-EMR exposure *(Kwee et al., 2001 and Leszcyzynski et al., 2002).*

In the present work, several ultrastructural changes were observed in the cerebellar cortex of the experimental group. These included nuclear alterations in the Purkinje cells such as shrunken nuclei especially of dark neurons. Also, dilatation of perinuclear cisternae was observed in many of the experimental group. According to *Cardozo-Pelaez et al. (1998)* these nuclear changes mostly reflect a lowering of the function of DNA transcription and gene expression with subsequent reduction of protein synthesis that influences the various functional activities of the neurons. It is reasonable to apply this explanation in the present study. In the current experiment , the nuclei of Purkinje cells appeared shrunken, irregular in outline with generalized increase in their electron density and complete nucleolar regression. According to the study reported by *Monteiro et al., (1994)* these histological features are suggestive of pyknotic nuclei. Similarly, pyknotic nuclei in the granular layer of the cerebellar cortex were observed. In addition, observations of the present study showed that some of the granular neurons, had severe signs of degeneration with marked rupture of the plasma membrane. Their nuclei were dark homogenous, with invisible nucleoli or chromatin and their cytoplasm contained few ill-defined organelles. These neurons represent degenerated neurons. Associated with these nuclear changes, there were associated alterations of RER in the neurons of the cerebellar cortex of the experimental animals. The RER cisternae appeared dilated with clear contents and this was especially obvious in the dark Purkinje neurons. The dilatation of RER cisternae, in association with the observed nuclear alterations, signifies the reduction in the ability of protein synthesis by these neurons which can influence the overall neuronal functions *(Monteiro, et al., 1994).* Also, in the present study, alterations of Golgi complex units were observed in the neurons of cerebellar cortex of the experimental animals. Golgi complex units of these experimental neurons appeared distorted and disintegrated with spherical or elliptical arrangement. The changes of Golgi complex, that were observed in the present work, are in agreement with the study of *Albert and Sherif (1988)* in which they described similar alterations of Golgi complex. In the present study, distortion of mitochondria was one of the most characteristic ultrastructural alterations in the Purkinje neurons of exposed animals. The distorted mitochondria appeared

swollen with destruction of their cristae. These alterations of mitochondria are indicative of mitochondrial degeneration and can be attributed to increased free radical reactions that trigger oxidative damage of mitochondrial membranes and mitochondrial DNA *(Cardozo-Pelaez et al., 1998).* Several studies have reported that the alterations in mitochondrial structure may result from the action of free radicals as the mitochondria are the major targets of free radical attack *(Mecocci, et a1.,1993, Ames et al. 1995* and *Sastre, et al., 1998). Southan et al. (1991)* concluded that mitochondrial membranes are more susceptible to free radical attack.

In conclusion The qualitative and quantitative data recorded in this study on both types of Purkinje cells may help us to raise hypothetical mechanisms about the stress-related Purkinje cell damage. These observations suggest that the nonionizing radiation of the mobile phone may interfere with the early genesis of cerebellar microneurons and may affect their migratory pattern and alter the metabolic status of Purkinje cells. But, they raise questions about the effects of irradiation such as; does irradiation interfere with genesis and/or maturation of Purkinje cells from neuropithelia? How these radiations affect the migratory pattern of these cells? Were the postmiotic Purkinje cells destroyed during early stages of formation?

References:

1. **Albers D S and Beal M F (2000):** Mitochondrial dysfunction and oxidative stress in aging and neurodegenerative disease, J. Neural. Transm., Suppl., 59: 133- 154.
2. **Albert E and Sherif M (1988):** Morphological changes in cerebellum of neonatal rats exposed to 2.45 GHz Microwaves. Prog. Clin Biol. Res;257:135-151.
3. **Ames B N, Shigenaga M K and Hagen T M (1995):** Mitochondrial decay in aging rats. Biohem. Biophys. Acta.; 1271: 165-70.
4. **Ammari M, Brillaud E, Gamez C, Lecomte A, Mohsen S, Abdelmelek H and Deseze R (2008):** Effect of a chronic GSM 900MHz exposure on glia in the rat brain. Biomedicine & Pharmacotherapy, 20:1-9.
5. **Bancroft J D and Gamble M (2002):** Theory and Practice of Histological Techniques, 5th Edition, Churchil, Livingstone, London.
6. **Cardozo-Pelaez F, Song S, Parthasarathy A, Hazzi C, Naidii K and Sanchez- Ramos J (1998):** Oxidative DNA damages in the aging mouse bain. Mov. Disord.; 14:972-980.
7. **Chou C K, Chan K W and Me Dougall L (1999):** Laboratory and quantitation of SAR distributions in rats comparable to human: A design and exposure methods. Bioelectromagnetics, 20: 21-39.
8. **Echlin P (1964):** Intracytoplasmic membranous inclusions in the blue green algae anacystis rudulans. Archiv. Fur Microbiologie, 49: 267.
9. **El-Abiad N. and Marzook E. (2005):** Effect of environmental microwave radiation exposure emitted from cellular phone base station on some biochemical parameters in rats. Sci. Me. J. ESCME. 17(1): 69-77.
10. **Feulgen R and Rossenbeck H (1924):** Mikroskopisch- chemischer nachweis einer nukleinsavre von typus der thymou ve and dara urberuhende elective farbung von zelkernum in mikroskopischen prepartem. Hopper Seyle S. Z. Physiol. Chem., 135: 203.
11. **Gona A. G., Yu M. C., Gona 0., al-Rabiai S., Von Hagen S. and Cohen E. (1993):** Effects of 60 Hz electric and magnetic fields on the development of the rat cerebellum. Bioelectromagnetics ,14(5):433-447.
12. **Hansson H (1988):** Effects on the nervous system by exposure to electromagnetic fields: Experimental and clinical studies. Prog. Clin. Biol. Res., 257:119-134.
13. **Kula B, Sobczak A, Grabowska R and Piskorska D (1999):** Effect of electromagnetic field on serum biochemical parameters in steelworkers. J. Occup. Health, 41 : 177-180.
14. **Kwee S, Raskmark P and Velizarov S (2001):** Changes in cellular proteins due to environmental non-ionizing radiation. I. heat shock proteins. Electro- and Magnetobiology, 20: 141-152.
15. **Lagroye I, Anane R, Wettring B A, Moros E G, Straube W L, Laregina M, Niehoff M, Pickard W F, Baty J and Roti Roti J L (2004)**: Measurement of DNA damage after acute exposure to pulsed- wave 2450 MHz microwaves in rat brain cells by two alkaline comet assay methods. Int. J. Radiat. Biol., 80(1): 11-20.
16. **Lai H and Singh NP (1995):** Acute low-intensity microwave exposure increases DNA single-srand breaks in Rat brain cells. Bioelectromagnetics 16:207-210.
17. **Lai H and Singh NP (1996):** Single- and double- strand DNA breaks in rat brain cells after acute exposure to radiofrequency electromagnetic radiation. Int. J. Radiat. Biol., 69: 513-521.
18. **Leszcyzynski D, Joenvaara S, Reivinen J and Kuokka R (2002):** Non-thermal activation of the hsp27/p38MARK stress pathway by mobile phone radiation in human endothelial cells: molecular mechanism for cancer- and

blood brain barrier- related effects. Differentiation, 70:120-129.

19. **Malayapa RS, Ahern EW, Bi C, Straube WL, La Regina M, Pickard WF and Roti Roti JL (1998):** DNA damage in rat brain cells after in vivo exposure to 2450 Mhz electromagnetic radiation and various methods of euthanasia. Radiat., Res., 149: 637-645.
20. **Mazia D, Brewer P A and Alfert M (1953):** The cytochemichal staining and measurement of protein with mercuric bromophenol blue. Biol. Bull., 104: 57-67.
21. **Mecocci P, MacGarvey U, Kaufmann A E, Koont D, Shoffner J M, Wallace D C and Beat M F (1993):** Oxidative damage to mitochondrial dna shows marked age-dependent increases in human brain. Ann. Neurol.; 34: 609-616.
22. **Mohamed M H (2003):** Changes produced by the exposure to microwaves of mobile phone on cerebellum of albino rat. Ph.D. Thesis, Anatomy Department, Faculty of Medicine, Assiut University.
23. **Monteiro R A, Rocha E and Marini-Abreu M M (1994):** Heterogenecity and death of purkinje cells of rat neocerebellum (crus i and crus ii): Hypothetic Mechanisms Based on Qualitative and Quantitative Microscopical Data., J. Brain. Res.; 35:205-222.
24. **Palay S L and Chan- Palay V (1974):** "Cerebellar Cortex, Cytology and Organization". New York, Heidelbeg, Berlin: Springer- Vergas, pp. 25- 28.
25. **Repacholi M H (1998):** Low-level exposure to radiofrequency electromagnetic fields: health effects and research needs. Bioelectromagnetics, 19: 1-9.
26. **Robenson D G, Ehlers U, Kerken R, Hermenn B, Mayer F and Schurman F W (1987):** Methods of Preparation for Electron Microscopy.Speringer, Verale, Berlin, Heidlberg, Germany.
27. **Sabatini D D, Bench K and Barnett R J (1963):** Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. J. Cell. Biol., 17: 19-58.
28. **Sastre J, Millan A, De La Asuncon J G, Pla R, Juan G, Pallardo F V, O'Connor E, Martin J A, Droy-Lefaix MT and Vina J (1998):** A ginkgo biloba extract (egh 761) prevents mitochondrial aging by protecting against oxidative stress. Free Rad. Biol. Med.; 24:298-304.
29. **Shkorffo M O (2007):** Histological and histochemical studies on the brain and kidney of mammals (rabbits) after exposure to the microwaves of the mobile phone. M.Sc. Thesis, Department of Biology, Faculty of Science, Al-Mergeb University, Libya.
30. **Southan, E; Thomas, P K; King, R H; Goss-Sampson, M A and Mutter, D P (1991):** Morphological and functional evidence of abnormal axonal transport secondary to free radical damage. Brain.; 114: 915-36.
31. **Walters T, Ryan k and Mason P (2001) :** Regional distribution of HSP-70 in the CNS of young and old food restricted rats following hyperthermia . Brain Res.Bull.,55:367 -374
32. **Valberg P, Kavet R and Rafferly C (1997):** Can low-level 50/60hz electric and magnetic fields cause biological effects?. Rad. Res., 148:2-21.

تأثير الإشعاعات غير المؤينة على مخيخ الفئران البيضاء حديثي الولادة

دراسة مورفولوجية ، هستوكيميائية وخلوية دقيقة

سمير عبد العظيم نصار

قسم علم الحيوان، كلية العلوم، جامعة الزقازيق، مصر

يعتبر الهاتف النقال أحد ملوثات البيئة المحيطة بالإنسان في الوقت الحالي بالرغم من فوائده العديدة وأهميته في توفير الوقت وإنجاز الأعمال. وتكمن خطورته في أن استعماله يستلزم اقترابه أو تلامسه مع رأس الإنسان بما تحويه من المخ وأعضاء الحس الأخرى. من هنا انبعثت فكرة الدراسة الحالية أن يكون **الهدف** هو بحث وتقييم الآثار السلبية للإشعاعات غير المؤينة المنبعثة من هذا الجهاز على خلايا القشرة المخيخية في المستوى الخلوي والتحت خلوي باستعمال المجهر الضوئي والإلكتروني.

استعملنا في هذا البحث مجموعتين من الفئران البيضاء حديثة الولادة. إحداهما اشتملت على خمسة فئران تجريبية تم تعريضها لإشعاعات الهاتف النقال (900– 1800 MHz ، معدل امتصاص نوعي = 0.92 وات/ كجم) في المرحلة الأخيرة قبل الولادة، المرحلة المبكرة بعد الولادة مباشرة. وكان التعرض لمدة ساعة واحدة في اليوم ولفترة 30 يوما متعاقبة. أما المجموعة الأخرى اشتملت على ستة فئران طبيعية استعملت كمجموعة نمطية ضابطة. ثم تم أخذ عينات المخيخ من كل الحيوانات وتثبيتها وإعدادها للفحص بالميكروسكوب الضوئي، الإلكتروني وجاءت النتائج كالآتي:

أظهر تعرض الجسم الكامل للفئران حديثة الولادة علامات مرضية كثيرة في المستوى الخلوي، التحت خلوي منها: نقص ملحوظ ذو دلالة إحصائية في عدد وتوزيع خلايا بيركنج وكذلك كمية أجسام نسل ونظام توزيعها في هذه الخلايا وكذا كمية الحامض النووي منقوص الأكسجين والمحتوى البروتيني لكل خلايا القشرة المخيخية. هذا بالإضافة إلى علامات مرضية اخرى تم تسجيلها من فحص الخلايا بالمجهر الإلكتروني منها تلف وتهتك في الأغشية الخلوية لخلايا الطبقة المحببة. تعرجات شديدة في الأغشية الخلوية، النووية لخلايا بيركنج وتكسير في أغشية الشبكة الإندوبلازمية لهذه الخلايا مع تمزق وانفجار لجدر الأجسام السبحية وتلف لأجهزة جولجي ونقص في محتوى الريبوسومات وأجسام نسل. كما ظهر أيضاً تلف واضح في الألياف العصبية والزوائد الشجيرية والخلايا العصبية للطبقة الجزيئية.

من هنا نخلص أن تعريض الجسم الكامل للفئران البيضاء حديثة الولادة لإشعاعات الهاتف النقال كانت له آثارا سلبية خطيرة على خلايا القشرة المخيخية في المستوى الخلوي والتحت خلوي. لذا نأمل أن يكون فى ذلك جرس إنذار للتوعية بكيفية استعمال هذا الجهاز وترشيد استخدامه.