Journal of Bioscience and Applied Research

JBSAR

WWW.JBSAR.com

Cerebellar histopathological and histochemical alterations induced by electromagnetic field exposure of mice

Somaia A. Negm[1], Amr M. Abd El-Hady [2] ,Noha N. Yassen [3]and Alhusain Nagm, M.D., M.Sc. [4]

1 Medical laboratory Technology Department, Faculty of Applied Medical Sciences, Misr University for Science and Technology (MUST), Cairo, Egypt, 2 Radiological Imaging Technology Department, Faculty of Applied Medical Sciences, Misr University for Science and Technology (MUST), Cairo, Egypt and 3 Pathology Department, National Research Centre, Dokki, Cairo, Egypt. 4Department of Neurosurgery, Shinshu University School of Medicine, Matsumoto, Japan,

(Corresponding author E-mail: somaia.negm@must.edu.eg **)**

Abstract

The current work aimed to clarify the health effects of the exposure to 900-1800 MHz radiofrequency electromagnetic fields (RF-EMF) emitted from mobile phones on histological and histochemical alterations in mice cerebellum. Thirty healthy adult male Swiss albino mice were divided into three groups, the first group served as the control, the second group exposed to mobile phone radiation for one month (45 min/day) and the third group exposed to mobile phone RF-EMF (45min/day) for one month and sacrificed one month following the last exposure day. The results showed histopathological changes represented by dystrophic changes in Purkinje cells layer, degenerated and accumulated granular layer cells with edematous spaces which appeared congested with blood. In addition, there were some histochemical and morphometric changes in the cerebellar tissue indicated by alterations in collagen, polysaccharides, total protein, DNA and amyloid-β protein contents of mice exposed to RF-EMF from the mobile phone as well as after one month following the end of the exposure. It was concluded that exposure of male mice to mobile phone radiation caused histopathological and histochemical disorders in the cerebellum of adult male mice.

Keywords: Electromagnetic field, Radiofrequency, Mobile phones, Brain, Histopathology, Histochemistry

1 Introduction

Exposure to electromagnetic field (EMF) is the result of the modern worldwide growing technologies. Increasing exposure to mobile phone radiation, together with other sources of non-ionizing radiations including Wi-Fi and microwaves has adverse health effects (Fragopoulou et al., 2012). Radiofrequency (RF) radiation emitted from mobile phones was reported earlier to be responsible for peripheral neurophysiological changes in some persons where these damaging effects occur at exposure levels below the present safety levels (Westerman and Hocking, 2004).

The brain is the most sensitive organ for detecting the damaging microwave radiation (MW) effects in mobile phone users where mitochondrial injury appears earlier than in other organs (Hao and Peng, 2015). Mobile phone EMF has adverse effects including oxidative stress in brain tissues (Meral et al., 2007), decreases Purkinje cell numbers in the female rat cerebellum (Sonmez et al., 2010), increased risk for brain tumor after 10 years (Hardell and Sage, 2008), increased the permeability of the blood-brain barrier (Nittby et al., 2009), induce irreversible destruction to the brain of adult mice (Rosli and Teoh, 2009). In the same direction, exposure of male rats to 900 MHz Radiofrequency radiation from digital mobile telephone can alter brain structure at tissue level but not at DNA level (Usikalu et al., 2012). Whole-body magnetic field exposure induced a direct effect on the human chorionic gonadotropin (hCG) stimulated steroidogenic response of mouse Leydig cells with no magnetic field exposure-related histopathological alterations in testis,

epididymes, adrenal, prostate and pituitary gland (Forgács et al., 2004).

The aim of the current work was to clarify the effect of RF-EMF emitted from the mobile phone on histological and histochemical changes in mice cerebellum.

2Material and Methods

Experimental animals:

Mice care and handling was done according to the approval from of ethics committee for animals care at the National Research Centre conformed to the "Guide for the care and use of Laboratory Animals" published by the US National Institutes of Health (NIH publication No. 85–23, 1996). Mice were delivered with tap water and commercial diets and accustomed to laboratory conditions for 15 days before commencement of the current experiment.

Animal groups:

Thirty healthy adult male Swiss albino mice approximately 3-5 months old ranging in weight (from 20-25 g, body weight) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and arbitrarily distributed to three equal groups 10 rats each:

I- Control group: Mice of this group received no treatment, anesthetized and sacrificed after one month of the experiment.

II- Irradiated group: Mice of this group exposed to mobile phone RF-EMF (45min/day) for one month. At the end of the last exposure day, mice were anesthetized and sacrificed.

III- Recovery group: Mice of this group exposed to mobile phone RF-EMF (45min/day) for one month in the same way as the second group, then anesthetized and sacrificed after one month following the last exposure day.

RF-EMF exposure facility:

EMF exposure of animals was performed by using a mobile phone radiation (Nokia, model 1280) at a specific absorption rate (SAR) of 0.78 W/Kg and frequencies from 900 to 1800 MHz at intensity 500μW/cm2, in connection with Egypt network (Vodafone, Egypt). The exposure process was performed at daily repeated series (45 min/ day) for 30 days. The mobile phone set on dialing mode and placed in a direct contact to the bottom of the exposure cage.

Histological and histochemical techniques

For the histopathological study, 10% neutral formalin fixed sagittal sections of cerebellum were embedded in paraffin wax, section processed to prepare 4 μ thick paraffin sections and stained with Harris hematoxylin and eosin and Mallory's trichrome stains according to the method of Bancroft and Gamble (2002). For histochemical studies, polysaccharides were detected by PAS (periodic acid-Schiff) method (Hotchkiss, 1948). Proteins were detected by mercuric bromophenol blue method (Mazia et al., 1953). Amyloid-β was detected by Congo red technique (Valle 1986) and DNA was visualized by using the standard Feulgen's method (Drury and Wallington, 1980).

Image analysis

Mean optical density (MOD) measurement of the studied histochemical components was performed using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England) of the Pathology Department, National Research Centre, Cairo, Egypt. All slides were examined at power magnifications 100× and 200×, and then MOD was measured in 10 fields. The final MOD value was calculated according to the software ranges from 0 (zero = most light stain, lowest expression), to 1 (one = most dense stain, highest expression) with illustrating histograms describe the number of cells taking such MOD value.

Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA) followed by LSD as post hoc test. The results obtained were expressed by mean ± standard deviation (SD). Differences were considered significant at P value $\leqslant$ 0.05 according to the method of George and William (1980).

3 Results

Histopathological findings

Light microscopic examination of the cerebellum tissue of group I (controls) showed normal cortical layers: molecular layer, granular layer, core of white matter and normal pyriform shape of Purkinje cells with strongly stained cytoplasm (Figs. 1A-1C) with thin bundles of collagen fibers in the cerebellar cortex layers (Figs. 2A-2C). In group II (irradiated group), the Purkinje cells showed dystrophic changes represented by a decreased of their number. Granular layer showed degeneration and nuclear accumulation with edematous spaces (Figs. 1D-1F),

Increased collagen fibres deposition was also noticed in the different cortical layers (Figs. 2D-2F). As regarding group III (recovery group), the cerebellar cortex showed decreased number of Purkinje cells, some of them appeared with karyolytic nuclei with thinning of the granular layer (Figs1G-1I). More or less normal collagen fibres deposition was detected in the cerebellar cortex especially in their white matter layers (Figs. 2G-2I).

Histochemical findings

Normal distribution of polysaccharides was noted in the cerebellar cortical layers of group I (control group) with densely stained Purkinje cells and granular layer cells (Figs. 3A, 3B). PAS negative stainability of the cerebellar cortical layers nuclei was noticed, indicating the lack of its mucopolysaccharides content. Increased PAS +ve materials were noticed in the different cerebellar cortical layers of the irradiated group (Figs. 3C,3D) with a slight reduction in their content in the recovery group especially in the destructed Purkinje cells and white matter layer (Figs. 3E,3F). Such histochemical observations were in accordance with the MOD values where highly significant increase (0.550 ± 0.027) was noted in the PAS values of irradiated group with non-significant reduction of their value (0.392 ± 0.028) in the recovery group as compared to the control group value (0.418 ± 0.058) charts (1-3).

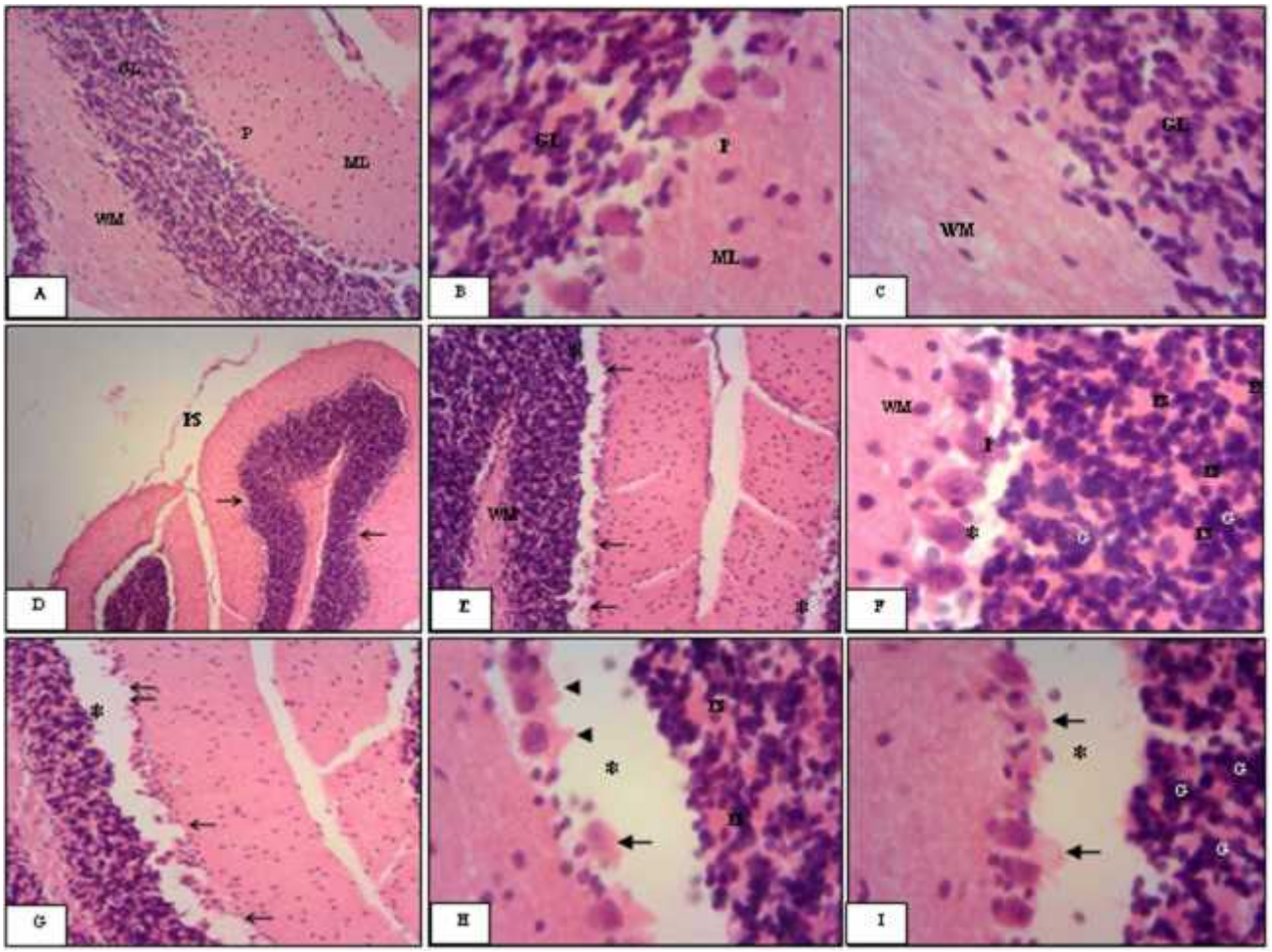

Figure 1. Photomicrographs of sections in cerebellar cortex of male mice of control and treated groups (H& E): (A) Showing the characteristic pattern of control cerebellar cortical layers; molecular layer (ML), Purkinje cells (P), granular layer (GL) and white matter (WM) core. (x200). (B, C) Showing the classical pyriform shape of Purkinje cells (P) with its strongly stained cytoplasm and normal appearance of the other three cortical layers; Molecular layer (ML), granular layer (GL) with its darkly-stained neurons and white matter (WM) layer. (x400). (D-F) Cerebellum of irradiated group showing focal loss of Purkinje cells (thin arrows), detached pial surface (DP), degenerated and accumulated granular cells (G) with edematous spaces (ES) appeared congested with blood and increased pericellular spaces (*) between granular and Purkinje cells layers. (x 200, 400). (G-I) Cerebellum of the recovery group showing mild thinning of granular layer (GL) and highly decreased number of Purkinje cells (thin arrows), some of them showed irregular or ruptured cellular membranes (▲) and others appeared with karyolytic nuclei (thick arrows). Granular layer cells appeared degenerated and accumulated with blood congested edematous spaces (ES) in between. (x 200, 400).

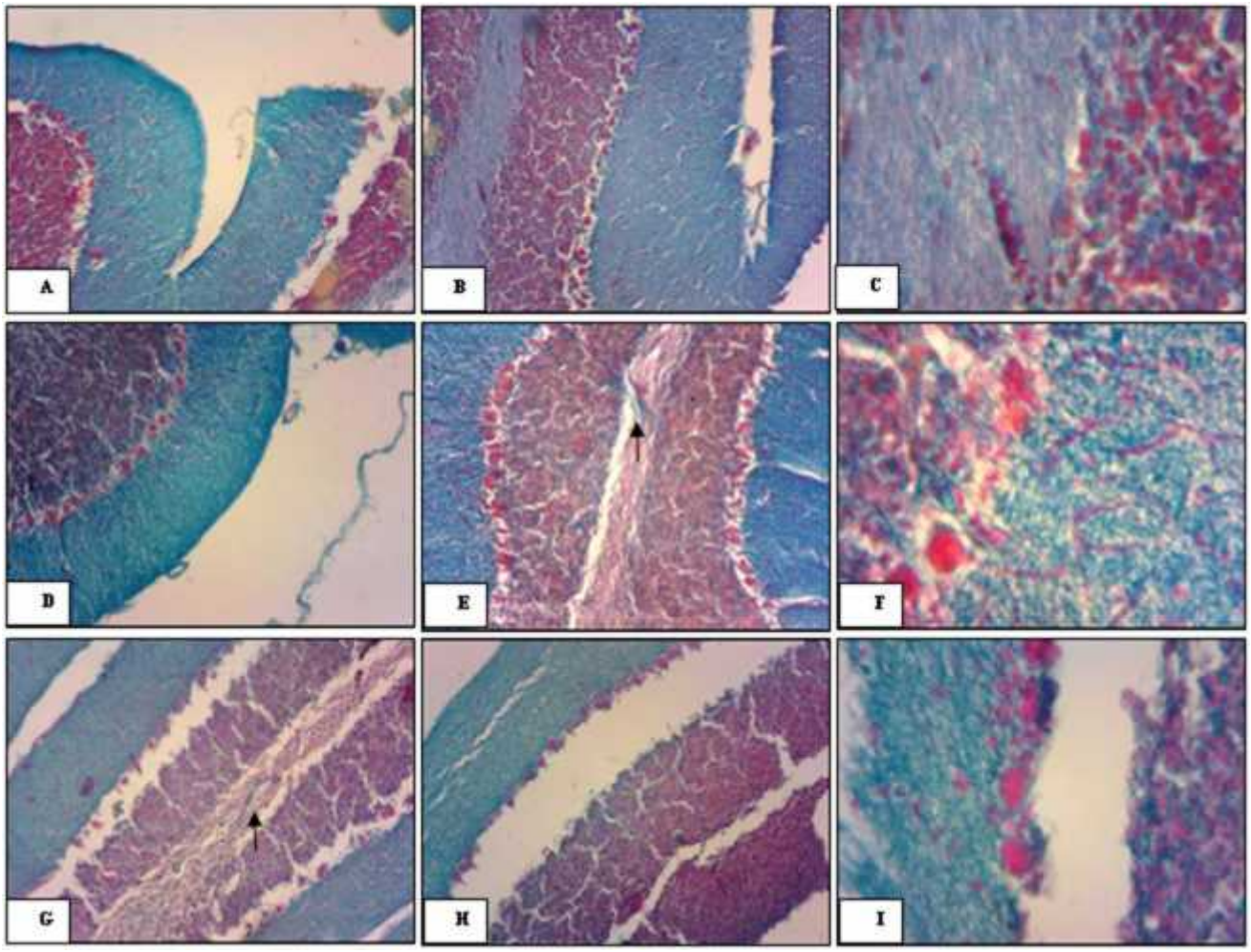

Figure 2. Photomicrographs of sections in cerebellar cortex of male mice of control and treated groups showing distribution of collagen (Mallory's trichrome stain, x 200 & 400): (A-C) Sections in control group showing thin bundles of collagen fibers in the cerebellar cortical layers. (D-F) Sections in irradiated groups showing increased blue collagen fibres deposition especially in the white matter (arrows) with more or less normal collagen fibres deposition in the recovery group (G-I).

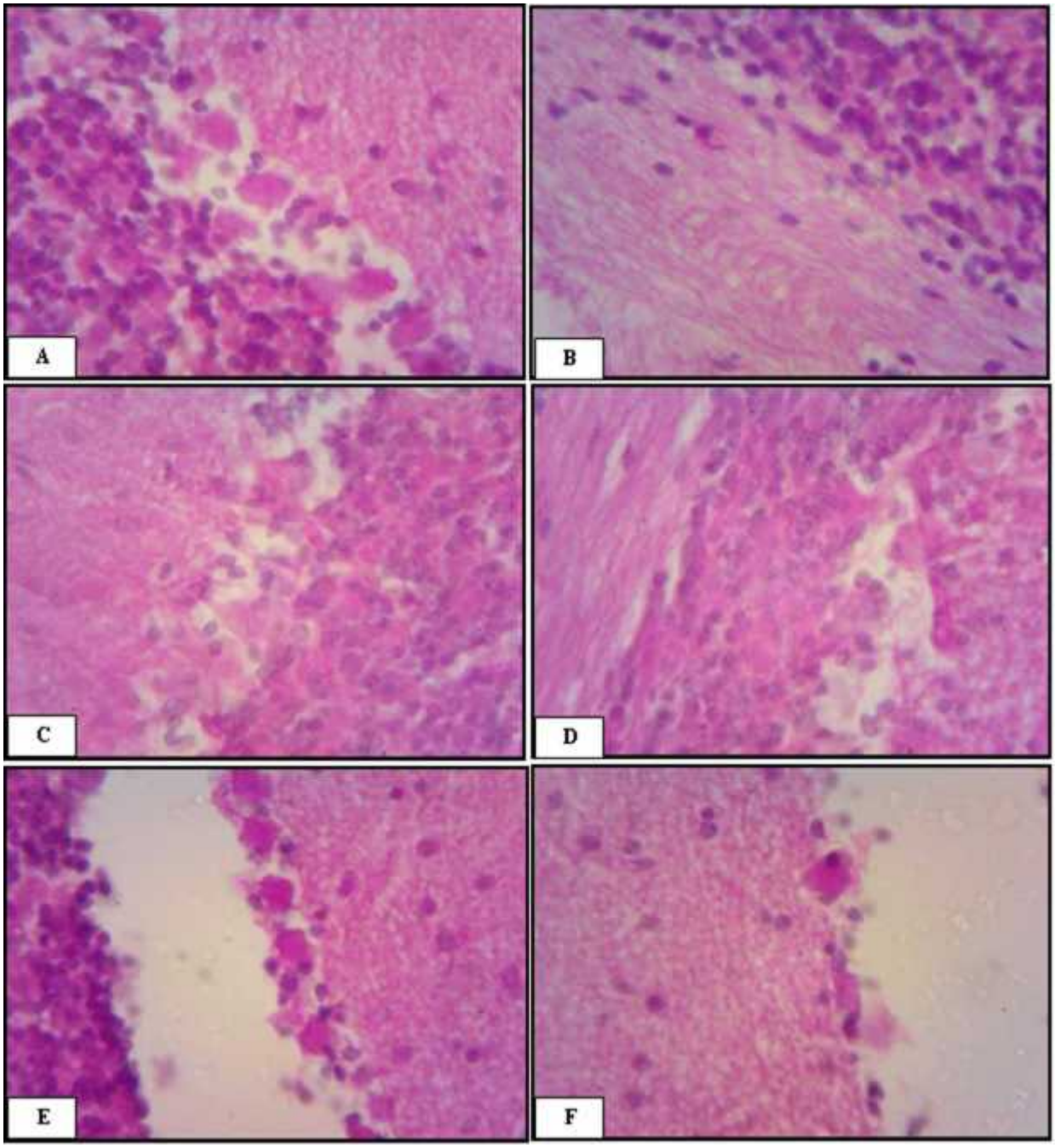

Figure 3. Photomicrographs of sections in cerebellar cortex of male mice of control and treated groups showing distribution of polysaccharides (PAS, x 400): (A, B) Control group showing normal distribution of PAS +ve materials in all the cortical layers with dense staining affinity in Purkinje cells and granular layer cells. (C-F) Irradiated and recovery groups showing mild increase in the PAS +ve materials especially in the destructed Purkinje cells and white matter layer cells of irradiated group (C,D) with a slight reduction in the PAS +ve stainability in the recovery group (E,F).

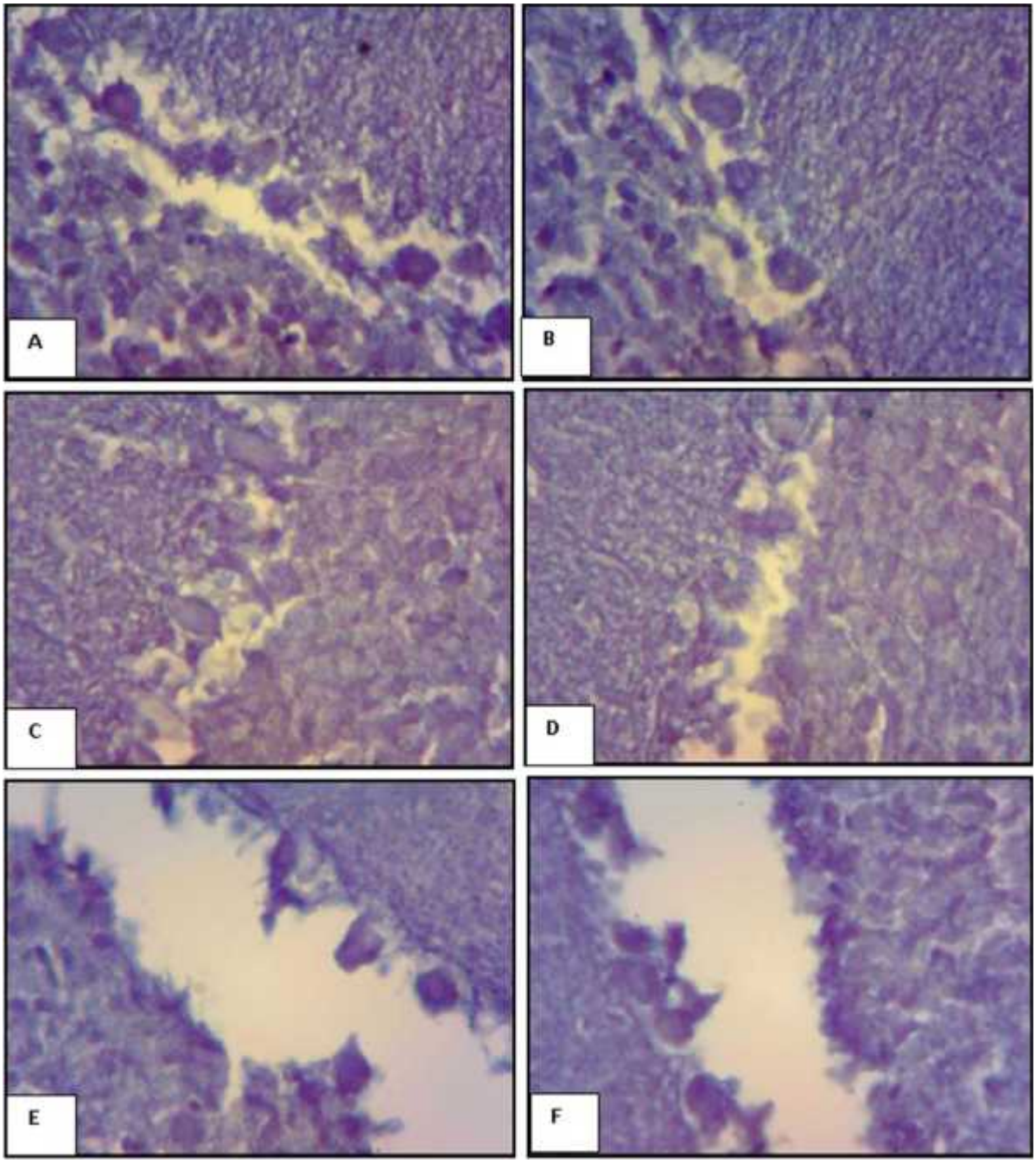

Figure 4. Photomicrographs of sections in cerebellar cortex of male mice of control and treated groups showing distribution of total protein (Mercuric bromophenol blue stain, x 400): (A, B) Control group showing dense to moderate stain affinity of total protein in the Purkinje cells and granular layer cells with less stained molecular layer. (C-F) Sections in irradiated and recovery groups showing reduced protein content in the different layers of cerebellum of irradiated group (C,D) with more or less normal protein content of the different cortical layers of the recovery group (E,F).

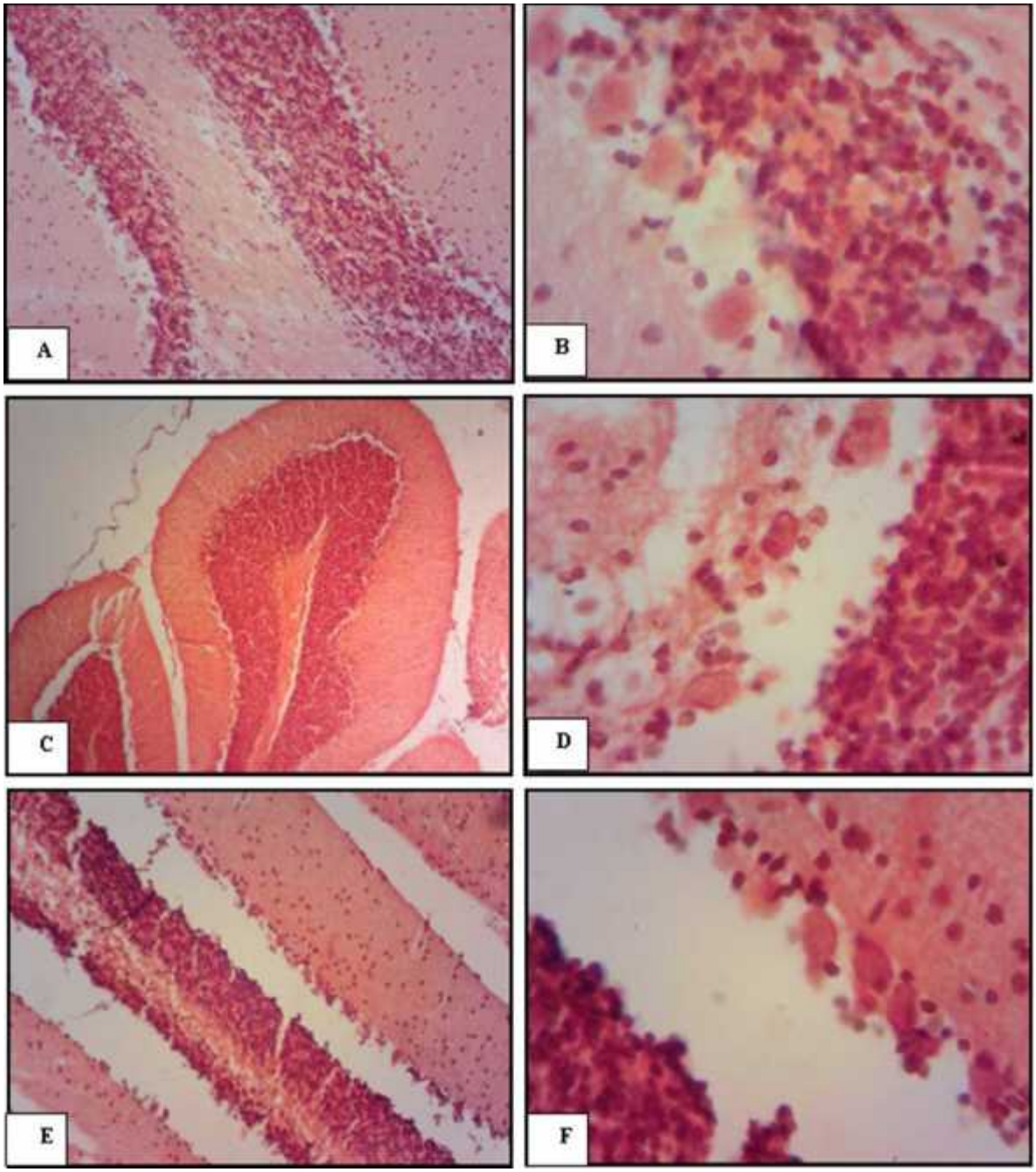

Figure 5. Photomicrographs of sections in cerebellar cortex of male mice of control and treated groups showing distribution of amyloid-β (Congo red, x 200, 400): (A, B) Control group showing slight deposition of amyloid β in the nuclei of Purkinje cells with negative Congo red reaction of the remaining layers of the cerebellar cortex. (C-F) Sections in irradiated and recovery groups showing decreased deposition of amyloid-β plaques in all layers of the cerebellum.

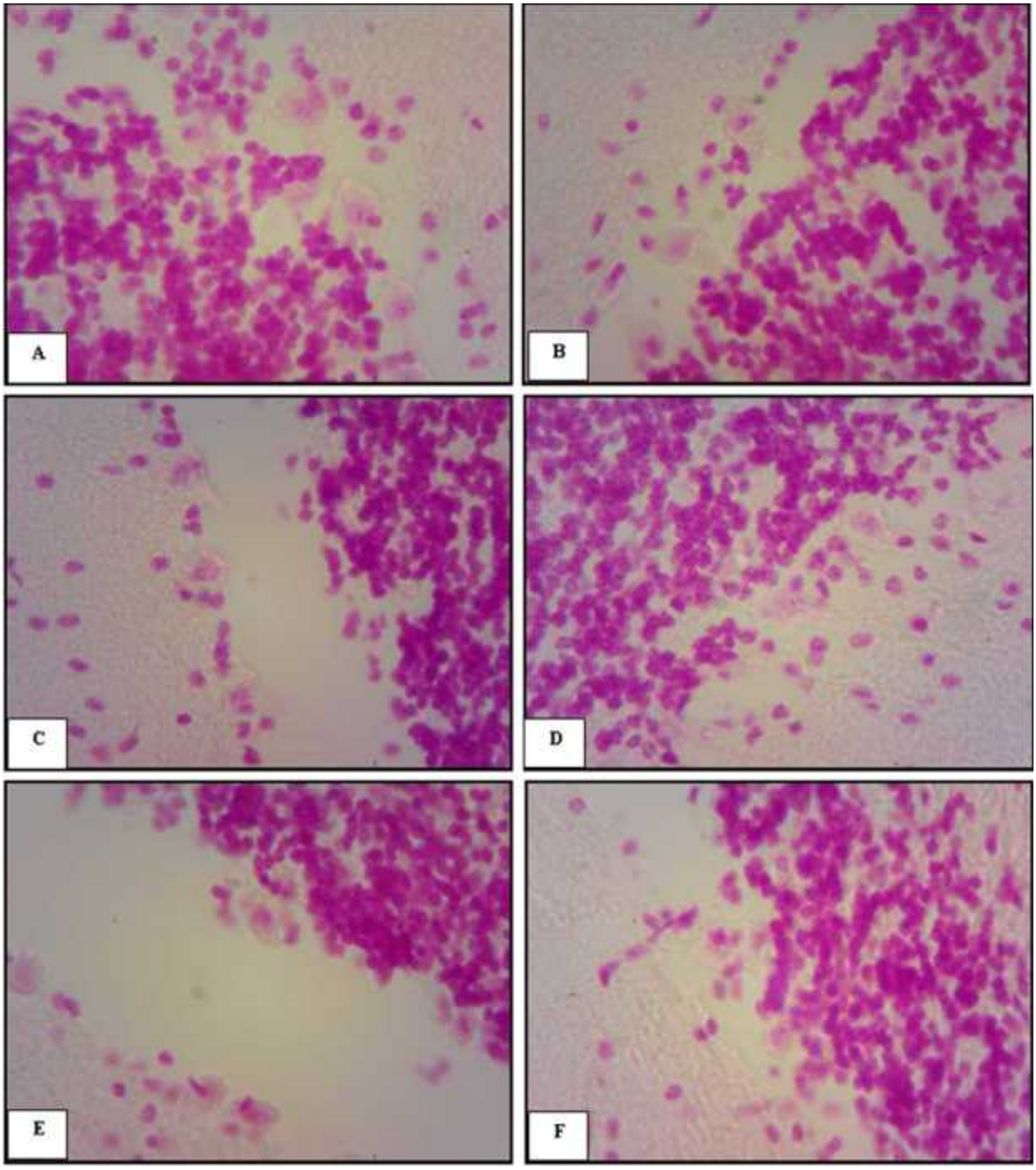

Figure 6. Photomicrographs of sections in cerebellar cortex of male mice of control and treated groups showing distribution of DNA (Feulgen's stain, x 400): (A, B) Control group showing normal distribution of the DNA materials in the nuclei of the different cerebellar cortical layers. (C-F) Sections in irradiated and recovery groups showing an increase in the DNA +ve materials of irradiated group (C,D) with more or less normal distribution of its content in the recovery group (E,F).

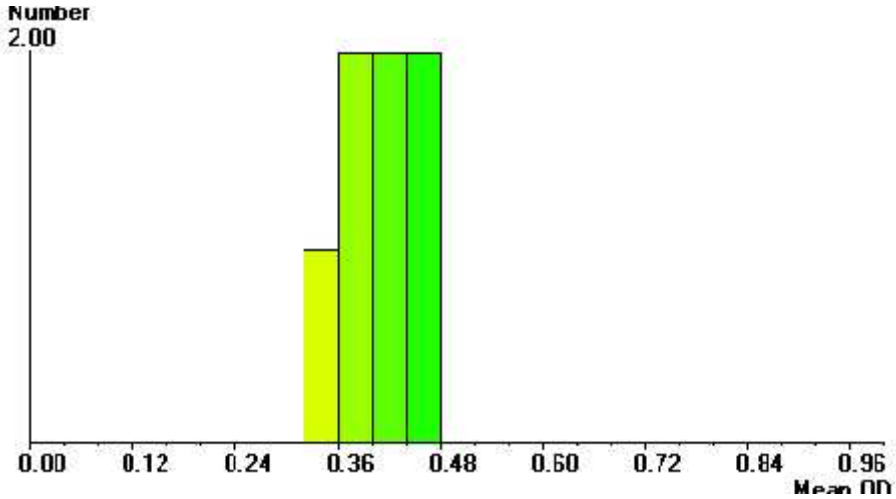

Chart 1. Histogram showing MOD of control cerebellar tissue stained by PAS showing distrubution of polysaccharides.

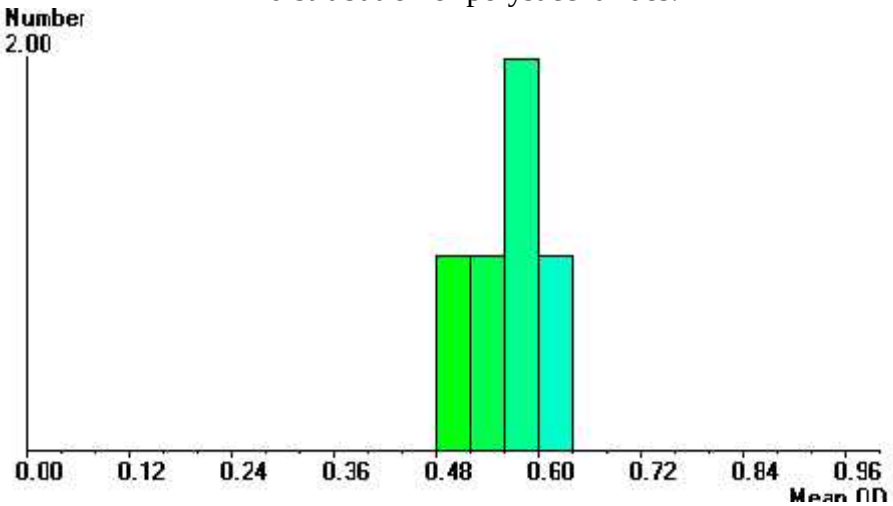

Chart 2. Histogram showing MOD of irradiated cerebellar tissue stained by PAS showing distrubution of polysaccharides as the gram deviated to the right as much more cells with higher expression to the stain (increased polysaccharides in the tissue).

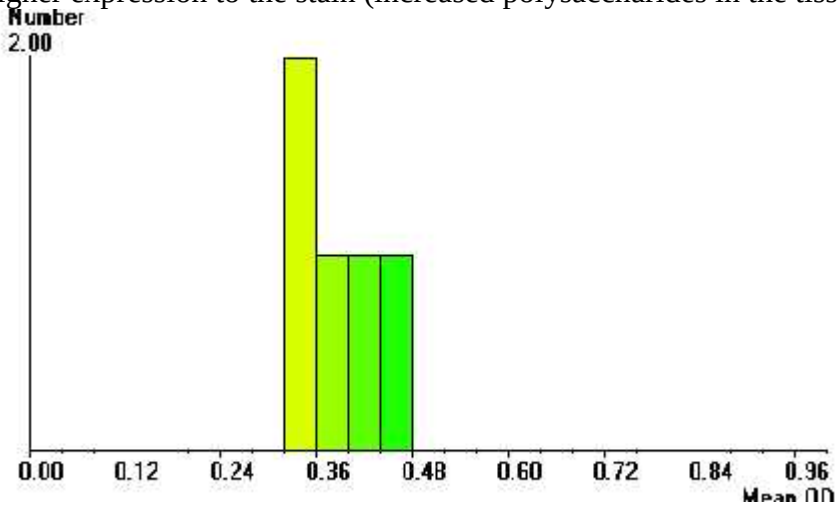

Chart 3. Histogram showing MOD of recovered cerebellar tissue stained by PAS showing distrubution of polysaccharides as the gram deviated to the left as much more cells with lower expression to the stain (recovered polysaccharides in the tissue).

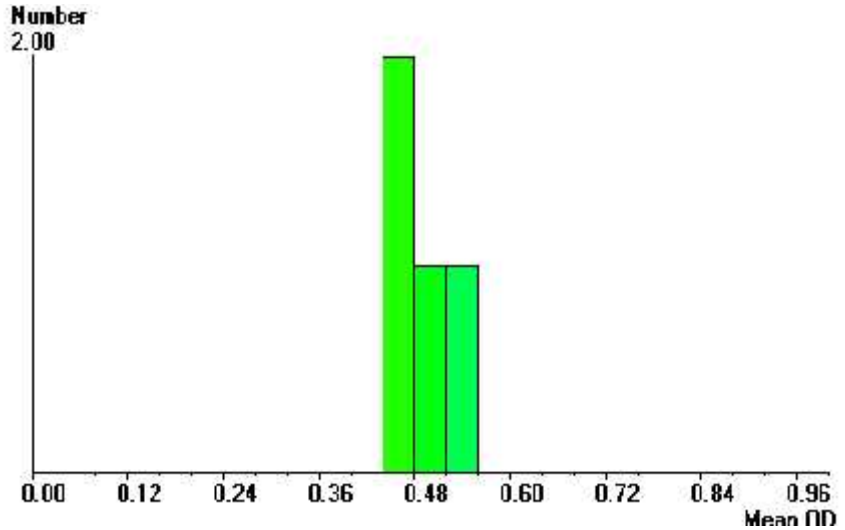

Chart 4. Histogram showing MOD of control cerebellar tissue stained by Mercuy bromophenol blue stain showing distrubution of protein.

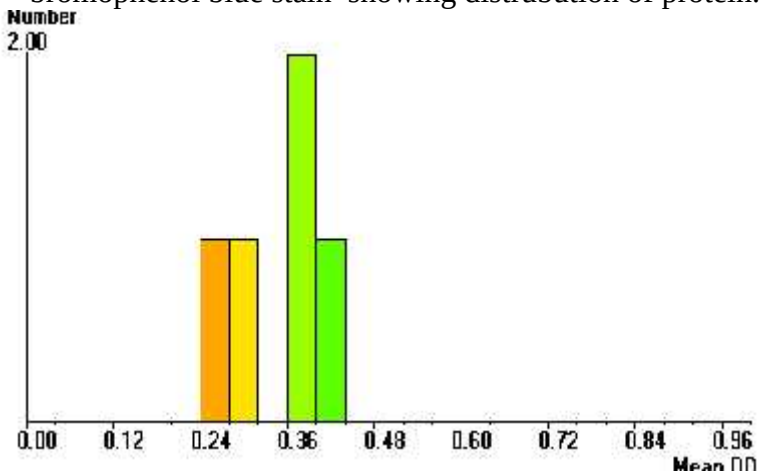

Chart 5. Histogram showing MOD of irradiated cerebellar tissue stained by Mercuy bromophenol blue stain showing distrubution of protein, the gram deviated to the left as much more cells with lowest expression to the stain (decreased proteins in the tissue, the breakdown of the tissue).

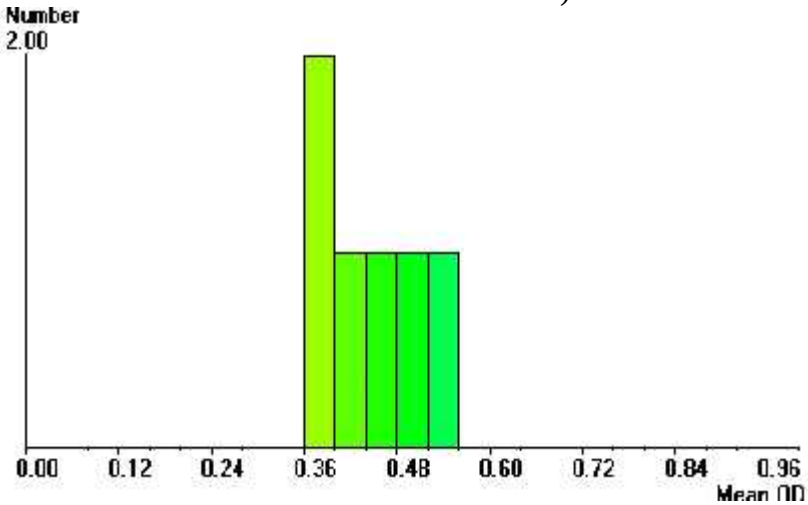

Chart 6. Histogram showing MOD of recovered cerebellar tissue stained by Mercuy bromophenol blue stain showing distrubution of protein, the gram deviated slightly to the right as more cells with slight increased expression of the stain (slightly increased proteins in the tissue, recovered tissues).

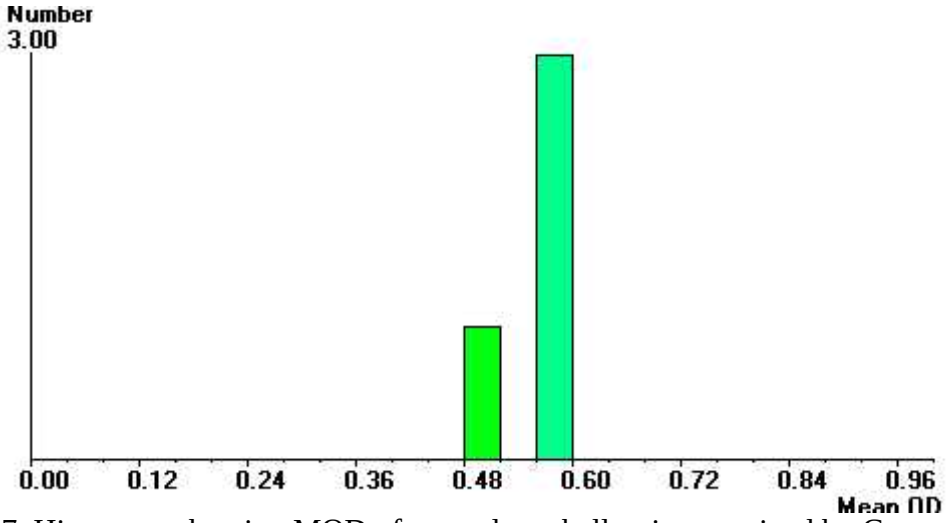

Chart 7. Histogram showing MOD of control cerebellar tissue stained by Congo Red stain showing distrubution of amyloid-ß .

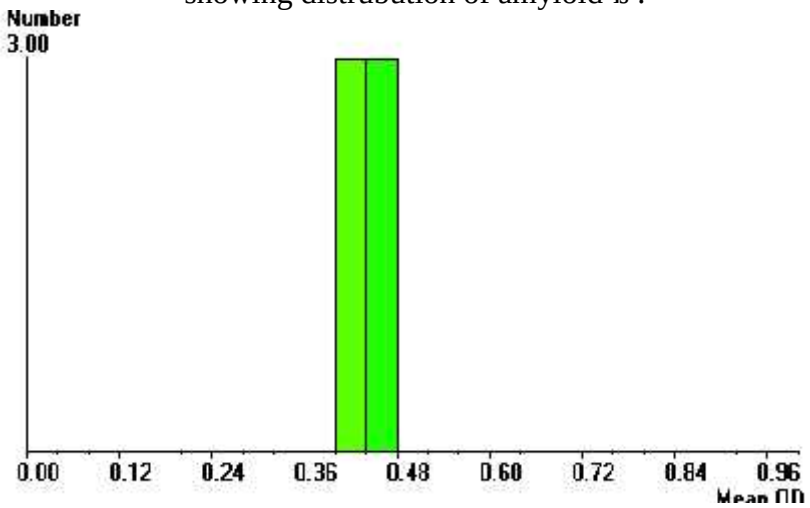

Chart 8. Histogram showing MOD of radiated cerebellar tissue brain tissue stained by Congo Red stain showing distrubution of amyloid-ß ,the gram deviated to the left as much cells with lowest expression to the stain (decreased amyloid-ß in the tissue).

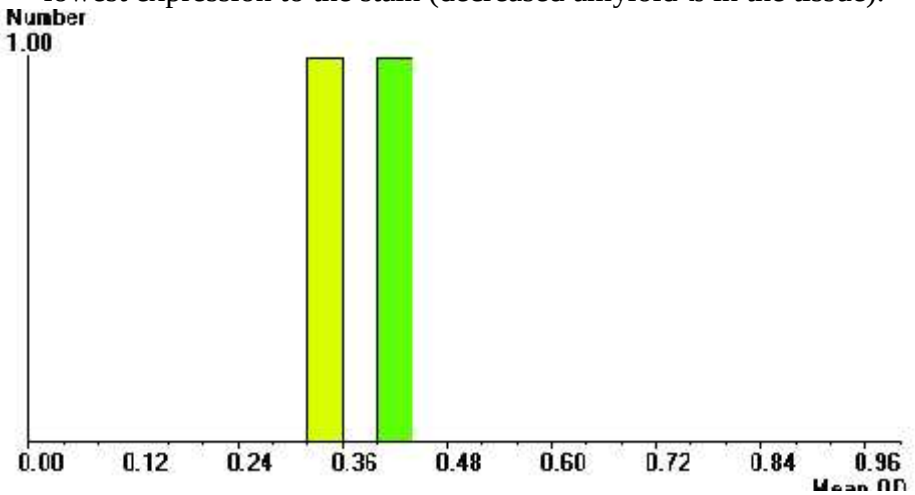

Chart 9. Histogram showing MOD of recovered cerebellar tissue stained by Congo Red stain showing distrubution of amyloid-ß , the gram deviated slightly to the left as some cells with lower expression to the stain (decreased amyloid-ß in the tissue).

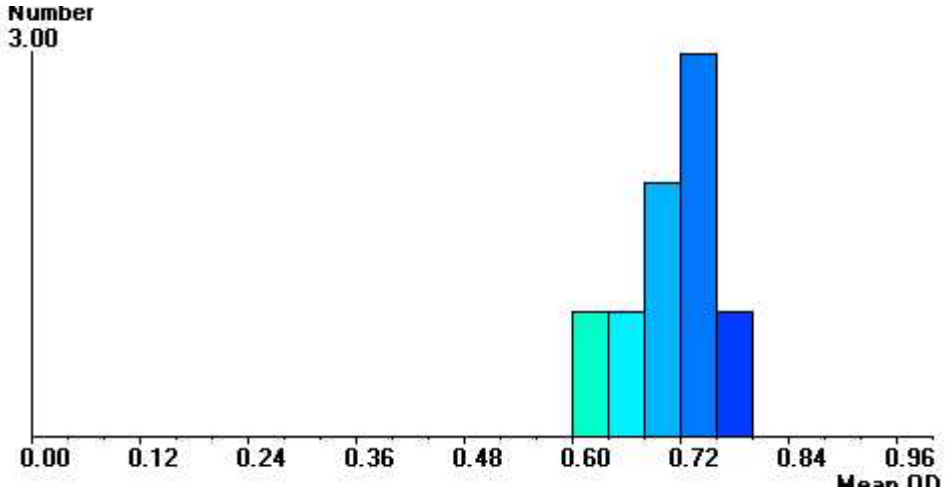

Chart 10. Histogram showing MOD of control cerebellar tissue stained by Feulgen`s stain showing distrubution of DNA.

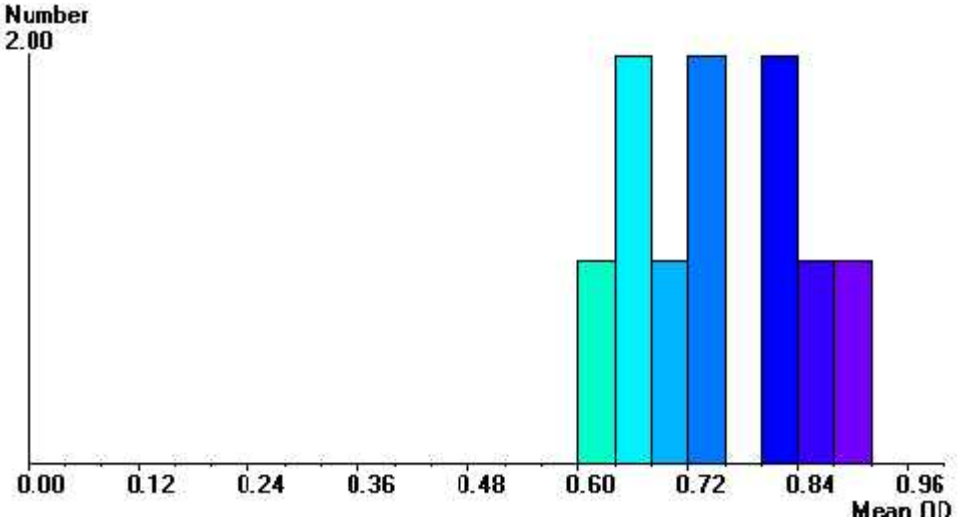

Chart 11. Histogram showing MOD of irradiated cerebellar tissue brain tissue stained by Feulgen`s stain showing distrubution of DNA , the gram deviated to the right as much cells with highest expression to the stain (increased DNA content).

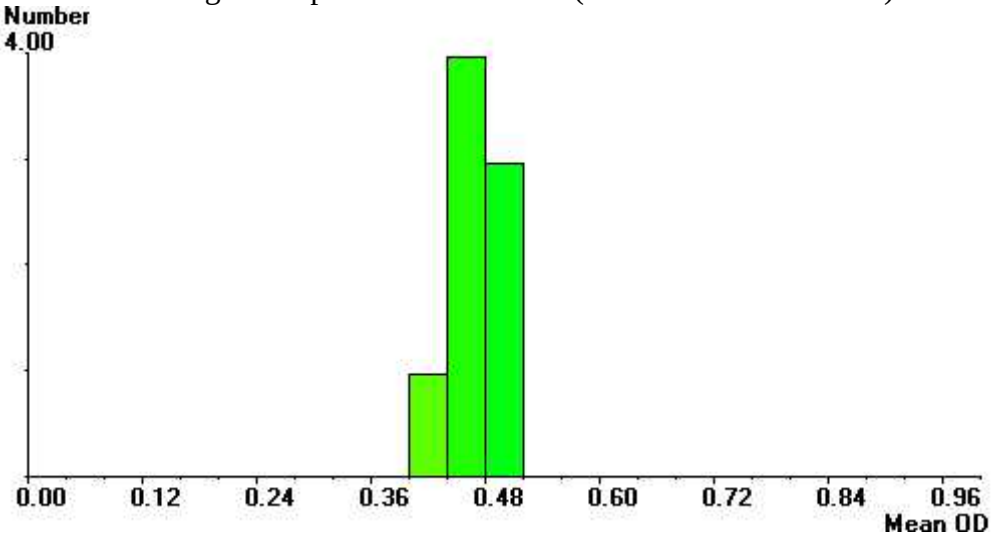

Chart 12. Histogram showing MOD of recovered cerebellar tissue stained Feulgen`s stain showing distrubution of DNA , the gram deviated more to the left as more cells with lower expression to the stain (clumbed DNA content).

Normal total protein content was seen in the cerebellar cortical layers of the control group illustrated by diffuse deeply blue stained granules throughout the cerebellar cytoplasm and nuclei (Figs. 4A, 4B). Decreased protein content was demonstrated in the different cerebellar cortical layers of the irradiated group (Figs. 4C, 4D) with a nearly normal appearance of the total protein content in the recovery group (Figs. 4E,4F). MOD values showed a highly significant decrease in the protein content of irradiated group (0.370 ± 0.056) with a significant decrease in its value (0.477 ± 0.085) in the recovery group as compared to the control group (0.497 ± 0.024) value, charts (4-6).

Negative reaction of Congo red was noticed in the different layers of the control cerebellar cortex except a slight deposition of amyloid-β in the nuclei of Purkinje cells which acquired orange stain affinity (Figs. 5A, 5B). Slightly decreased deposition of amyloid-β plaques in all layers of the cerebellum was detected in both irradiated and recovery groups (Figs. 5C- 5F). Such decrease was realized by MOD values where amyloid-β decreased significantly to (0.425 ± 0.019) and (0.428 ± 0.065) in irradiated and recovery groups respectively in comparison with those of the control group (0.452 ± 0.031) value, charts (7-9).

The normal distribution of DNA materials was noticed in the form of moderate reaction in the nuclei of the cerebellar cortical layers especially the granule layer nuclei (Figs. 6A, 6B). Exposure of mice to the EMF of mobile phone increased the DNA content of irradiated group nuclei (Figs. 6C, 6D) with an obviously decrease in the recovery group (Figs. 6E, 6F). The image analysis results of the histochemical staining of DNA slides supported the qualitative findings where there was high significant increase in the MOD value of irradiated group (0.755 ± 0.069) with highly significant decrease in its value to reach (0.481 ± 0.025) as compared to that of the control group (0.565 ± 0.035) value, and charts (10-12).

4 Discussion

The focus of the majority of animal studies has mainly been on effects of the EMF on the brain tissue because of the close vicinity of mobile phones during calls. The cerebellum was selected for this work because it is one of the most important regions of the brain with respect to the development. It was reported before that during the normal use, mobile phones emit EMFs, which are absorbed into the brain of the user and disrupt its functions (Marino et al., 2003).

As regarding the current experiment, exposure of male mice to mobile phone radiation (900- 1800 MHz) 45 min/day for one month showed some dystrophic changes in Purkinje cells layer represented by decreased number, degenerated and accumulated granular layer cells with edematous spaces which appeared congested with blood. Increased pericellular spaces were also depicted between granular and Purkinje cells layers. In the recovery group, cerebellar tissue showed mild thinning of their granular layer with a reduction in the number of Purkinje cells. Granular layer cells degeneration and accumulation were also detected with blood congested edematous spaces in between. These observations are consistent with those reported before where EM radiation caused significant functional and structural disorders of brain cells in mice (Krstic et al., 2005). Also, low EMF can induce irreversible destruction to the brain of mice, specifically the reduction of the Purkinje cells with thinning of the granular layer (Rosli and Teoh, 2009). As regarding the current findings, epithelial pial surface detachment and degeneration of Purkinje cells comes in consonance with those reported earlier (Nassar and Shkorfo, 2009). In contrast to this study, Khalil et al. (2012) recorded that 900 MHz GSM-like RF radiation did not cause significant histopathological changes in the brain tissues. Increased collagen fibres deposition was noticed in the different cortical cerebellar layers of the exposed group especially in their white matter layer which comes in coincidence with those reported by Seyhan and Canseven (2006) where extremely low-frequency EMFs increased collagen synthesis in Guinea pigs brain tissue. George et al. (2001) declared that decreased collagenolytic enzymes synthesis by the impaired cells could be contributed to the accumulation of collagen fibres.

The disturbed histochemical pattern of polysaccharides, total proteins, amyloid β-proteins, and DNA reflected the hazardous effects of EMF in the biochemical and histochemical aspects which preceded the pathological signs. Both histochemical observations and MOD measurements showed a mild increase in the PAS +ve materials in EMF exposed group with a slight decrease in its content in the recovery group, such increase could be attributed to glucose uptake following extremely low-frequency EMF exposure (Sieron et al., 2007). In the same direction Anan et al. (2012) attributed the post EMF exposure increase in PAS +ve materials to the adaptive changes of irradiated cells as a response to the stress prompted by the non ionizing radiation which accompanied by increased secretion of cortisol and accumulation of glycogen in the exposed cells.

The decrease in PAS +ve reaction one month following the onset of EMF exposure during the current experiment comes in line the results of Lotfi et al. (2011) where the authors recorded a lower blood glucose level of mice (0 to 20 days) following extremely low-frequency electromagnetic field (ELF-EMF) exposure and attributed it to the increase in glucose uptake through increased pancreatic secretion of insulin and also by increasing affinity of insulin receptors and/or increasing signal transduction in the target cells, and insulin transporter function (Sieron et al., 2007),whereas some other reports confirmed decreased glucose-stimulated insulin secretion, membrane depolarization, and cytosolic free calcium ion concentration post ELF-EMF exposure (Sakurai et al., 2005).

As regarding the present experiment, the detectable decrease in both total protein and amyloid-B in both irradiated and recovery groups was noticed earlier (Hamzany et al., 2013; Dragicevic et al., 2011). Such decrease may be due to the damaged cells which leak into

circulation following the exposure process. Also, EMF exposure could reduce the brain amyloid-β deposition through the decreased aggregation of amyloid-β and the increase in soluble amyloid-β levels (Soderqvist et al., 2010).

The current results also showed a highly significant increase in the DNA content of irradiated group followed by a high significant decrease in the recovery group which could be due to increasing reactive oxygen species (ROS) production as a result of the pathological processes driven by mobile phone exposure. ROS was reported before to be directly involved in oxidative damage process of cellular macromolecules including lipids, proteins and nucleic acids post exposure to electromagnetic radiation from cellular phones (Irmak et al., 2002; Ilhan et al., 2004).

Conclusion: One month exposure to 900- 1800 MHz RF-EMF emitted from mobile phone induced many histopathological and histochemical disorders in the cerebellum of adult male mice. No complete recovery signs were noted even after 30 days following the onset of EMF exposure which could be due to the limited recovery time (one month only). Further studies are still needed to clarify the confusing findings from the current study.

5 References

Anan H. H., Gawish M.F., Amer M.G. and Ibrahim N.E. (2012): Effects of low magnetic irradiation on morphology and ultrastructure of parotid glands in rats and amelioration by vitamin E. J Cytol Histol 2012, 3:2 http://dx.doi.org/10.4172/2157-7099.1000139.

Bancroft, J.D. and Gamble, M. (2002): Theory and Practice of Histological Techniques. 5th ed., Churchill livingstone. London. pp: 150-152.

Dragicevic, N. , Bradshaw, P.C. , Mamcarz, M. , Lin, X. , Wang, L. , Cao, C. and Arendash, G.W. (2011): Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice: a mechanism for electromagnetic field-induced cognitive benefit?. Neuroscience. 30: 135-149.

Drury and Wallington (1980): Carleton's Histological Technique. 4th ed. Oxford Univ. Press. New York.

Forgács Z., Somosy Z., Kubinyi G., Sinay H., Bakos J., Thuróczy G., Surján A., Hudák A., Olajos F., and Lázár P. (2004): Effect of whole body 50 Hz magnetic field exposure of mouse Leydig cells. Sci. World J., 4(S2):83-90.

Fragopoulou, A.F. , Samara, A. , Antonelou, M.H. , Xanthopoulou, A. , Papadopoulou, A. , Vougas, K. , Koutsogiannopoulou, E. , Anastasiadou, E. , Stravopodis, D.J. , Tsangaris, G.Th. and Margaritis, L.H. (2012): Brain proteome response following whole body exposure of mice to mobile phone or wireless DECT base radiation. Electromagnetic Biology and Medicine, 1:25-36.

George I., Ramesh k., Stem R. and Chandrakasan G. (2001): Dimethyl nitrosamine induced liver injury in rats: the early deposition of collagen. Toxicology, 156: 129-138

George, W. and William, G. (1980): Statistical methods. 7th edition, pp. 217.

Hamzany, Y. , Feinmesser, R. , Shpitzer, T. , Mizrachi, A. , Hilly, O. , Hod, R. , Bahar, G. , Otradnov, I. , Gavish, M. and Nagler, R.M. (2013): Is human saliva an indicator of the adverse health effects of using mobile phones?. Antioxid. Redox. Signal, 18 (6): 622 - 627.

Hao YH. and Peng RY. (2015): Effects of microwave radiation on brain energy metabolism and related mechanisms. Hao et al. Military Medical Research (2015) 2:4 DOI 10.1186/s40779-015-0033-6

Hardell, L. and Sage, C. (2008): Biological effects from electromagnetic field exposure and public exposure standards. Biomed. Pharmacother., 62(2):104-109.

Hotchkiss R.D. (1948): A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations. Arch. Biochem., 16: 131-132.

Ilhan, A. , Gurel, A. , Armutcu, F. , Kamisli, S. , Iraz, M. , Akyol, O. and Ozen, S. (2004): Ginko biloba prevents mobile phone-induced oxidative stress in rat brain. Clin. Chim. Acta, 340: 153-162.

Irmak, M. K. , Fadillioglu, E. , Gulec, M. , Erdogan, H. , Yagmurca, M. and Akyol, O. (2002): Effects of electromagnetic radiation from a cellular telephone on the oxidant and antioxidant levels in rabbits. Cell. Biochem. Funct., 20: 279-283.

Khalil, A. , Al-Adhammi, M. , Al-shara, B. , Gagaa, M. , Rawshdeh, A. and Alshamli, A. (2012): Histological and ultrastructural analyses of male mice exposed to mobile phone radiation. J. Toxicology Review, 1(1): 1-6.

Krstic, D.D., Ind, I.C. , Sokolovic, D.T. , Markovic, V.V. , Petkovic, D.M. and Radic, S.B. (2005): The results of experimental exposition of mice by mobile telephones. In: Microwave Review. T.E.L.S.I.K.S. Conference, Serbia and Montenegro, pp: 34-37.

Lotfi, A. , Ahadi, F. , Shahryar, H. A. and Chekani-Azar, S. (2011): Effects of exposure to constant or pulsed 50 Hz magnetic fields on body weight and blood glucose concentration of BALB/C Mice. Int. J. Agric. Biol., 13: 148-150.

Marino, A.A., Nilsen, E. and Frilot, C. (2003): Nonlinear changes in brain electrical activity due to cell phone radiation. Bioelectromagnetics, 24: 339-346.

Mazia D, Brewer P.A. and Alfert M. (1953): The cytochemical staining and measurement of protein with mercuric bromophenol blue. Biol. Bull., 104:57-67.

Meral, I., Mert, H., Mert, N. and Deger, Y. (2007): Effects of 900 MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels. Brain Res. J. Brainres, 3:7-15.

Nassar, S.A. and Shkorfo, M.O. (2009): Changes produced in cerebellum and kidney of rabbit after chronic exposure to mobile phone microwaves. The Egyptian. of Hospital Medicine, 34:164-182.

Nittby, H., Brun, A., Eberhardt, J., Malmgren, L. , Persson, B.R. and Salford, L.G. (2009): Increased blood–brain barrier permeability in mammalian brain 7 days after exposure to the radiation from a GSM-900 mobile phone. Pathophysiology, 16(2-3):103-112.

Rosli, Y. and Teoh, P.J. (2009): The effect of low electromagnetic field in the cerebellar layers of mice. J. Biol. Sci., 9:601-606.

Sakurai T., Koyama S., Komatsubara Y., Jin W. and Miyakoshi J. (2005): Decrease in glucose-stimulated insulin secretion following exposure to magnetic fields. Biochem. Biophys. Res. Commun., 332(1):28-32.

Seyhan N., Canseven A.G. (2006): In vivo effects of ELF MFs on collagen synthesis, free radical processes, natural antioxidant system, respiratory burst system, immune system activities, and electrolytes in the skin, plasma, spleen, lung, kidney, and brain tissues. Electromagn. Biol. Med.; 25 (4): 291-305.

Sieron A., Brus H., Konecki J., Cieślar G., Szkilnik R., Nowak P., Noras L, Kwieciński A., Kostrzewa R.M. and Brus R. (2007): Effect of low frequency electromagnetic fields on [3 H] glucose uptake in rat tissues. Polish J.Environ. Stud. Vol. 16, No. 2 : 309-312.

Soderqvist, F. , Hardell, L. , Carlberg, M. and Mild, K.H. (2010): Radiofrequency fields, transthyretin, and Alzheimer's disease. J. Alzheimers Dis., 20 (2):599-606.

Sonmez, O.F., Odaci, E. , Bas, O. and Kaplan, S. (2010): Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 MHz electromagnetic field. Brain Res., 1356:95-101.

Usikalu M. R., Rotimi S. O. and Oguegbu A. E. (2012): Effect of exposure of 900 MHz radiofrequency radiation on rat brain. European Journal of Experimental Biology, 2 (6):2499-2504.

Valle S. (1986): Special stains in microwave oven. J. Histotechnol., 9:237-248.

Westerman, R. and Hocking, B. (2004): Diseases of modern living: neurological changes associated with mobile phones and radiofrequency radiation in humans. Neuroscience Letters, 361 (1-3):13-16.