

Effects of Daily Exposure to Microwave Radiation on Biophysical Properties of Rat's Cornea

Rana Mohamed¹, Aida Salama¹, Mona Gamal² and Sahar Awad¹

¹Biophysical Branch, Collage of Science, Al- Azhar University, Cairo and ² Biophysics and Laser Science Unit, Research Institute of Ophthalmology, Giza, Egypt

THE present study was conducted to investigate the harmful effects of daily exposure to microwave radiation on the cornea of rats. Forty eight, healthy, mature male Wister Albino rats (180-200 g) were classified into 6 groups of 8 rats each. Group (I) was used as control group which was sham exposed. Groups (II, III, IV, V and VI) were exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for one, three, five, seven and nine days respectively. All rats were decapitated and rat's eyes were enucleated immediately at the end of exposure period. Total soluble protein content, Fourier transform infrared (FTIR) spectroscopy analysis and histopathological examination were carried out on cornea. The results obtained showed that the total soluble protein content of cornea decreased by increasing exposure time to microwave radiation. The most prominent changes in FTIR were detected in NHOH, CH stretching and fingerprint regions after irradiation with microwave for 9 successive days (8 hours/day). Histopathological examination of cornea revealed that rats exposed to 1.944×10^{-2} mW microwave radiation for 1 and 3 days show no change in all corneal layers. The pronounced change was observed after 5, 7 and 9 days of daily exposure to microwave radiation. Finally, it is concluded that daily exposure to microwave radiation has the potential to cause biological effects in corneal tissue.

Keywords: Microwave radiation, Cornea, The total soluble protein content, Fourier transform infrared spectroscopy and histopathological examination.

Introduction

Microwaves are a form of electromagnetic radiation with frequencies ranging from 300MHz to 300GHz and wavelengths ranging from 1 meter to 1 millimeter (Sri Nageswari, 2003). Microwave radiation is emitted by many modern instruments, including cell phone transmitters and receivers, radars, radio and TV transmitters, visual display unit terminals and microwave ovens (George, 2008). Microwave (MW) radiation has been shown to have a negative impact on biological systems. The impact of microwave radiation on biological systems can be divided into two types: thermal and non-thermal effects (Scenih, 2006). Microwave thermal effects are caused by prolonged exposure to electromagnetic radiation energy, which result in a rise in body mass temperature of more than 1 °C due to absorption of these radiations. Non-thermal effects, on the

other hand, occur as a result of electromagnetic radiations energy directly interacting with cell molecules rather than a result of a rise in body mass temperature (Sienkiewicz, 2013). Microwave radiation absorption has the potential to alter cell propagation rates, enzyme activity, and gene expression in cells' DNA (Ali, 2013).

The dielectric constant and conductivity of a material determine how much microwave energy is absorbed. Because both values for water are very high, it's apparent that tissues and organ systems with high water content are the most affected by microwave radiation. Similarly, heat will not be released fast in places of the body where there isn't much blood flow, causing severe injury (Baranski and Czernski, 1976). Since the eyeballs have high water content, very little blood circulation and hence have a weak cooling mechanism, they are especially susceptible

to thermal damage from microwave radiation exposure (Davias *et al.*, 1999). The transparent cornea is the front part of the eye's outer covering and it serves two purposes: it protects the interior contents of the eye and it provides roughly two-thirds of the eye's refractive power. (Hayashi *et al.*, 2002). Microwave radiation effects on eye were reported in many studies. (Kues and Monahan, 1992) showed that exposure to low level of microwave radiation can cause considerable visual alterations in nonhuman primates. These alterations range from cellular damage to impaired visual function. At 10 mW/cm², they found corneal endothelial lesions, increased iris vascular permeability, and degenerative alterations in iris and retina cells. (Akdag *et al.*, 2002) investigated the effect of low-intensity microwave radiation (MW) on rat eyes and the protective effect of some vitamins against the damage induced by microwave. The rats were exposed to a 9450 MHz microwave for 21 days (1 hours/day). In the cornea of experimental groups, cellular loss, shape and size differences among cells and lack of polarity were noticed compared to control group. Slight edema, congestion, desquamation in epithelial cells, separation between layers in some areas and alteration in pigment cells of retina were also determined in experimental group rats compared to control groups. (Kojima *et al.*, 2004) subjected rabbit eyes to 2.45 GHz microwaves for 60-20 minutes (300 mW/cm²; 108 W/kg) with and without systemic anaesthesia, and used a fluoroptic thermometer to quantify the temperatures within the eye during microwave exposure. The findings revealed corneal edema, meiosis, conjunctival congestion, and inflammation of the anterior segments. According to (Balci *et al.*, 2007), mobile phone radiation causes oxidative stress in corneal and lens tissues, and antioxidants such as vitamin C can aid to attenuate these effects.

We believe more research efforts are needed to understand the effects of microwaves, thus the present study was designed to assess the molecular changes of rats' cornea after daily exposure to microwave radiation.

Materials and Techniques

Experimental Animals

Forty eight healthy, mature, male Wister Albino rats (180-200 g) were randomly selected from the animal house at the Research Institute of Ophthalmology, Giza, Egypt. All rats were housed in special designed cages in a room temperature

of 22 - 25°C and a light/dark cycle (12/12 hrs.) environment. Animals were fed on a laboratory balanced diet with free access to water and food. Use of animals in the present study was in agreement with the guidelines from the association of research in vision and ophthalmology (ARVO) statement and approved by the Research Institute of Ophthalmology ethical committee (RIO—ethical committee). The animals were classified into 6 groups of 8 rats each as follows:

Group (I) was used as control group which was sham exposed. Groups (II, III, IV, V and VI) were exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for one, three, five, seven and nine days respectively.

At the end of each exposure period, the rats were decapitated. The eyes were enucleated and four eyes were subjected to histopathological examination. The rest of eyes were opened carefully by corneal section through the ora serrata. The cornea was separated and prepared for the measurements of total soluble protein content and FTIR.

Microwave exposure system

The microwave system shown in Fig. 1 consists of Gunn Diode Transmitter which was adjusted to provide 1.944×10^{-2} mW of coherent, linearly polarized microwave output ($I = 36 \mu\text{A}$, $V = 0.54 \text{ v}$). The unit consists of a Gunn diode in a 1.525 GHz resonate cavity, a microwave horn to direct the output, and an 18 cm stand to help in reducing top reflections. The Transmitter may be powered directly from a standard 220/240 V AC, 50/60 Hz by using the provided power supply connected to a jack on transmitter panel. The Transmitter contains a LED power indicator light to indicate the unit is on, and a rotational scale on a base wooden board to adjust the distance between the Transmitter and Receiver. The output is linearly polarized along the axis of the diode and the attached horn radiates a strong beam of microwave radiation centered along the axis of the horn. The Microwave Receiver provides a meter for reading the output power ($P = VI = 1.944 \times 10^{-2}$ mW) proportional to the incident microwave signal. It also has a microwave horn identical to that of the transmitter's which collects the microwave signal. The animals were placed in a plastic cage to prevent the scattering or diffraction of microwave radiation emitted from the transmitter. The cage has a small opening for breathing. The cage is placed on a small wooden base in the area between the transmitter and receiver.

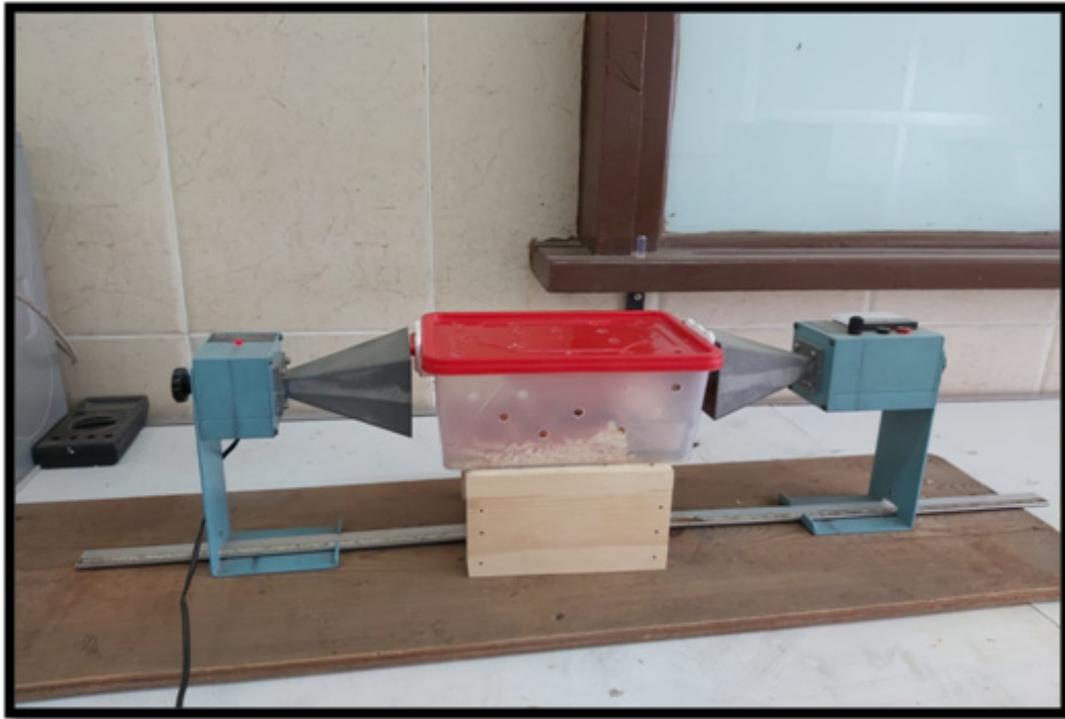


Fig.1. Microwave exposure system.

Experimental measurements

Determination of total soluble protein content: The corneas were removed from the anterior chamber of the eye, weighted and homogenized in bi-distilled water and then centrifuged at 8,000(rpm) for 30 minutes. The supernatant were separated for the determination of total soluble protein according to the method of (Lowry et al., 1951)

Fourier transform infrared (FTIR) spectroscopy analysis: FTIR spectra of pool samples of rat's cornea were recorded by (Thermo Scientific Nicolet iS5) FTIR spectrometer, USA, in the 4,000 – 1,000 cm^{-1} range. To eliminate interference from atmospheric carbon dioxide and water, the spectrometer is operated under a continuous dry nitrogen gas purge. Before Fourier transformation, the data is baseline corrected and smoothed using Savitzky Golay to remove noise. Each interferogram is made up of 100 scans with a resolution of 2 cm^{-1} . To produce the KBr discs for FTIR analysis, corneas are weighed, lyophilized, and then mixed with KBr powder (98mg KBr: 2mg cornea). Using Origin Pro 9.3 software, the average of spectra for each group is calculated.

Histopathological examination: Four eyes from each of the rat groups were used for studying the structure of the cornea. Results from the

groups exposed to microwave radiation were compared to that from the control group. At the end of each period, rats were sacrificed; the eyes were enucleated, immediately immersed and injected at the corneoscleral junction with 4% glutaraldehyde buffered at PH 7.3 with 0.1 M phosphate buffer containing 5.4% glucose at 4°C. After half an hour, the eyes were opened by corneal section through the ora serrata. The retina and lens were removed and the cornea was immersed again in fresh glutaraldehyde buffered solution. After half an hour, the cornea was cut into small pieces (about 1 mm^3) and then further fixed for another six hours in fresh glutaraldehyde buffered solution. Then, the specimens were washed for one hour with several changes of phosphate buffer at 4 °C. They were further washed in distilled water, dehydrated in cold alcohol series (50%, 70%, 80%, 90% and 96%). The specimens were then placed in propylene oxide 10 minutes then in Araldite. The specimens were embedded in rubber boats filled with freshly prepared araldite mixture and the blocks were polymerized at 70°C for 48 hours. Semi-thin sections blocks were sectioned by LKB ultratome, mounted on a glass slide and stained with Toluidine blue. The slides were examined by a light microscope (Glauert 1965).

Statistical analysis: The results were statistically evaluated according to the “students” t- test (Bell, 1995). The results were presented as the mean \pm the standard deviation (S.D.). The significance level was set at $p < 0.05$.

Results

Total soluble protein content

Table 1 illustrates the total soluble protein content of corneas from control rats and rats irradiated with 1.944×10^{-2} mW microwave radiation (8 hours/day) for successive 1, 3, 5, 7 and 9 days. The total soluble protein content from control rats' cornea was 72 ± 3.52 mg/g wet weight. The data indicated non-significant decrease in the level of total soluble protein content after exposure to microwave radiation for 1 and 3 days compared to control group however, there is a significant decrease in the level of total soluble protein content after exposure for 5, 7 and 9 days compared to control group as shown in Table 1.

Ftir Spectroscopy

The spectrum analysis was carried out in three distinct frequency ranges: NH–OH region ($3800\text{--}3000\text{ cm}^{-1}$), CH stretching region ($3000\text{--}2800\text{ cm}^{-1}$), and fingerprint region ($1800\text{--}900\text{ cm}^{-1}$) (Akkas *et al.*, 2007).

a-NH-OH Region

Figure 2 illustrates the stretching NH–OH region ($3800\text{--}3000\text{ cm}^{-1}$) for cornea from control rats and those exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for 1, 3, 5, 7 and 9 days respectively. The main bands of control rat were resolved into three structural components centered at 3559 cm^{-1} ($_{\text{str}}\text{OH}$), 3428 cm^{-1} ($_{\text{str}}\text{OH}_{\text{asym}}$) and 3350 cm^{-1} ($_{\text{str}}\text{NH}_{\text{asym}}$) (Dovbeshko *et al.*, 2000). When rats exposed to 1.944×10^{-2} mW microwave radiation for 1 and 3 days the IR pattern illustrated no significant change in band position and/or bandwidth. However, there was a significant decrease in frequency of $_{\text{str}}\text{OH}$ and $_{\text{str}}\text{NH}_{\text{asym}}$ bands when rats exposed to microwave radiation for 5 days compared to control (Table 2). After exposure of rats to 1.944×10^{-2} mW microwave radiation for 7 and/or 9 days, $_{\text{str}}\text{NH}_{\text{asym}}$ band disappeared while a new band of $_{\text{str}}\text{OH}_{\text{sym}}$ appeared at wave number 3274 cm^{-1} and 3270 cm^{-1} respectively. Also, there was a significant change in $_{\text{str}}\text{OH}_{\text{asym}}$ band position and width compared to control.

b-CH Stretching Region

The CH region ($3000\text{--}2800\text{ cm}^{-1}$) shown in Table 3 and Fig. 3 indicates the presence of 4 bands in control rats cornea centered at 2961 cm^{-1} ,

2924 cm^{-1} , 2882 cm^{-1} , and 2852 cm^{-1} which corresponds to CH_3 asymmetric, CH_2 asymmetric, CH_3 symmetric and CH_2 symmetric respectively as previously described by (Severcan *et al.*, 2000). When rats were exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for 1, 3, 5 and 7 days, the IR pattern illustrated no significant change in band position and/or bandwidth compared to control. The main change in CH region is obvious in rats exposed to microwave radiation for 9 days where a new band of CH_{str} was detected at 2900 cm^{-1} compared to control.

c-Finger Print Region

The Fingerprint region of the normal rats' cornea was characterized by 8 absorption bands: (1)- Ester C=O at 1740 cm^{-1} ; (2)- Amide I at 1657 cm^{-1} (Dovbeshko *et al.*, 1997); (3)- Amide II at 1541 cm^{-1} (Palaniappan *et al.*, 2009); (4)- bend CH_2 1454 cm^{-1} ; (5)- COO^- sym at 1398 cm^{-1} ; (6)- Amide III band components of protein at 1316 cm^{-1} (Yang *et al.*, 2005); (7)- PO_4^{2-} asymmetric phosphate at 1234 cm^{-1} ; and (8)- PO_4^{2-} symmetric phosphate at 1080 cm^{-1} (Table 4).

Figure 4 (a, b, c, d, e and f) illustrates the fingerprint region ($1800\text{--}1000\text{ cm}^{-1}$) of cornea from control rats and cornea from rats irradiated with 1.944×10^{-2} mW microwave radiation 8 hours a day for 1, 3, 5, 7 and 9 days respectively. There is no change in IR bands of fingerprint regions in rats irradiated for 1 and 3 days. After 5 days of daily (8 hours/day) exposure to microwave radiation, amide III disappeared. When rats irradiated with the same field for 7 days, Ester C=O disappeared. After 9 days of daily (8 hours/day) exposure to microwave radiation, Ester C=O, $\text{COO}^-_{\text{sym}}$ and amide III bands disappeared.

Histopathological examination

The cornea of control rats is composed of epithelial layer (Ep), Bowman's layer (B), stroma (S), descemet's membrane (D) and endothelial layer (En) (Fig. 5). When rats were exposed to 1.944×10^{-2} mW microwave radiation 8 hours/day for 1 day (Fig. 6) and for 3 days (Fig. 7) the rats' cornea showed no change in all corneal layers. The epithelial layer showed slight vacuolation after exposure to microwave for 5 days (Fig. 8). The remaining layers of the cornea appear normal. After exposure of rats' cornea to 1.944×10^{-2} mW microwave radiation for 7 days (8 hours/day), the epithelium layer shows dilatation of intracellular spaces between cells. Moreover, edema of endothelial cells was detected (Fig. 9).

The histological examination of cornea from

TABLE 1. Total soluble protein content of rat cornea from all the Studied groups (mg/g wet wt.):

Groups	Mean \pm S.D.	P Value	% change
Control	72 \pm 3.52		
1-day	71 \pm 3.05	NS	1.3 %
3-days	61 \pm 2.27	NS	15.3 %
5-days	56 \pm 2.21	S	22.2 %
7-days	51 \pm 1.21	S	29.2 %
9-days	46 \pm 1.11	S	36.1 %

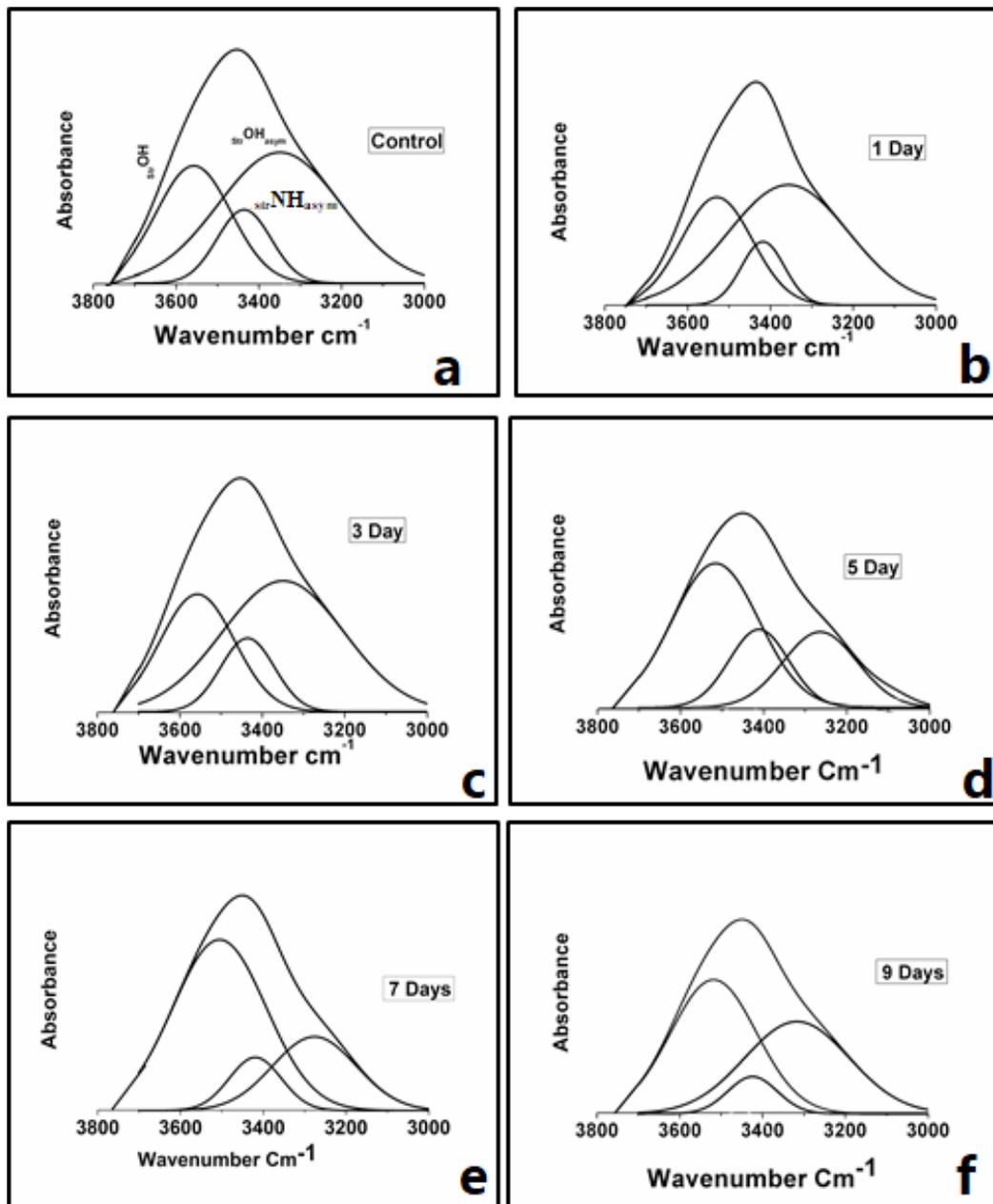


Fig. 2 (a, b, c, d, e and f): NH-OH region (3800-3000cm⁻¹) of rat's cornea from control rats and cornea from rats exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for 1,3,5,7 and 9 days respectively.

TABLE 2. NH-OH region (3800-3000) cm^{-1} of rat's cornea from control rats and cornea from rats exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for 1,3,5,7 and 9 days.

Groups	Peaks assignment			
	OH _{str}	OH _{asvm}	NH _{asvm}	OH _{svm}
Control	3559 ± 8 54 ± 1.6	3428 ± 6 42 ± 1.2	3350 ± 5 76 ± 2.3	
1-day	3558 ± 7 54 ± 1.8	3428 ± 5 42 ± 1.5	3350 ± 5 76 ± 2.2	
3-days	3519 ± 8* 54 ± 2.2	3420 ± 3 39 ± 1.8	3350 ± 9 76 ± 3.0	
5-days	3508 ± 4* 65 ± 1.9*	3420 ± 6 32 ± 1.9	3312 ± 9* 69 ± 2.6	
7-days	3508 ± 7* 66 ± 1.4*	3420 ± 10 41 ± 1.6		3274 ± 5 51 ± 2.4
9-days	3508 ± 6* 65 ± 2.7*	3420 ± 6 41 ± 2.1		3270 ± 7 51 ± 2.1

First line indicates the wavenumber of the band in cm^{-1} , while and the second line indicates the band width

*Statically significant.

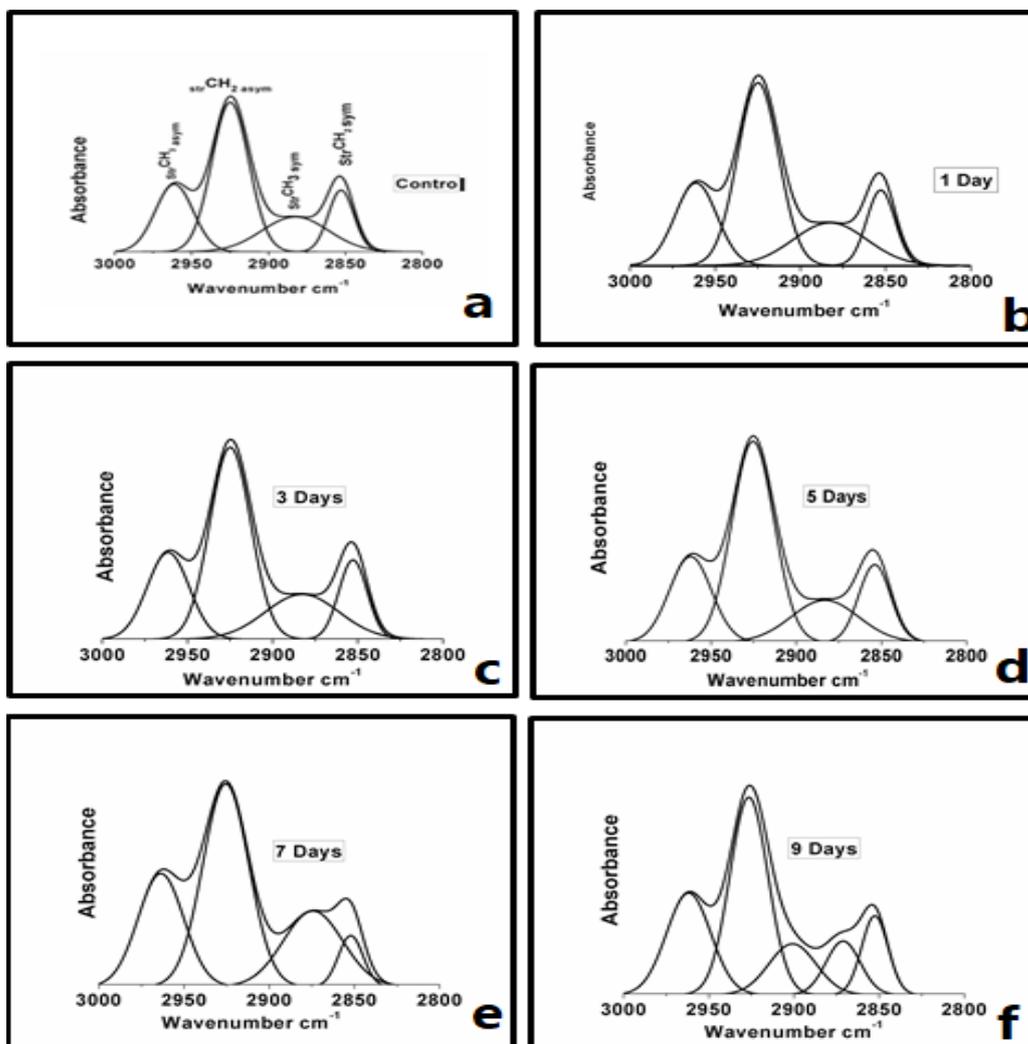


Fig. 3. (a, b, c, d, e and f): CH region (3000-2800 cm^{-1}) of rat's cornea from control rats and cornea from rats exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for 1,3,5,7 and 9 days

TABLE 3. CH stretching region (3000-2800) cm⁻¹ of rat's cornea from control rats and cornea from rats exposed to 1.944×10⁻² mW microwave radiation 8 hours a day for 1,3,5,7 and 9 days.

Groups	Peaks assignment				
	str CH ₃ asym	str CH ₂ asym	str C-H	str CH ₃ sym	str CH ₂ sym
Control	2961 ± 7 56 ± 2.3	2924 ± 5 78 ± 2.1		2882 ± 4 39 ± 1.2	2852 ± 8 44 ± 2.1
1-day	2961 ± 6 56 ± 1.9	2924 ± 6 78 ± 1.8		2882 ± 3 39 ± 1.8	2852 ± 6 44 ± 2.2
3-days	2961 ± 7 55 ± 1.7	2924 ± 5 78 ± 2.9		2882 ± 6 38 ± 2.1	2852 ± 5 44 ± 1.7
5-days	2962 ± 8 55 ± 1.9	2925 ± 7 78 ± 3.1		2883 ± 5 39 ± 1.7	2853 ± 7 45 ± 2.6
7-days	2963 ± 7 62 ± 2.1	2925 ± 8 78 ± 2.7		2874 ± 7 44 ± 2.4	2852 ± 6 44 ± 2.2
9-days	2961 ± 4 66* ± 1.4	2926 ± 6 79 ± 2.2	2900 ± 7 37 ± 2.1	2871 ± 4 34 ± 1.5	2852 ± 5 45 ± 1.9

First line indicates the wavenumber of the band in cm⁻¹, while and the second line indicates the band width

*Statically significant.

TABLE 4. Fingerprint region (1800-1000) cm⁻¹ of rat's cornea from control rats and cornea from rats exposed to 1.944×10⁻² mW microwave radiation 8 hours a day for 1,3,5,7 and 9 days.

Groups	Peaks assignment							
	Ester C=O	Amide I	Amide II	Bend CH ₂	COO ⁻ sym	Amide III	Po2 asym	Po2 sym
Control	1740 ± 6 23 ± 0.7	1657 ± 8 66 ± 1.8	1541 ± 6 62 ± 2.1	1454 ± 5 25 ± 1.1	1398 ± 4 49 ± 1.7	1316 ± 5 39 ± 1.7	1234 ± 3 49 ± 1.7	1080 ± 5 51 ± 2.2
1-day	1740 ± 4 23 ± 0.9	1657 ± 5 65 ± 2.5	1541 ± 4 62 ± 2.4	1454 ± 6 25 ± 0.7	1398 ± 3 42 ± 2.2	1316 ± 4 40 ± 2.1	1233 ± 7 46 ± 2.1	1080 ± 6 56 ± 2.8
3-days	1740 ± 7 23 ± 1.2	1656 ± 6 62 ± 2.7	1541 ± 5 64 ± 2.8	1454 ± 4 27 ± 0.9	1398 ± 6 49 ± 1.8	1321 ± 5 38 ± 2.0	1230 ± 4 34 ± 1.3	1080 ± 6 56 ± 1.9
5-days	1740 ± 6 26 ± 1.1	1656 ± 7 63 ± 1.9	1541 ± 4 62 ± 2.0	1454 ± 6 25 ± 1.5	1398 ± 5 50 ± 2.4		1229 ± 5 38 ± 1.5	1080 ± 6 51 ± 1.6
7-days		1657 ± 6 62 ± 2.0	1541 ± 8 64 ± 1.9	1454 ± 7 29 ± 1.1	1399 ± 5 48 ± 1.2	1335 ± 6* 36 ± 1.3	1246 ± 6 42 ± 1.3	1080 ± 7 59 ± 2.1
9-days		1653 ± 7 85 ± 2.3*	1541 ± 7 66 ± 2.9	1454 ± 9 52 ± 2.5*			1249 ± 3 44 ± 1.4	1080 ± 6 60 ± 1.5

First line indicates the wavenumber of the band in cm⁻¹, while and the second line indicates the band width

*Statically significant

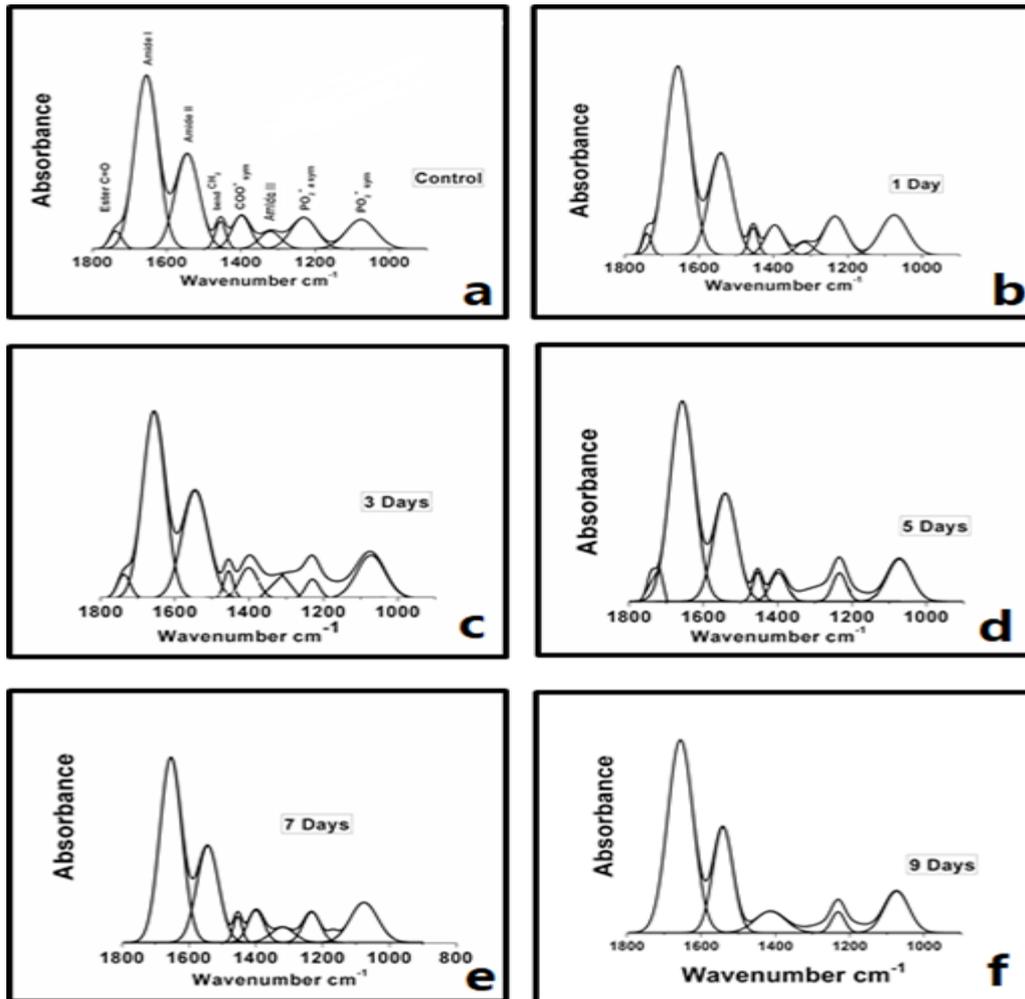


Fig. 4 (a, b, c, d, e and f): FTIR(Fingerprint region (1800-1000) cm^{-1}) of rat's cornea from control rats and cornea from rats exposed to 1.944×10^{-2} mW microwave radiation 8 hours a day for 1,3,5,7 and 9 days respectively.

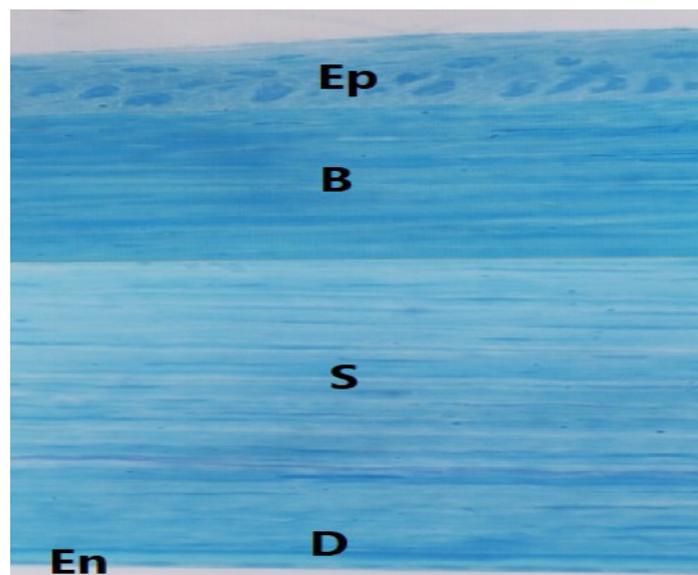


Fig.5. Light micrograph of control rat cornea showing; epithelial layer (Ep), Bowman's layer (B), stroma (S), descemet's membrane (D) and endothelial layer (En). (Toluidine blue X500).



Fig.6. Light micrograph of cornea from rats exposed to $1\ 944\times 10^{-2}$ mW microwave radiation for 1 day showing no change in all corneal layers.

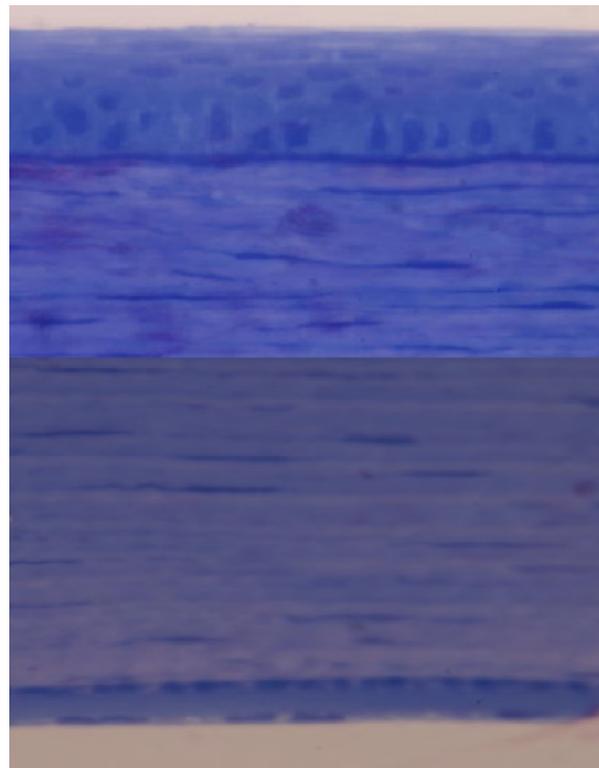


Fig.7. Light micrograph of cornea from rats exposed to 1944×10^{-2} mW microwave radiation for 3 days showing no change in all corneal layers.



Fig.8. Light micrograph of cornea from rats exposed to 1.944×10^{-2} mW microwave radiation for 5 days showing slight vacuolation of epithelial layer (black arrow).



Fig.9. Light micrograph of cornea from rats exposed to 1.944×10^{-2} mW microwave radiation for 7 days showing dilatation of intracellular spaces between epithelial cells (black arrow) and edema of endothelial cells (black star).

rats exposed to 1.944×10^{-2} mW microwave radiation for 9 days showing dilatation of epithelial intracellular spaces, and vascularization

of anterior stroma. The endothelium displayed edema of its cells (Fig. 10, 11).

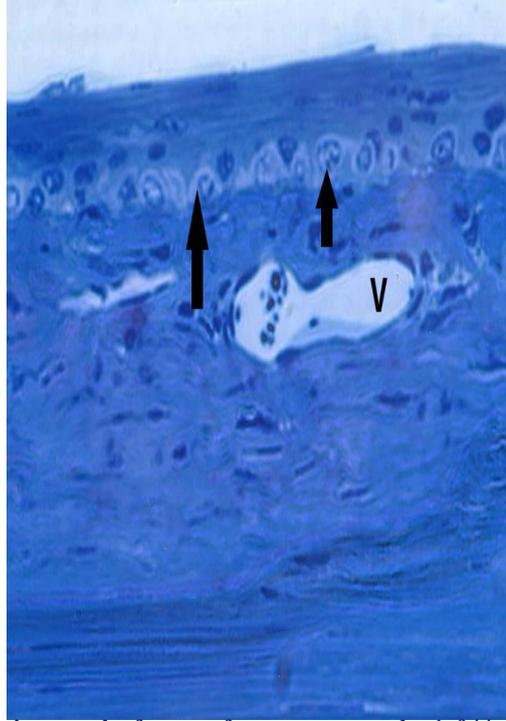


Fig.10. Light micrograph of cornea from rats exposed to 1.944×10^{-2} mW microwave radiation for 9 days showing dilatation of epithelial intracellular spaces, and vascularization of anterior stroma (V).



Fig.11. Light micrograph of cornea from rats exposed to 1.944×10^{-2} mW microwave radiation for 9 days showing edema of endothelial cells.

Discussion

In the present study, the total soluble protein content of cornea decreased by increasing exposure time to microwave radiation and this decrease may be due to denaturation of corneal protein as a result of heat generation. Our analysis is in agreement with (Krstic *et al.*, 2005) who found that electromagnetic fields induced an increase in the levels of protein structural change, which resulted in severe functional and structural abnormalities in several mice cells. (Hyland, 2000) demonstrated that the eyes are one of the most thermally susceptible areas of the body due to their limited blood supply.

FTIR spectroscopy is a well-established technique for determining the structure of proteins, DNA, and RNA in biological tissues (Tajmir-Riahi *et al.*, 2009). The NH stretched region is mainly used for characterizing the lipid and protein structure within the biological tissue. The change detected in this region after exposure to microwave for 7 and 9 days displayed corneal sensitivity to microwave radiation. Moreover, the change in the vibrational frequencies of OH_{str} and OH_{sym} indicated that the hydrogen bond has been destructed and/or weakened as described previously by (Haris and Chapman 1994).

The cornea provides two-thirds of the eye's overall refractive power. Physiological and morphological changes in the cornea can develop as a result of mechanical, thermal, chemical, or radiation damage due to its external placement (Kenchegowda, 2010). The change detected in FTIR patterns of rats' cornea after microwave exposure may be due to the local production of elevated temperature.

Infrared spectroscopy's CH region can be utilized to determine lipid levels in biological tissues. Because they are good monitors of acyl chain modifications, CH₂ stretching vibration provides useful information about the state order of hydrocarbon tails in lipids (Toyran *et al.*, 2008). The formation of the new band CH_{str} after daily exposure to microwave for 9 days indicate that long microwave exposure leads to disorder in the lipid hydrocarbon chains.

COO_{sym} band is vibrational mode owing to amino acid side chain and fatty acid. The disappearance of this band after 9 days of exposure to microwave may indicate change in amino acid side chains of corneal tissue and change in fatty acid tail of membrane. Also, the change in

vibrational motion around the ester C=O (due to lipid) may reflect the changes in lipids of rat cornea after microwave exposure.

The band observed at 1316 cm^{-1} corresponds to amide III components of protein (Fujioka *et al.*, 2004). The disappearance of amide III band after daily exposure to microwave radiation (for 5 and 9 days) reflects alterations in the composition of protein secondary structure. The decrease of total protein content in rat cornea after radiation exposure confirms this finding. The decrease in protein may be due to its denaturation by heat as described by (Wirbelauer *et al.*, 2000) who investigated that, the heating of the cornea is a major problem when exposed to microwave radiation; the cornea is an avascular structure with no heat disposal due to circulation. For 10 and 60 seconds of hyperthermia, the thresholds for 50 percent chance of ocular injury were found to be 65 and 59°C, respectively.

The change in cornea detected in FTIR after exposure to microwave radiation for 5, 7 and 9 days is documented by histopathological examination of cornea tissue. The changes in the corneal layers due to microwave radiation exposure documented by the histological examination are in agreement with (Ringvold *et al.*, 2003) who proposed that the corneal epithelium played an important role in protection against ambient radiation. (Kojima *et al.* 2004) investigated that, in ocular systems subjected to high level microwave radiation corneal edoema, meiosis, conjunctival congestion, anterior segmental inflammation, endothelial cell loss, and vacuolization were seen.

Conclusion

In conclusion, the daily use of microwave instruments and technological appliances emitting low level of microwave radiation may have harmful effects on cornea. The results of this study indicated that the exposure of rats to microwave radiation caused some alterations in corneal protein which may lead to functional disorders. Therefore, low level microwave exposure may lead to changes in membrane structure of corneal cells and destructed or weakened hydrogen bond. In addition Histopathological examination of cornea showing slight vacuolation of epithelial layer, dilatation of intracellular spaces between epithelial cells, edema of endothelial cells and vascularization of anterior stroma which may affect corneal function.

References

1. Akdag M.Z. , Sert C., Erdinc L., Desdag S., Buyukbayram H., Celik M.S. and Cakmak S.S. (2002): "The Evaluation of the Role of Microwave and Vitamins on Rat Eyes Related to Lipid Peroxidation and Tissue Damage" *Biotechnology & Biotechnological Equipment*; 16(1): 131-137.
2. Akkas S. B., Severcan M., Yilmaz O. and Severcan F. (2007): "Effects of lipoic acid supplementation on rat brain tissue" An FTIR spectroscopic and neural network study. *Food Chemistry*, 105, 1281–1288.
3. Ali F. Md. (2013): "SAR Analysis for Handheld Mobile Phone Using DICOM Based Voxel Model". *Journal of Microwaves Optoelectronics and Electromagnetic Applications*;12(2): 363-375.
4. Balci M.,Devirm E., and Durak I. (2007): "Effects of Mobile Phones on Oxidant/Antioxidant Balance in Cornea and Lens of Rats". *Current Eye Research*; 32:21–25.
5. Baranski S. and Czerski P. (eds.) (1976): "Biological effects of Microwaves". Dowden, Hutchinson and Ross, Inc. Stroudsburg, PA.
6. Bell F.D. (1995): "Basic Biostatistic, concept for the health science". Wm. C Brown Communication, Inc. pp.113-123.
7. Davias N. and Grüiffin D.W. (1999): "Effects of Metal-Framed Spectacles on Microwave Radiation Hazards to the Eye of Humans". *Med. and Bio. Eng. and Comput.*; 27: 191-197.
8. Dovbeshko G.I., Gridina N.Y., Carmona P., Navarro R. and Hernanz A. (1997): "Spectroscopy of Biological Molecules". *Modern Trends*, Kluwer Academic, Dordrecht, PP. 451.
9. Dovbeshko G. I., Gridina N. Y., Kruglova E. B., and Pashchuk O. P.(2000): "FTIR spectroscopy studies of nucleic acid damage". *Talanta*; 53(1), 233-246.
10. Fujioka N., Morimoto Y., Arai T., and Kikuchi M. (2004): "Discrimination between normal and malignant human gastric tissues by Fourier transforms infrared spectroscopy". *Cancer Detection and Prevention*, 28(1), 32-36.
11. George D.F., Bilek M.M. and McKenzie D.R. (2008): "Non-Thermal effects in the microwave induced unfolding of proteins observed by chaperone binding". *Bioelectromagnetics*.
12. Glauert A. M. (1965): "The fixation and embedding of biological specimens. In *Techniques for Electron Microscopy*". Oxford: Blackwell, 2nd edition. (ed. Kay D. H.), pp. 166-212.
13. Haris P. and Chapman D. (1994): "Analysis of polypeptide and protein structures using Fourier transform infrared spectroscopy". *Methods Mol Biol*; 22: 183–202
14. Hayashi S., Osawa T. and Tohyama K.(2002): "Comparative observations on corneas,with special reference to Bowman's layer and Descemet's membrane in mammals and amphibians". *J. Morphol.* 254, 247e258 .
15. Hyland G. J. (2000): "Physics and biology of mobile telephony". *The Lancet*; 356: 1833-1836.
16. Kenchegowda S. and Bazan H.E.P.(2010): "Significance of lipid mediators in corneal injury and repair". *Journal of Lipid Research* 51:879–89 .
17. Kojima M., Hata I., Wake K., Watanabe S., Yamanaka Y., Kamimura Y., Taki M. and Sasaki K. (2004): "Influence of anesthesia on ocular effects and temperature in rabbit eyes exposed to microwaves". *Bioelectromagnetics* 25:228–233.
18. 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.
19. Kues H.A. and Monahan J.C. (1992): "Microwave –Induced changes to the primate eye". *Johns Hopkins APL Technical Digest*; 13(1):244-255.
20. Lowry O. H., Rosebrough A. L., Fan A.L. and Randall, R.J. (1951): "Protein measurement with the folin phenol reagent". *J. Biol. Chem.* 193, 265– 275.
21. Palaniappan P. L. R. M. and Vijayasundaram V. (2009): "The FTIR study of the brain tissue of *Labeo rohita* due to arsenic intoxication". *Microchemical Journal*; 91(1):118–124.
22. Ringvold A., Anderssen E. and Kjonniksen I. (2003): "Impact of the environment on the mammalian corneal epithelium". *Investigative Ophthalmology & Visual Science* 44:10–15.

23. **Severcan F., Toyran N., Kaptan N., Turan B.(2000):** “ Fourier transform infrared study of the effect of diabetes on rat liver and heart tissues in the C–H region”. *Talanta* 53, 55–59.
24. **Scenihl (2006):** “Scientific Committee On Emerging And Newly Identified Health Risks”. possible effects of Electromagnetic Fields (EMF) on Human Health.
25. **Sienkiewicz Z. (2013):**” Risk analysis of human exposure to electromagnetic Fields”. European Health Risk Assessment Network on Electromagnetic Fields Exposure.
26. **Sri Nageswari K. (2003):** “Biological Effects of Microwaves and Mobile Telephony”. International Conference on Non-Ionizing Radiation at UNITEN (ICNIR 2003) Electromagnetic Fields and Our Health, October, 1, 11.
27. **Tajmir-Riahi H. A., N’soukpoé-Kossi C. N. and Joly D.(2009):** “Structural analysis of protein–DNA and protein–RNA interactions by FTIR, UV-visible and CD spectroscopic methods”. *Spectroscopy*; 23:81–101.
28. **Toyran N., Severcan F., Severcan M. and Turan B. (2008):**”Effects of selenium supplementation on rat heart apex and right ventricle myocardia by using FTIR spectroscopy: A cluster analysis and neural network approach”. *Food Chem.*; 110: 590-597.
29. **Wirbelauer C., Scholz C. , Hoerauf H. ,et al .,(2000):**”Examination of the cornea using optical coherence tomography” .*Ophthalmologist* ,In press ;97.
30. **Yang Y., Sulé-Suso J., Sockalingum G. D., Kegelaer G., Manfait M., and El Haj A. J. (2005):**”Study of tumor cell invasion by Fourier transform infrared microspectroscopy”. *Biopolymers: Original Research on Biomolecules*, 78(6), 311-317.

تأثير التعرض اليومي لأشعة الميكروويف على الخواص الفيزيائية الحيوية لقرنية الفئران

رنا محمد^١ , عايدة سلامة^١ , منى جمال^٢ . سحر عوض^١

^١ . فرع الفيزياء الحيوية ، كلية العلوم ، جامعة الأزهر ، القاهرة و^٢ . وحدة الفيزياء الحيوية وعلم الليزر ، معهد بحوث طب العيون ، الجيزة ، مصر

هدفت هذه الرسالة الي دراسة الاثار الضاره لأشعة الميكروويف علي قرنية عين الفئران التي تم تعريضها يومياً لـ $1,944 \times 10^{-1}$ ملي وات من اشعاع الميكروويف ٨ ساعات في اليوم لمدة ١ و ٣ و ٥ و ٧ و ٩ أيام متتالية. تم استخدام ثمانية وأربعين من الذكور الناضجين من الفئران البيضاء في هذه الدراسة. تم استخدام ثمانية فئران كمجموعة ضابطة. تم تصنيف بقية الفئران إلى ٥ مجموعات تتعرض لإشعاع الميكروويف. تم دراسة تأثير اشعة الميكروويف عن طريق قياس محتوى البروتين الكلي وقياس طيف الاشعه تحت الحمراء بالإضافة إلى فحص التغيرات النسيجية من أنسجة القرنية . اشارت النتائج الي انخفاض كبير في مستوى البروتين الكلي للقرنيه وهذا التأثير ملحوظ مع زيادة وقت التعرض . كما كشف نمط طيف الأشعة تحت الحمراء عن حدوث تغيرات في المجموعات الوظيفية التي تنتمي إلى مكونات الأنسجة مثل الدهون والبروتينات والأحماض النووية للقرنية. هذه التغيرات تم تأكيدها ايضاً بواسطة الفحص النسيجي للقرنيه الذي اظهر تغيرات في أنسجة القرنيه. وبهذا يمكن الاستنتاج أن اشعة الميكروويف لها تأثير سلبي على العين لأنه يسبب تلف حراري للقرنية .