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Effect of Red, Yellow and White Light Emitting Diode (LED) on Rat's Retina

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ABSTRACT

Light-emitting diodes (LEDs) are the basic lighting components in screens of PCs, phones and TV sets; hence it is so important to know the implications of LED radiations on the human visual system. The present study is designed to evaluate the influence of Red, Yellow and White LEDs on rat's retina. Sixty-six female Wistar albino rats weighing 250-300 g classified into 4 groups. Group (1) served as control. Group (II) exposed to (645 nm) red LED (12 hr light - dark cycle) for 3, 7, 14 and 21days. Group (III) exposed to (588 nm) yellow LED (12 hr light - dark cycle) for 3, 7, 14 and 21days and Group IV exposed to (the wavelength peaks are 448.7 nm and 561 nm) white LED (12 hr light - dark cycle) for 3, 7, 14 and 21days. At the end of each period, electroretinogram (ERG) has been carried out. The results indicated that there was no significant effect for yellow LED on the ERG. After exposure of rat's retina to red and white LED, there is reduction in a- and b-wave amplitudes. The data suggest that the red and white LED may causes retinal toxicity. Therefore, it is very important to recognize the hazard of LED radiations that affects vision and take appropriate precautions for the eye safety.

Keywords: Light Emitting Diodes, Electroretinogram

I. INTRODUCTION

E The exposure to intense natural or artificial light can be detrimental to eye tissues by causing photo-chemical damages. Many studies have been conducted to evaluate the effect of light on the evolution of pre-existing retinopathy [1]. The retinal damages induced by light depend on radiation intensity, radiation wavelength, and time of exposure [2], [3].

Light-induced retinal lesions are characterized by a degeneration of photoreceptors outer segments leading to their death by apoptosis [4], [5] and, light damage in mice has long been used as a model system to study retinal degeneration [2].

Photo-oxidative stress has been implicated in light damage pathogenesis. Immunohistochemistry has demonstrated labeling for markers of oxidative damage [6]. Several antioxidant genes are upregulated following photic injury, including heme oxygenase [7], thioredoxin [6], glutathione peroxidase [8] and ceruloplasmin [9]. Further, exogenous antioxidants protect the rodent retina from photic injury [10], [11]. Photochemical retinal injury resulting from a cumulative effect is caused by free radicals generated from retinal tissue through continuous light exposure [12].

Recently, the use of new technologies in domestic lighting induced a renewal of the interest on the effects of light on the retina. Among these new devices, light emitting diodes (LEDs) are at the canter

of this interest. From a technical point of view LEDs have many advantages such as low energy consumption, high mechanical strength, and, especially, long life. One of the major concerns with the use of this technology is the emission spectrum of LEDs. The spectrum of LEDs is characterized by an intense blue light component absent in the day light spectra [13].

LED (or solid-state) lighting sources are designed to emit all energy within the wavelength range of human vision, making LEDs the most energy-efficient commercially manufactured light. However, many current "white light" LED designs emit much more blue light than conventional lamps, which has a number of health implications, including disruption of circadian rhythms [14]. The most popular LED lighting product, a phosphor conversion (PC) LED, is an LED chip that emits blue light, which passes through a yellow phosphor-coating layer to generate the ultimate white light [15]. Although the white light generated from LEDs appears normal to human vision, a strong peak of blue light ranging from 460 to 500 nm is also emitted within the white light spectrum; this blue light corresponds to a known spectrum for retinal hazards [13]. Some epidemiological studies have suggested that short-wavelength light exposure is a predisposing cause for age-related macular degeneration (AMD) [16].

Animal models have also been used to determine that excessive exposure to blue light is a critical factor in photochemical retinal injury targeting photoreceptors and the retinal pigment epithelium (RPE) [17].

The aim of the present work is to investigate the effects of different exposure protocols. Twenty-four hours exposure emitted from Viva lamp (V BULB), model E27 (220 V, 9 W, Egypt) is compared to a chronic cyclic (dark/light) at domestic levels for 3, 7, 14 and 21 days using different LEDs (red, yellow and white).

II. METHODS AND MATERIAL

A. Animals

Sixty-six female Wister albino rats weighing 250-300 g are selected from the animal house of Research Institute of Ophthalmology, Giza, Egypt. The rats are maintained in a standard 12 hr light - dark cycle with free access to water and balanced diet at a temperature of 22 ± 2°C, 50% humidity. All procedures are conducted according to the principles enunciated in the guide for care and use of laboratory animals. The rats are classified into 4 groups according to the following:

Group I: contain six rats and used as control. Each of the remaining three groups (II, III and IV) contains 20 rats exposed to red, yellow and white LEDs light respectively. Each group was subdivided into three subgroups (5 rats and 10 eyes each) decapitated after 3, 7, 14 and 21 days of LED exposure.

B. Light sources

Single-wavelength red LED (645 nm), yellow LED (588 nm) and white LED (the wavelength peaks are 448.7 nm and 561 nm) are custom-made for the exposure experiments (Fig. 2). The spectrum distributions and total intensities for all light sources are detected by Ocean Optics USB4000 Spectrometer (Detector: Toshiba TCD1304AP linear CCD array, detector range: 200-1100 nm, grating options: 14 gratings/mm, UV through shortwave NIR, optical resolution: 0.3-10.0 nm FWHM).

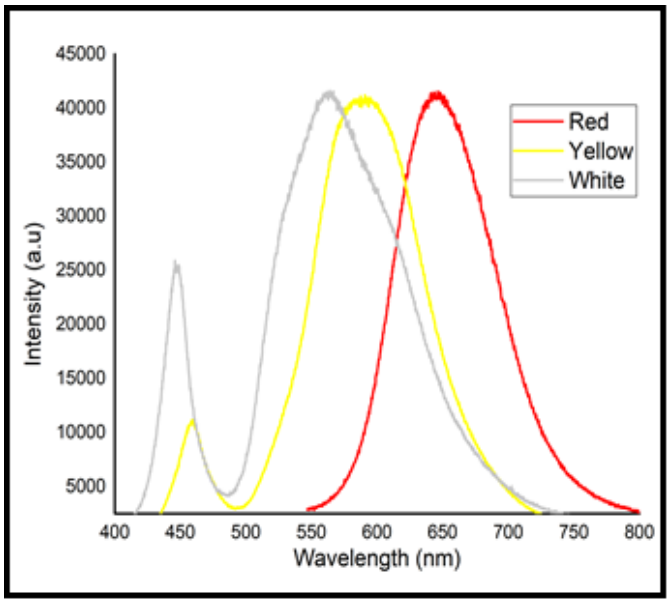

Figure 1: spectral irradiance of red, ywllow and white LED source.

C. Light exposure

Each group of rats is maintained in a 12-hours light-dark cycle in transparent poly carbonate cages with dimensions of (50 cm × 27 cm × 18 cm). Red, yellow and white LED light sources {Viva lamp (V BULB), model E27 (220 V, 9 W, Egypt)} are set on the top of each cage and are measured 25 cm away from the rats with total exposure duration of 3, 7, 14 and 21 days.

D. Electroretinography (ERG)

Animal preparation

The animals are dark adapted for 3 hours before the electrophysiological recording. They are anesthetized intramuscularly by xylazine (21 mg/kg of body weight) as muscle relaxant, and ketamine hydrochloride (45 mg/kg). After establishing the anesthesia, animals are placed on the pad of an operating table where their body temperature was maintained at 37 ºC. Each rat is positioned with its head resting to one side and local anesthetizing eye drops are also applied. The pupil of the recorded eye is dilated with topical 1% mydriacyl.

Electrophysiological recording

A white flash with fixed intensity 4 lux and duration 0.2s is applied. The electroretinogram is recorded by using sensor PS-2111 and its electrodes (PASCO, Roseville, CA) which connect to PASPORT interface direct to the computer. One electrode is placed at the corneal periphery as active electrode; the other electrode is placed on the skin of the lower eyelid as a reference one. The last electrode is placed on the ear as an earthed one. The electrodes are placed on the skin after removal the hair Fig. (3). The result of electrophysiological signals is collected and analyzed by data studio 1.9.8 software (PASCO, Roseville, CA).

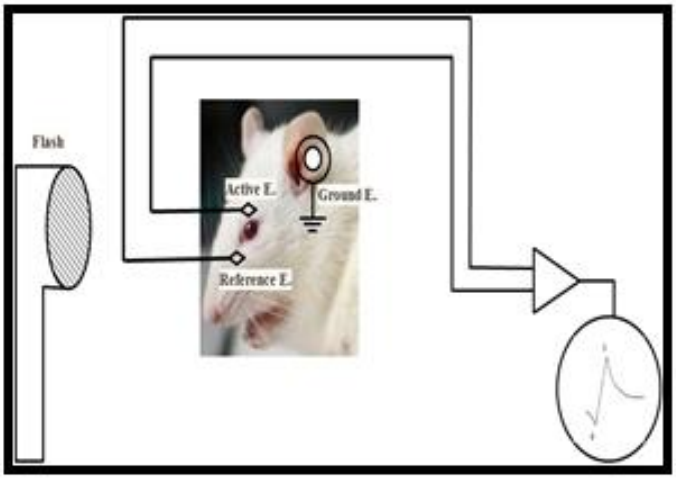

Figure 2: system for recording ERG of rats.

E. Statistical analysis

Data are presented as mean ± SD, data are analyzed by using student t-test and difference between the mean of different groups is considered significant at a level of $p < 0.05$. The statistical program applied is Statistical Package for the Social Sciences (SPSS).

III. RESULTS AND DISCUSSION

A. ERG analysis

The baseline of the ERG is the standing potential of the eye. The amplitude of a-wave is measured from the baseline to the a-wave trough while b-wave is measured from the peak of the a-wave to the peak of the b-wave. Typical records of ERG for control group and after exposure to red, yellow and white LED are shown in Fig. (3). The amplitude of a- wave has mean

values of 0.958 ±0.029 mV while the b-wave is 1.769 ± 0.053 mV for control group. The obtained data are summarized in tables (1 and 2).

The obtained results are summarized in table (1) and table (2). It lists the mean and standard deviation of the amplitude of both a-and b-waves for the control and exposed eyes to different wavelength of LED for different periods.

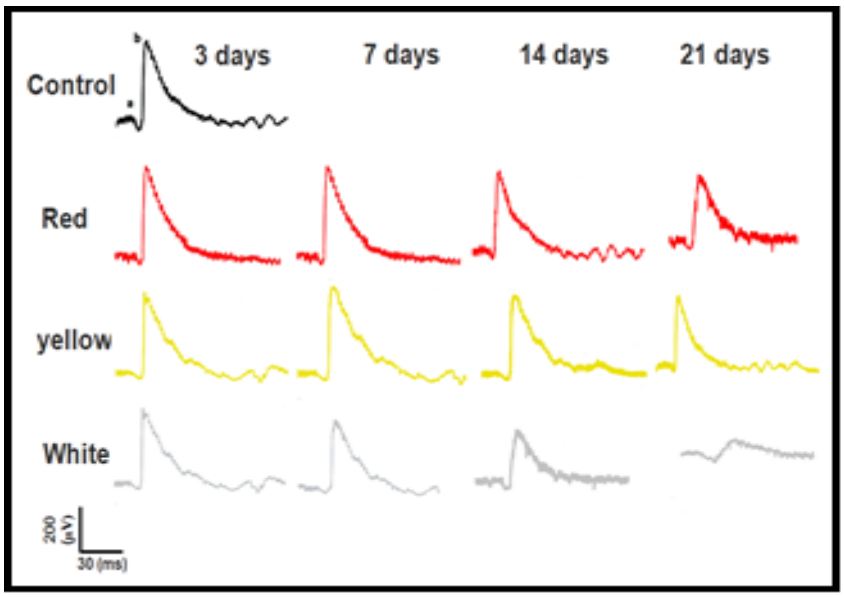

Figure. 3: ERG records of control rats and which exposed to red, yellow and white LED for 3,7,14,and 21 days.

It is noticed that there was not any effect to the yellow LED light on the ERG up to 14 days fig. (4 & 5), slight changes appeared on the 21st day fig. (4 & 5).

Table (1): Amplitude and % change of a-wave for control and rats exposed to red, yellow and white LEDs.

	Amplitude a wave (mv)							
	3 D		7 D		14 D		21 D	
	Mean±SD	% change	Mean±SD	% change	Mean±SD	% change	Mean±SD	% change
Control	0.958±0.029	-	0.958±0.029	-	0.958±0.029	-	0.958±0.029	-
Red	0.929±0.026	3.0%	0.901±0.017	5.9%	0.832±0.035	13.2%	0.733±0.21	23.5%
Yellow	0.956±0.014	0.21%	0.934±0.026	2.5%	0.929±0.012	3.0%	0.859±0.31	10.3%
White	0.922±0.022	3.8%	0.867±0.025	9.5%	0.711±0.034	25.8%	0.523±0.025	45.4%

After exposure to red and white LED, there is reduction in a- and b- wave amplitudes, Figs. (5 & 6). This reduction is increased with time of exposure in comparison with control group. The percentage difference clarified that a-wave is more affected than b-wave (tables 1 & 2).

The b/a ratio is serving as a quantitative index and an examination to the vitality of the retina. In the present study, the calculation of b/a ratio for control group is 1.85. For the exposed groups, this ratio shows enhancement throughout the time of experiment performance (Fig. 6).

Table (2): Amplitude and % change of b-wave for control and rats exposed to blue and green LEDs.

Amplitude b wave (mv)								
	3 D		7 D		14 D		21 D	
	Mean±SD	% change	Mean±SD	% change	Mean±SD	% change	Mean±SD	% change
Control	1.769±0.053	-	1.769±0.053	-	1.769±0.053	-	1.769±0.053	-
Red	1.756±0.025	0.7%	1.696±0.034	4.1%	1.598±0.035	10%	1.421±0.035	20%
Yellow	1.756±0.034	0.7%	1.742±0.026	1.5%	1.744±0.032	1.4%	1.619±0.024	8.5%
White	1.742±0.051	1.5%	1.684±0.033	5%	1.443±0.024	18%	1.233±0.052	30%

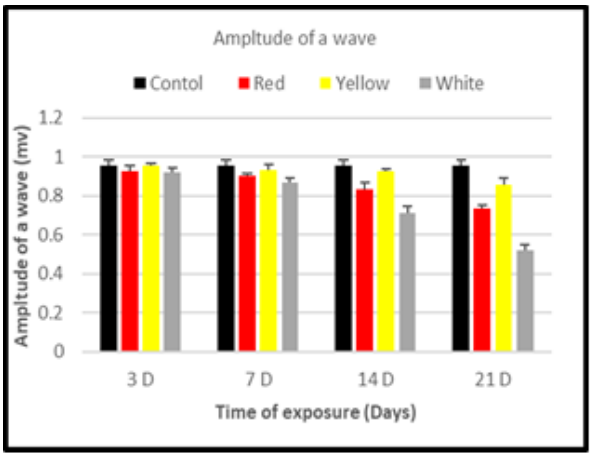

Figure 4: Amplitude of a-wave (mV) of the control and rats exposed to red, yellow and white LED for 3,7,14 and 21 days.

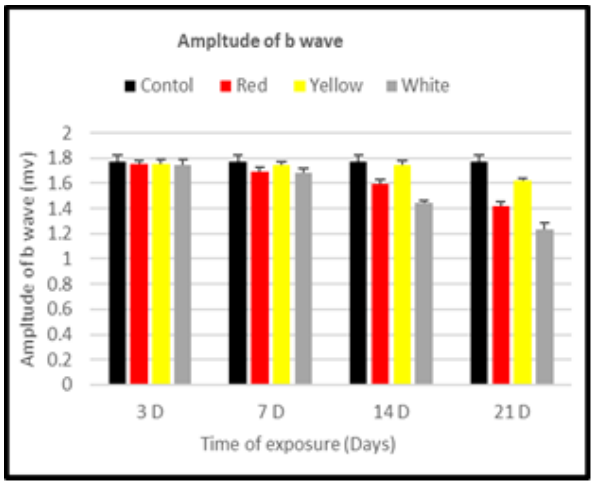

Figure 5: Amplitude of b-wave (mV) of the control and rats exposed to red, yellow and white LED for 3,7,14,and 21 days.

The b/a ratio is serving as a quantitative index and an examination to the vitality of the retina. In the present study, the calculation of b/a ratio for control group is 1.85. For the exposed groups, this ratio shows enhancement throughout the time of experiment performance (Fig. 6).

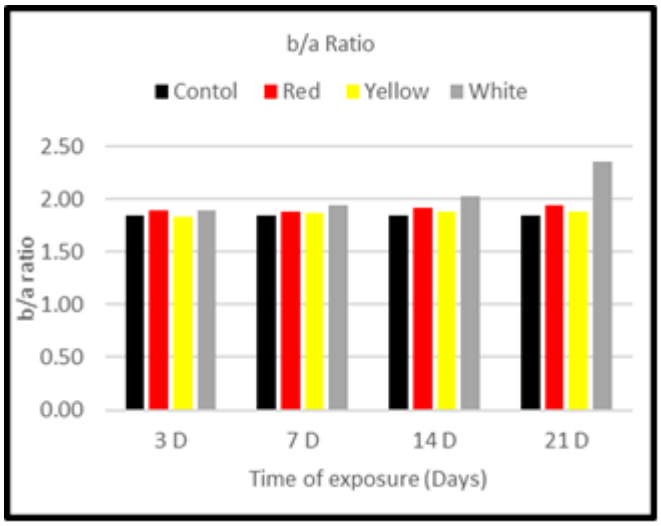

Figure 6: b/a Ratio of the control and rats exposed to red, yellow and white LED for 3,7,14 and 21 days.

Retinal light damage depends on the duration of exposure and the light level reaching the retina (retinal irradiance). The pathological process is also wavelength dependent [18]. The results of the present study indicate that exposure to LED light in this Wistar albino rat model can induce retinal damage as evidenced by the functional ERG study. In the present work, the ERG records are recorded for female Wistar albino rats before and after 3,7,14 and 21 days of exposure to red, yellow and white LEDs.
The results show that the obtained ERG waves for control animals consist of the two well-known waves; namely a- and b- waves. Their amplitudes are used to determine retinal function. However, an additional parameter for the ERG analysis is the b/a (R) value becomes most useful in evaluating the loss of retinal function [19].

The results also suggest that this retinal damage could be related to light–induced oxidative stress within the retinal tissues, as evidenced by the ROS generated in the retina after LED light exposure. The exposure to yellow LED light indicated insignificant effects on the ERG record along the time of experiment except under the yellow LED on the 21st day fig. (4). Exposure to white LED, significantly reduced a- and b- wave amplitudes. This reduction is accompanied by an increase in the (R) value (Fig. 6). The above results are in agreement with those obtained by Shang et al., (2014) [20] who indicated that White LED group demonstrated a significant decrease of b-wave amplitude in rats at days 9 and 28 of light exposure.

The ERG results also, show that red LED groups demonstrated a significant decrease in the a and b wave amplitude at 3, 7, 14 and 21 days after light exposure. In order to clarify the obtained results, firstly the photochemical damage process should be explained. For a photochemical damage to occur; it is necessary that the light must be transmitted to tissues with high oxygen content and then be absorbed by a chromophore [21]. By applying the previously mentioned idea on retina and light photochemical damage will occur. Photochemical damage is theorized to result due to generation of free radicals after exposure. While the retina possesses inherent mechanisms to protect itself against such insult, it is thought that damage may occur once these protective mechanisms have been overcome [12], [22], [23].
Once generated, free radicals can attack many types of molecules, thereby causing damage and rendering them inactive. Photoreceptor cells which possess a dense of cell membranes are particularly vulnerable to free radical's effects; the attack of free radicals on polyunsaturated fatty acids results in lipid peroxidation that breaks down membranous structure [21]. The a-wave reflects the photoreceptor activity. The b-wave is generated in the middle retinal layer in which the blood supply is provided mainly by the retinal circulation. The b-wave amplitude depends on the a-wave, on retinal circulation, and on the functional integrity of the interactions between the a- and b- wave generators [24].

Accordingly, the b-wave is believed to be a good indicator of the middle retina and retinal circulation [24]. So, this explanation may give an intuitively idea about the deformation obtained in the ERG. Therefore, the accumulation of free radicals after the longest exposure period to blue color may be the reason for the appearance of the negative ERG which reflects the non-vitality of the retina in the form of

blocking the signal transmission from photoreceptors to second and higher order retinal neurons [25].

As regard to the middle of the retina, the damage is in the form of swollen nuclei of the bipolar, horizontal, and amacrine cells and their cytoplasm is vacuolated. The damage increases through time of exposure. Under the effect of white and red LED light exposure, the results of ERG indicate that they are strongly dependent on both duration of exposure and wavelength of light.

IV. CONCLUSION

LEDs are expected to become the primary domestic light sources in the near future. Certain amounts of LED light exposure may induce retinal damage, and this animal model provides comparative measures of damage from different wavelength of commercial LED. Photochemical damage to retinal tissues occurs when the incidence light has a wavelength in the high energy portion of the visible spectrum. The present results suggest that oxidative stress is an early step leading to cellular damage by White LED exposure. The free radicals generated due to high energy light attack polyunsaturated fatty acids which results in lipid peroxidation that breaks down membranous structure, especially in photoreceptor region. Finally, the wavelength and time of exposure should be considered carefully when switching to LED lighting applications.

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