PROTECTIVE ROLE OF GRAPE SEED EXTRACT AGAINST THE EFFECT OF BLUE LIGHT ON THE RETINA

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Abstract. The aim of the present study is to investigate the effect of blue light (403 nm) on electroretinogram (ERG) and retinal rhodopsin. Also, the possible protective role of grape seed extract (GSE) was tested. Newzealand albino rabbits were used in this study. The rabbits were exposed to blue light with and without GSE supplementation for 48 hours, one week, two weeks and 3 weeks respectively. At the end of each period, the electroretinogram (ERG) was recorded. The rabbits were decapitated; the rhodopsin was extracted, malondialdehyde (MDA), total antioxidant capacity (TAC) and fourier transform infrared spectrum (FTIR) of bleached rhodopsin was measured. The data indicates changes in ERG, MDA, TAC and FTIR spectra after blue light exposure. Supplementation of GSE improves the ERG parameters, leads to decrease of MDA level and increase of the TAC with respect to non supplemented antioxidant groups. It is concluded that long exposure to blue light affects retina and GSE may have protective effects that help in protecting the retina from blue light damage.

Key words: retina, electroretinogram, rhodopsin, blue light, FTIR, MDA, TAC.

INTRODUCTION

The retina is the innermost layer of the eyeball and it is made up of cells with vastly different functions. The outer monolayer is known as the retinal pigment epithelium (RPE), and inside of this is the inner neurosensory retina, which consists of photoreceptor cells, bipolar cells, ganglion cells, horizontal cells, amacrine cells, and interplexiform cells. Two classes of photoreceptor cells, rods and cones, are present in the retina. The visual pigment in the rod is rhodopsin, which consists of opsin and the vitamin A aldehyde, 11-*cis*-retinal.

People exposure to blue light (380–500 nm) has increased dramatically [6]. *In vitro* studies showed that blue light irradiation of retinal explants made early

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ultra-structural changes, as well as damage that leads to cell death in photoreceptor cell [19]. Researches also indicated that high illumination levels of blue light can be toxic to retina and results in reactive oxygen species production in photoreceptor cells after 30 min of blue light exposure. Recent studies indicate that monochromatic blue light (403 nm) can induce what is known as the photoreversal of rhodopsin bleaching *in vivo* [7]. Logvinov *et al*. [14] reported that the continuous irradiation of rats with a light for 48 hours led to destruction of neurosensoric cells.

In recent years, a considerable emphasis has been focused on the importance of the naturally available botanicals that can be consumed in an individual's everyday diet. A wide variety of botanicals, mostly dietary flavonoids or polyphenolic substances, have been reported to possess substantial anticarcinogenic and antimutagenic activities because of their antioxidant and anti-inflammatory properties [17]. Proanthocyanidins are considered as one of them. The seeds of the grape are a particularly rich source of proanthocyanidins. The grape seed proanthocyanidins (GSPs) are mainly dimers, trimers and highly polymerized oligomers of monomeric catechins. GSPs have been shown to be potent antioxidants and free radical scavengers, being more effective than either ascorbic acid or vitamin E. Hanneken *et al*. [8] and Yamakoshi *et al*. [25] identified a selected group of flavonoids that protect retinal pigment epithelium cells from oxidative-stress–induced death with a high degree of potency and low toxicity.

So, the aim of present work is to investigate whether the dietary intake of grape seeds can help as an antioxidant and a neuroprotective to retinal rhodopsin from the hazards of exposure to blue light. This may help prevent or delay the progression of eye diseases.

MATERIALS AND METHODS

MATERIALS

In accordance to the ARVO resolution to the use of animals in vision research, thirty five New Zealand albino rabbits weighted 2–2.5 kg were used in this study. The animals were selected from the animal house of the Research Institute of Ophthalmology and fed on the laboratory balanced diet and in a central temperature of 20–25^{O} C. The rabbits were classified into three groups I, II and III according to the following:

Group I: contains six rabbits and is used as control group.

Group II: contains 16 rabbits and is subdivided into four subgroups (4 rabbits and 8 eyes each). All subgroups were exposed to blue light and decapitated after 48 hours, one week, two weeks and 3 weeks respectively.

Group III: Contains 16 rabbits and is subdivided into four subgroups (4 rabbits and 8 eyes each). All rabbits were supplemented with 10 mg/kg body weight grape seed extract (GSE) two weeks before exposure to blue light. GSE supplementation was continued till decapitation. Rabbits were decapitated after 48 hours, one week, two weeks and 3 weeks of exposure to blue light respectively.

IRRADIATION PROTOCOL

All animals were housed two to three animals per cage at temperature of 23 ± 1^0 C with a regular 12 hours dark-blue light cycle. The blue light was emitted from Sylvania lamp, model E27 (220 V, 60 W, United Kingdom). The lamp was calibrated at Photometry Department, National Institute of Standards, Giza, Egypt. The wavelength emitted from the anterior surface of the lamp is 403 nm.

ERG RECORDING

The rabbits were dark adapted for 2 hours before ERG recording and anesthetized by intramuscular injection using ketamine hydrochloride. The ERG was recorded by using three Ag-Ag CL skin electrodes. The active electrode was placed near the margin of the lower eyelid, the reference electrode was placed on the forehead and the earth electrode was inserted into the ear. Fifty flashes were used with a flash energy of 0.2 joule and a flash frequency of 1 Hz, i.e. one flash per second and background intensity of zero. The flashes were derived from a computerized system (EREV 99, Lace Eletronica, Italy).

EXTRACTION OF RHODOPSIN

At the end of each period, the albino rabbits were maintained for 12 hours in dark and were decapitated; the eyes were enucleated (removed from orbits) and then the cornea and lens were carefully removed and the posterior of the eye containing the retina was used for preparation of the rhodopsin sample. Extraction of rhodopsin was carried out [5]. All operations were carried out either in dim red light or in total darkness.

LIPID PEROXIDATION (MDA)

Quantitative determination of malondialdehyde (MDA) for retinal rhodopsin was carried out colorimetrically using kit purchased from Biodiagnostic Co., Egypt, according to the method described by Martinez *et al*. [15].

TOTAL ANTIOXIDANT CAPACITY (TAC)

Calorimetric method was performed for determination of the total antioxidant capacity of retinal rhodopsin using the kit purchased from Biodiagnostic Co., Egypt, according to the method described by Koracevic *et al*. [12].

FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

200 μL of rhodopsin were lyophilized and then mixed with KBr powder to prepare the KBr disks for FTIR analysis. FTIR spectra were measured using a Shimadzu infrared spectrometer with effective resolution of 2 cm^{-1}. Hundred sample interferograms were recorded for each spectrum. The spectrometer is operated under a continuous dry nitrogen gas purge to remove interference from atmospheric carbon dioxide and water. The date was baseline corrected and smoothed by Savitzky-Golay to eliminate the noise before Fourier transformation. The average of three spectra for each group was obtained using Origin Pro 7.5 software.

STATISTICS AND DATA ANALYSIS

Statistical comparison was performed between exposed and unexposed eyes using the Student's t-test. The results were presented as the mean ± SD and studies were repeated at least four times independently. Differences were considered not significant (NS) when $P > 0.05$; significant (S) when $P < 0.05$; highly significant (HS) when $P < 0.01$ and very highly significant (VHS) when $P < 0.001$.

RESULTS

The electroretinogram (ERG) has proven to be an objective and useful tool to assess retinal function. Typical records of control and treated rabbits ERG are shown in Fig. 1.

The amplitude and implicit time of a- wave have mean values of 80.73 μV and 26.4 ms while those of the b-wave are 200.72 μV and 52.4 ms, respectively. Table 1 lists the amplitude and the implicit time of the a- and b-waves for the control and blue light treated eyes, with and without GSE supplementation. It is clear that the ERG was strongly affected by exposure to blue light. The results showed variations in the ERG waveform, amplitude and implicit time for all components.

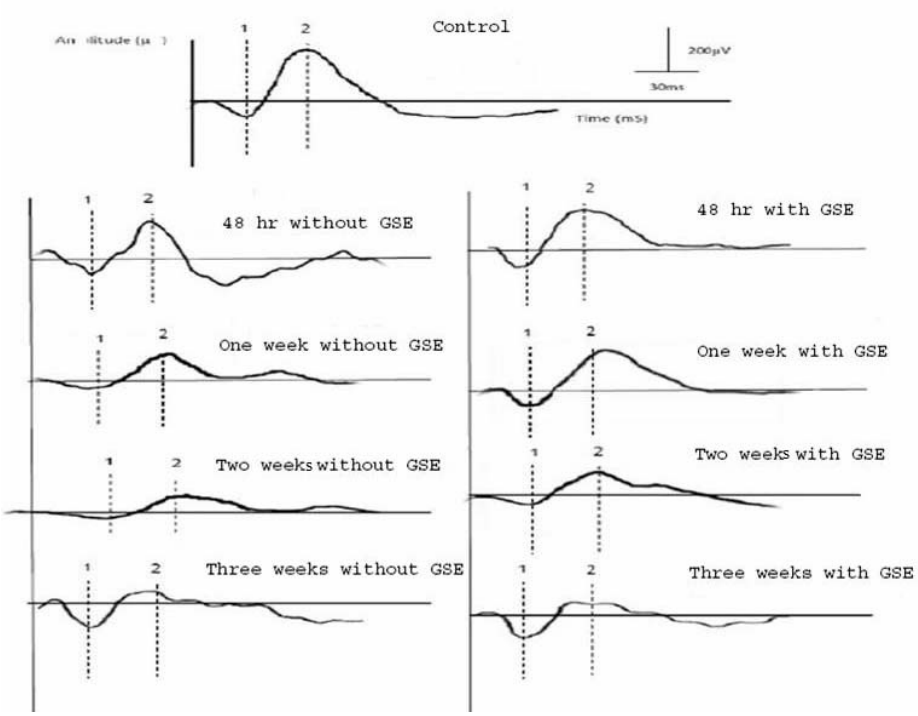

Fig. 1. ERG recorder of control, 48 hours, one, two and three weeks of exposure to blue light with (right) and without (left) GSE supplementation. (cursor 1 and 2 points to a-wave and b-wave respectively).

Table 1

The amplitude and implicit time of a- and b-waves for all the studied groups

Time after exposure	Amplitude of a-wave (µV)		Implicit time of a-wave (ms)		Amplitude of b-wave (µV)		Implicit time of b-wave (ms)	
	w/o	w	w/o	w	w/o	w	w/o	w
control	80.73± 0.26	80.73 ±0.26	26.4 ±1.3	26.4 ±1.3	200.72 ±0.62	200.72 ±0.62	52.4 ±2.1	52.4 ±2.1
48 h	70.37 ±0.3	90.39 ±0.23	29.59 ±0.9	27.36 ±08	180.51 ±0.5	190.01± 0.5	54.95 ±2.4	52.06 ± 1.9
One week	50.37 ±0.19	70.38 ±0.11	32.41 ±1.3	28.18 ±1,1	140.76 ±0.4	180.78± 0,4	59.18 ±1.5	56.36 ±2.2
Two weeks	20.01 ±0.1	40.70 ±0.02	35.67 ±1.2	29.59 ±0,9	80.72 ±0.5	110.40± 0.4	63.41± 1.9	59.18 ± 1.3
Three weeks	110.41 ±0.2	110.5 ±0.3	26.12 ±1.3	26.83 ±1.1	50.21 ±0.4	50.68 ±0.1	56.36± 2.2	56.13 ± 1.1

After exposure to blue light for 48 hours, one week and two weeks, the amplitudes of the a- and b-waves significantly decreased and the implicit time increased with respect to the control group. However, the most striking feature in

the ERG waveform variations appeared after exposure with blue light for 3 weeks where a negative ERG is recorded. The percentage difference for amplitude was –31% and implicit time was 1% for the a-wave.

The behavior of the b-wave was found to follow another pattern where there was a significant reduction in its amplitude and implicit time and the percentage differences for both values were 75% and –31% respectively. In case of the negative ERG it is useful to calculate the (b/a) ratio to examine the vitality of the retina and it was found to be 0.45 (Table 2).

Supplementation of GSE improved the ERG parameters in different grades. The highly significant improvement appeared in case of exposure to blue light for 48 hours. The protective role of GSE decreased with increased exposure period to blue light, but after exposure to 3 weeks no effect for GSE appeared. The percentage difference of amplitude is –32% and implicit time is 1.5% for a-wave and regarding to b-wave, 73% is for its amplitude and –24% is for its implicit time. Finally the b/a ratio is 0.49. It is noticed that the last group (three weeks) have the same results before and after supplementation of GSE.

Table 2

The amplitude and implicit time of a- and b-waves for all the studied groups

Time	Without antioxidant	With antioxidant
	b/a	b/a
Control	2.373	2.373
48 h	2.511	2.024
One week	2.748	2.544
Two weeks	4.338	2.425
Three weeks	0.456	0.493

MALONDIALDEHYDE (MDA)

The malondialdehyde (MDA), which is a by-product of lipid peroxidation, for rhodopsin extracted from control rabbit's retina was 10.64±0.443 μM/L. When the rabbits were exposed for 48 hours to blue light, there was a highly significant increase in the MDA value. There was a very highly significant increase in MDA after exposure to blue light for one, two and three weeks (Table 3).When the rabbits were supplemented with antioxidant and exposed to blue light, there was a non-significant increase in the malondialdehyde for 48 hours exposure period and very highly significant increase in MDA for the other periods (Table 3). However, the MDA increase in these cases with antioxidant is less than that without using the antioxidant.

Table 3

Effect of blue light with and without GSE on the MDA of rabbit's eye rhodopsin (µM/L)

With antioxidant	Without antioxidant			With antioxidant		
	Mean±SD	*P*-value	%change	Mean±SD	*P*-value	%change
Control	10.64±0.44			10.64 ±0.44		
48 h	13.25±0.980	HS	24.53%	11.48±0.876	NS	7.89%
One week	18.55±0.753	VSH	74.34%	17.34±0.338	VHS	62.96%
Two weeks	21.64 ±0.371	VHS	103.38%	20.19±0.611	VHS	89.75%
Three weeks	26.85±0.435	VHS	152.34%	22.06±0.788	VHS	107.33%

TOTAL ANTIOXIDANT CAPACITY (TAC)

Table 4 lists the effect of blue light with and without GSE on the TAC of rabbit's eye rhodopsin. The data indicated a non-significant decrease in the total antioxidant capacity after exposure to blue light for 48 hours. However, exposure to blue light for one, two and three weeks led to significant, highly significant and very highly significant decrease in total antioxidant capacity, respectively. When the rabbits were supplemented with antioxidant then exposed to blue light for 48 hours and one week, there was a non-significant decrease in the total antioxidant capacity. After two and three weeks of GSE supplementation and blue light exposure, there were highly significant decreases in total antioxidant capacity.

Table 4

Effect of blue light with and without GSE on the TAC of rabbit's eye rhodopsin (mM/L)

With antioxidant	Without antioxidant			With antioxidant		
	Mean±SD	*P*-value	% change	Mean±SD	*P*-value	% change
Control	2.74 ± 0.311			2.74± 0.311		
48 h	2.55± 0.38	NS	6.93%	2.56 ± 0.398	NS	6.56%
One week	1.99 ± 0.39	S	26.50%	2.29 ± 0.332	NS	16.42%
Two weeks	1.78 ± 0.206	HS	35.03%	1.97 ± 0.305	HS	28.10%
Three weeks	1.64 ± 0.24	VHS	40.14%	1.86 ± 0.296	HS	32.11%

(FTIR) OF BLEACHED RHODOPSIN

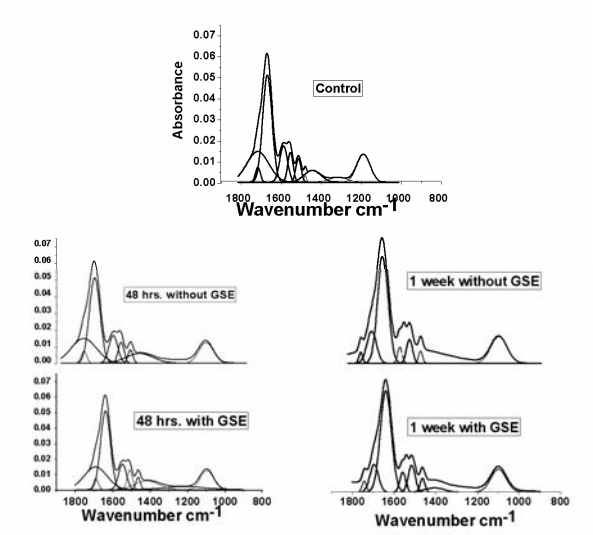

Fig. 2. FTIR of bleached rhodopsin extracted from rabbit's retina after 48 h and one week of blue light exposure, with and without GTE supplementation, in fingerprint region (1800–800 cm^{-1}).

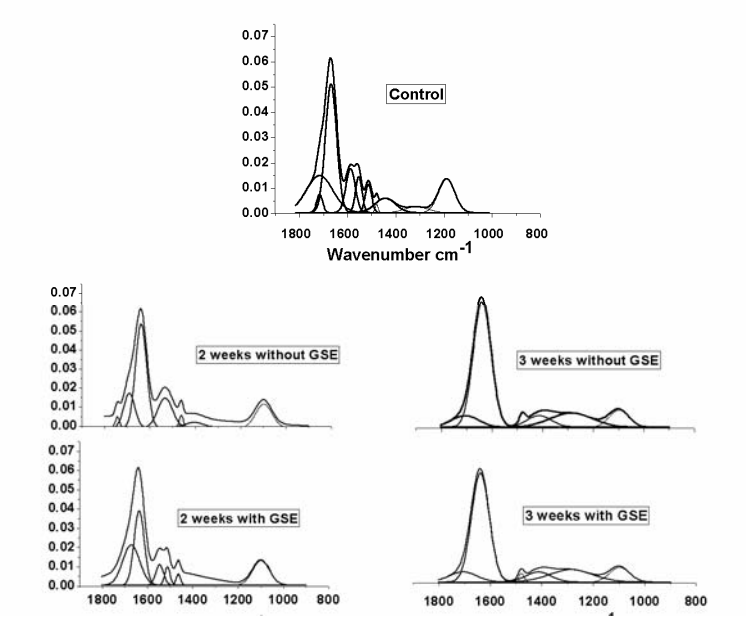

Fig. 3. FTIR of bleached rhodopsin extracted from rabbit's retina after two and three weeks of blue light exposure, with and without GTE supplementation, in fingerprint region (1800–800 cm^{-1}).

Fig. 2 illustrates the infrared spectra of final decay product of rhodopsin (bleached) extracted from normal rabbit's retina. Analysis of normal pattern revealed the presence of 10 bands in the finger print region at: 1692 cm^{-1}, 1691 cm^{-1}, 1638 cm^{-1}, 1547 cm^{-1}, 1508 cm^{-1}, 1463 cm^{-1}, 1452 cm^{-1}, 1385 cm^{-1}, 1238 cm^{-1}, and 1098 cm^{-1} (Table 5). The band at 1638 cm^{-1} assigned for amide I and the band at 1547 cm^{-1} originate from the amide II vibration of peptide group. The band centered around 1238 cm^{-1} arises from changes in retinal geometry and retinal-protein interaction reflected in C–C stretching vibrations. The band frequencies for groups exposed to blue light for 48 hours with and without GTE supplementation remain in the control range (Fig. 2). The band centered around 1452 cm^{-1} disappeared in both supplemented and non supplemented GSE groups.

Table 5

Wavenumber and bandwidth of fingerprint region for control and groups exposed to blue light with and without GSE supplementation

Peaks	Control	48 h with GSE	48 h without GSE	1 week with GSE	1 week without GSE	2 weeks with GSE	2 weeks without GSE	3 weeks with GSE	3 weeks without GSE
ster C=O				1742 19	1742 14	1742 20	1743 19	1742 17	
Amide I	1692 27	1692 27	1692 27						
Amide I	1691 123	1691 124	1691 124	1691 127	1691 123	1690 98	1673* 125	1690 120	1672* 98
Amide II	1638 52	1638 52	1638 52	1638 51	1639 49	1638 49	1637 48	1638 48	1635 42
Amide II	1547 43	1547 42	1547 42	1547 40	1547 36	1545 35	1542 34	1545 35	1542 31
Amide III	1508 29	1509 29	1509 29	1510 27	1510 24	1511 23		1511 23	1512 18
CH_2 Bending	1463 29	1463 24	1463 24	1463 23	1462 19	1462 19	1460 17	1462 19	
CH2 bending	1452 14								
CH_3 bending	1385 33	1419* 31	1419* 31	1420* 29					
C=C Stretching	1238 27	1238 26	1238 26						
C=C Stretching	1098 75	1098 75	1098 74	1098 74	1098 75	1098 75	1097 72	1098 75	1099 75

First line indicates the frequency of the band in cm^{-1}, while the second line indicates the bandwidth in cm^{-1}.

* Significant change.

When the rabbit's eye is exposed to blue light for one week, with and without GSE supplementation, a new peak appears at 1742 cm^{-1}. The bands at frequencies 1452 cm^{-1} and 1420 cm^{-1} disappeared in both supplemented and non-supplemented GSE group (Fig. 2 and Table 5). There is a shift in the band at frequency 1385 cm^{-1} in the rhodopsin of the group supplemented with GSE and this band has disappeared in non supplemented GSE groups.

After two weeks from blue light exposure, the new peak at 1742 cm^{-1} is still found (Fig. 3). The peaks at 1452 cm^{-1}, 1385 cm^{-1} and 1420 cm^{-1} disappeared in both supplemented and non-supplemented GSE groups. The peak at 1508 cm^{-1} disappeared in non-supplemented GSE group. The FTIR spectra of finger print region for rabbit's eye exposed to blue light for 3 weeks, with and without GTE supplementation indicate the presence of seven bands for GSE supplemented group and six bands for the non supplemented group (Figure 3 and Table 5).

DISCUSSION AND CONCLUSIONS

Rhodopsin is the only photoreceptor protein (a visual pigment) in the outer segment of rod visual cell responsible for twilight vision. It has 11-*cis* retinal as its chromophore, which is embedded inside a single peptide transmembrane protein called opsin [11]. As the light is transmitted through the retina, then absorbed by rhodopsin, it creates reactive oxygen species, which are deleterious to a variety of cellular organelles, and induces oxidative stress in photoreceptor cells. Once free radicals are generated, they can attack many molecule types, thereby causing damage and rendering them inactive. Photoreceptors cells in which there is a large concentration of cell membranes are particularly vulnerable to free radicals; the attack of free radicals on polyunsaturated fatty acids results in lipid peroxidation that breaks down the membranous structures.

The present results indicate a significant increase in MDA content (a lipid peroxide which is produced when the free radicals attack on the polyunsaturated fatty acids) after exposure to blue light and its content increases with increasing period of exposure. As Shan *et al*. [21] reported, MDA content is a sensitive indicator which can be used to measure the free radicals damage and the free radical metabolism. The inner segments of photoreceptor cells synthesize the components for the renewal of the outer segments, and their mitochondria provide the energy required for the phototransduction, so lipid peroxidation occurred in photoreceptor cells will affect the regeneration of rhodopsin. This is in line with the work of Boulton *et al*. [3] and Rozanowska *et al*. [20] who reported that the formation of reactive oxygen species increases with decreasing wavelength of light.

Grape Seeds are one of the richest sources of proanthocyanins; a class of biologically active flavonoids found throughout the plant kingdom. Grape seed extract (GSE) has received much attention due to its numerous biological activities and antioxidant effects [1]. This protective flavonoid has the ability to function

within cell membranes and is one of the powerful free radical scavengers. It also stimulates cells to produce detoxifying enzymes, most notably glutathione peroxidase. Proanthocyanins are useful to support vision health. It has been demonstrated that consumption of GSE decreases the incidence of cataracts in the eyes of hereditary cataractous rats [25]; they regenerate reduced glutathione from oxidized glutathione and are also used to treat macular degeneration and diabetic retinopathy [23].

The eye has a number of antioxidants to minimize free radical induced damage and to repair damage which does occur. So after the exposure to blue light, the results show a decrease in the total antioxidant capacity, which represents the sum of endogenous and food-derived antioxidants. This is supported by the work of Kovacic and Jacintho [13] and Ridnour *et al*. [18], who reported that the harmful effect of free radicals causing potential biological damage termed oxidative stress and this occurs in biological systems when there is an overproduction of reactive oxygen species on the one side and a deficiency of antioxidants on the other. So, in this study when the rabbits were supplemented with GSE for two weeks before exposure to blue light and continued GSE supplementation till decapitation, the results indicate some sort of increase in total antioxidant capacity with respect to the blue light group. This agrees with the *in vitro* studies by Kalt *et al*. [10], who demonstrated that anthocyanins and other flavonoids interact directly with rhodopsin and modulate visual pigment function. Also Matsumoto *et al*. [16] reported that anthocyanins stimulate the regeneration of rhodopsin. These findings support the obtained results of MDA, which illustrate a decrease in its level compared to the blue light group.

In the present study, the electrophysiological study shows a decrease of the amplitude of both a- and b- waves accompanied with increase in their implicit time. These changes may be attributed to damage of photoreceptors from which the a-wave originates, while the continuous decrease in b-wave amplitude and increase in its duration may be due to that the damaged photoreceptor cells would decrease the integral pulse fed to the bipolar cells and delay the normal activation processes necessary for completing the b-wave. Wenzel *et al*. [24] reported that exposure to blue light was found to severely damaged rod photoreceptors. Youssef *et al*. [26] reported that photochemical damage is associated with both long-duration exposure times as well as lower-wavelength (higher-energy) light exposure. So, in the present work, the accumulation of free radicals after the longest exposure period to blue light may be the cause in the appearance of the negative ERG which reflect the non-vitality of the retina in the form of blocking the signal transmission from photoreceptors to second and higher order retinal neurons.

Fourier transform infrared spectroscopy is used to study the conformational changes that occur in rhodopsin after blue light exposure. The region between 850 and 1680 cm^{-1} (fingerprint region) contains information about the retinal chromophore. Besides bands attributed to C=C stretching vibrations and located between 1500 and 1580 cm^{-1}, this region also contains chromophore bands (above

1600 cm^{-1}) attributed to vibrations of the aldimine (C=NH) group of the Schiff base. C–C and C–C–H stretching vibrations are localized in the fingerprint region (between 1100 and 1400 cm^{-1}). Frequencies of all types of deformational vibrations are located in the region below 1000 cm^{-1} [22].

In the present study, the amide I band centered around 1638 cm^{-1} and amide II band centered around 1547 cm^{-1}. In a study about signaling state of rhodopsin, Bartel *et al*. [2] detected protein bands**,** amide I and II bands at 1700–1620 cm^{-1} and 1570–1500 cm^{-1}. The band centered around 1238 cm^{-1} arises from changes in retinal geometry and retinal-protein interaction reflected in C–C stretching vibrations.

The present data illustrate that there was no significant change in the final decay product of rhodopsin after exposure to blue light for 48 hours. When blue light exposure increases to one, two or three weeks, a new peak appears at 1742 cm^{-1}. This peak assigned to change in the protonation state or hydrogen-bonding of aspartic and / or glutamic group [4] Also, there was a decrease in the band width of protein bands (amide I and II). Large spectral changes in the 1600–800 cm^{-1} originate from vibrational bands of the retinal chromophore such as C=C stretch (1600–1500 cm^{-1}), C–C stretch (1300–1100 cm^{-1}) and hydrogen out-of-plane vibrations (1000–800 cm^{-1}) [11]. Heck *et al*. [9] detected retinal bands in finger print region at 1238 cm^{-1} and retinal-related bands in the 1550–1570 cm^{-1} region (C–C stretching vibration), thus interfering with the amide II bands. In the present study, retinal bands at 1238 cm^{-1} disappear by increasing blue light exposure time to 1, 2 and 3 weeks. This finding is confirmed by MDA and TAC content and the negative ERG which recorded after exposure with blue light for 3 weeks. Supplementation of GSE improved FTIR spectra in different grades. The highly significant improvement appeared in case of exposure to blue light for 48 hours.

In conclusion, flavonoids provide protection against rhodopsin loss after blue light exposure. The improvement may be due to the newly regenerated photoreceptors, which are continuously replenishing themselves. So, the antioxidant treatment could improve the progression of some retinal disorders.

REFERENCES

1. BAGCHI, D., M. BAGCHI, S.J. STOHS, D.K. DAS, S.D. RAY, C.A. KUSZYNSKI, S.S. JOSHI, H.G. PRUESS, Free radicals and grape seed proanthocyanidin extract: importance in human health and disease prevention, *Toxicol*., 2000, **148**, 187–197.
2. BARTEL, F.J., E. RITTER, K.P., HOFMANN, Signaling states of rhodopsin, *Biochem.*, 2001, **276**, 30161–30166.
3. BOULTON, M., A. DONTSOV, J. JARVISEVANS, M. OSTROVSKY, D. SVISTUNENKO, Lipofuscin is a photoinducible free-radical generator, *J. Photochem. Photobiol.*, 1993, **19**, 201–204.
4. DeGRIP, W.J., J. OLIVE, P.H.M. BOVEE-GEURTS, Reversible modulation of rhodopsin photolysis in pure phosphatidylserine membranes, *Biochim. Biophys. Acta*, 1983, **134**, 168–179.

5. DELMELLE, M., W.K. NOELL, D.T. ORGANISCIAK, Hereditary retinal dystrophy in the rat: rhodopsin, retinol, vitamin A deficiency, *Exp. Eye Res*., 1975, **21**, 369–380.
6. ELAINE, K., The effects of blue light on ocular health, *J. Vis. Impair. Blind.*, 2000, **6**, 399–411.
7. GRIMM, C., C.E. REME, P.O. ROL, T. P. WILLIAMS, Blue light's effects on rhodopsin: photoreversal of bleaching in living rat eyes, *Invest. Ophthalmol. Vis. Sci*., 2000, **4**, 3984–3990.
8. HANNEKEN, A., F. LIN, J.P. JOHNSON, P. MAHER, Flavonoids protect human retinal pigment epithelial cells from oxidative-stress-induced death, *Invest. Ophthalmol. Vis. Sci.*, 2006, **7**(7), 3164–3177.
9. HECK, M., S.A. SCHADEL, D. MARETZKI, F.J. BARTL, E. RITTER, K. PALCZEWSKI, K.P. HOFMANN, Signaling states of rhodopsin, *J. Biol. Chem.*, 2003, **278**(5), 3162–3169.
10. KALT, W., A. HANNEKEN, P. MILBURY, F. TREMBLAY, Recent research on polyphenolics in vision and eye health, *J. Agric. Food Chem*., 2010, **58**, 4001–4007.
11. KANDORI, H., Y. SHICHIDA, T. YOSHIZAWA, Photoisomerization in rhodopsin, *Biochemistry* (Moscow), 2001, **66** (11), 1197–1209.
12. KORACEVIC, D., G. KORACEVIC, V. DJORDJEVIC, S. ANDREJEVIC, V. COSIC, Method for the measurement of antioxidant activity in human fluids, *J. Clinical Pathology*, 2001, **54**, 356–361.
13. KOVACIC, P., J.D. JACINTHO, Mechanisms of carcinogenesis: Focus on oxidative stress and electron transfer, *Curr. Med. Chem*., 2001, **8**, 773–796.
14. LOGVINOV, S., A. POTAPOV, D.A. DROBATULINA, A. MILLER, Effects of combined influence of ionizing radiation and light on the retina, *Radiats. Biol. Radioecol.*, 2004, **44**(6), 666–671.
15. MARTINEZ, R., K. QUINTANA, R. NAVARRO, C. MARTIN, M.L. HERNANDEZ, I. AURREKOETXEA, J.I. RUIZ-SANZ, M. LACORT, M.B. RUIZ-LARREA, Pro-oxidation and antioxidant potential of catechol estrogens against ferryl-myoglobin-induced oxidative stress, *Biochim. Biophys. Acta*, 2002, **1583**, 167–175.
16. MATSUMOTO, H., Y. NAKAMURA, S. TACHIBANAKI, S. KAWAMURA, M. HIRAYAMA, Stimulatory effect of cyanidin 3-glycosides on the regeneration of rhodopsin, *Agric. Food Chem.*, 2003, **51**, 3560–3563.
17. NANDAKUMAR, V., T. SINGH, S.K. KATIYAR, Multi-targeted prevention and therapy of cancer by proanthocyanidins, *Cancer Lett*, 2008, **269**(2), 378–387.
18. RIDNOUR, L.A., J.S. ISENBERG, M.G. ESPEY, D.D. THOMAS, D.A. ROBERTS, Wink nitric oxide regulates angiogenesis through a functional switch involving thrombospondin-1, *Proc. Natl. Acad. Sci.*, 2005, **102**, 13147–13152.
19. ROEHLECKE, C.U., M.A. SCHUMANN, L. KNELS, H. RICHARD, Influence of blue light on photoreceptors in a live retinal explants system, *J. Molecular Vision.,* 2011, **17**, 876–884.
20. ROZANOWSKA, M., J. WESSELS, M. BOULTON, Blue light induced singlet oxygen generation by retinal lipofuscin in nonpolar media, *Free Radic. Biol. Med*., 1998, **24**, 1107–1112.
21. SHAN, Y., X. YE, H. XIN, Effect of the grape seed proanthocyanidin extract on the free radical and energy metabolism indicators during the movement, *Scien. Res. Essay,* 2010, **5**, 148–153.
22. TERPUGOV, E.L., O.V. DEGTYAREVA, FTIR emission spectra of bacteriorhodopsin in a vibrational excited state, *Biochem.* (Moscow), 2001, **66**(11), 1315–1322.
23. THOMAS, G., Free radicals, antioxidants and eye diseases. Not as incurable as we once thought, *The standard*, 1999, **2**(1), 1–7.
24. WENZEL, A., C. GRIMM, M. SAMARDZIJA, C.E. REME, Molecular mechanisms of light-induced photoreceptor apoptosis and neuroprotection for retinal degeneration, *Progr. Ret. Eye Res.*, 2005, **24**, 275–306.
25. YAMAKOSHI, J., M. SAITO, S. KATAOKA, M. KIKUCHI, Safety evaluation of proanthocyanidins-rich extract from grape seeds, *Food Chem. Toxicol.*, 2002, **40**, 599–607.
26. YOUSSEF, P., N. SHEIBANI, D. ALBERT, Retinal light toxicity, *Eye*, 2011, **25**, 1–14.