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Light-Emitting Diode Laser Therapy for Hyperoxia-Induced Retinal Abnormalities

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Abstract

Introduction: Hyperoxygenation is linked to numerous effects in a variety of organ systems. It can cause tissue damage by generating reactive oxygen species (ROS), increasing oxidative stress, and inducing cell death by apoptosis. The present study aimed to evaluate the effects of low-level laser therapy on the retina in response to acute hyperoxia in animals.

Methods: A total of 70 Wistar albino rats were evaluated in the present study: 10 rats were designated as a control group, and the rest were exposed to hyperoxia (O_2, 90%) for 3 days, 1 week, and 2 weeks (20 rats each). Each group was divided into two subgroups (n=10), one of which was designated as hyperoxia only. The other was treated with a 670 nm light-emitting diode laser (2 sessions/one week, ~ 9.0 J/cm^2) in each eye. The animals were euthanized, and their retinas were dissected for analysis of protein content, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), total antioxidant capacity (TAC), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), and histological examination.

Results: We found that two weeks of hyperoxia induced an increase in retinal protein content ($P<0.001$), an alteration in the intensities and molecular weights of protein fractions, a significant decrease in the TAC level ($P<0.01$), and a noticeable increase in H_2O_2 and MDA levels ($P<0.001$). Histological examination revealed fragmentation of the photoreceptors and neovascularization in the outer and inner plexiform layers. Furthermore, the data showed remarkable improvement in the retinal protein contents, oxidative state, and retinal structure after light-emitting diode laser therapy.

Conclusion: Light-emitting diode laser therapy was found to be a useful treatment paradigm for reducing hyperoxia-induced retinal damage.

Keywords: Hyperoxia; Light-emitting diode laser therapy; Retinal protein; Oxidative stress; Histological examination.

Introduction

A suitable level of cellular oxygen is essential for normal vital processes in living systems. Decreasing the oxygen (O_2) level is life-threatening, especially in severe conditions, such as in the setting of coronavirus disease 2019 (COVID-19).[1] Supplemental oxygen is required in hypoxemic patients to improve cell metabolism and function while limiting organ dysfunction. Nevertheless, in patients suffering from hypoxemia, increasing the oxygen concentrations delivered throughout the body can, in turn, cause harmful hyperoxia outcomes.[2] Additionally, hypoxemia creates an endogenous damage-associated molecular structure that causes vasoconstriction and inflammatory responses.[3]

Bronchopulmonary dysplasia (BPD) is a neonatal disease that affects preterm infants and occurs as a result of arrested lung development.[4,5] Furthermore, Preterm infants with BPD and exposed to supplemental oxygen are at risk of retinopathy of prematurity (ROP).

ROP is the most well-known disease leading to blindness in childhood. ROP is strongly related to elevated oxygen levels, which can cause weakened retinal vessels, retinal detachment, and visual impairment.[6]

Moreover, a reduction in retinal blood flow in response to hyperoxia has been well described in an animal model.[7] Oxygen-induced retinopathy was occurred in mice (at day 7 postnatal) exposed to hyperoxia for five days. These mice develop vaso-obliteration followed by pre-retinal neovascularization.[8,9] In the rat model of OIR, exposed to fluctuation between 50% and 10% oxygen for 14 days, retinal neovascularization developed between avascular and vascular retina.[10] Rats exposed to hyperoxia (75% oxygen) from day 4 to day 14 postnatal, followed by 14 days in room air, decreased lung alveolarization and

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reduced retinal thickness.[11]

Systemic hyperoxic stress causes the caliber of retinal vessels to change by approximately 14% and 9% in healthy adults due to vasoconstriction of the veins and arteries respectively. [12]

Photobiomodulation (PBM), or irradiation with wavelengths from 600-1000 nm (far-red to near-infrared), has been shown to be beneficial in the treatment of several types of tissue damage,[13-15] including inflammation, tissue injuries, diabetic ulcers, radiation-induced ulcers, and neurodegenerative diseases.[16-18]

PBM has been used successfully in the treatment of several retinal problems, such as retinitis pigmentosa and age-related macular degeneration (AMD), as well as in animal models of diabetic retinopathy (DR).[19,20] It has been confirmed that PBM at a wavelength of 670 nm decreases the damaging effects of laser-induced injuries in the retinas of elderly patients, as well as methanol toxicity in rat retinas.[21,22] Additionally, 670 nm light can significantly protect photoreceptors in the retina against light damage.[13,23] The most beneficial outcome of using 670 nm light in the treatment of retinal diseases, including AMD and DR, is the marked reduction of oxidative stress and the inflammatory response.[24,25]

The present study aimed to investigate the potentially hazardous side effects of hyperoxia on the rat's retina and to explore the experimental retinopathy that developed in this model after exposure to 90% of O_2. We focused on retinal protein content and structure, oxidative stress, and retinal histological structure since the retinal vessels are highly vulnerable to oxygen up-take levels. Moreover, we evaluated whether the low-level light-emitting diode (LED) laser therapy would preserve the retina against this severe hyperoxia-induced disruption in the retina or whether these retinal changes reached the point of no recovery.

Materials and Methods

Experimental Animals

A total of 70 female Wistar rats (12 weeks old, weighing 200 ± 20 g) were kept in a constant 12-hour light/dark cycle with a balanced diet and free access to water.

Experimental Groups Designation

The rats all underwent slit-lamp biomicroscopy prior to exposure to hyperoxia to ensure that there were no signs of intraocular edema or inflammation. Of the 70 experimental rats, 10 were designated as the control group, which did not receive any treatment. The remaining 60 rats were divided into three separate groups and then each group was kept in a plexiglass chamber with dimensions of 40 cm × 60 cm × 50 cm (width *length * height). The rats were exposed to hyperoxia (90% O_2) for 20 hours/day, for 3 days, 1 week, and 2 weeks (n = 20 for each group). For each group, the rats were divided into two subgroups, one that did not receive treatment (n = 10) and one that was exposed to low-level LED laser therapy (n = 10).

Hyperoxia Exposure

The O_2 in the plexiglass chamber was regulated by a medical gas regulator (YR-88; China). A gas bubbler was utilized to humidify the O_2 entering the chamber (Figure 1). An Ultrasonic Oxygen Sensor Module (Gasboard 7500E, Wuhan Cubic Optoelectronics Co., Ltd.) was simply fixed inside the plexiglass chamber and checked three times daily to indicate the oxygen concentration (0 ~ 100% ± 1.8%). The sensor had dimensions of 12 cm × 2.2 cm × 1.4 cm (L*W*H) and operated at 12.0 ± 0.5 VDC, < 50 mA and Pmax 0.6 W. The chamber was opened daily to feed and water the rats, and the temperature inside the chamber was maintained at 25 ± 1°C, with humidity not exceeding 23%.

Light-Emitting Diode Laser Treatment

The hyperoxia group was treated with 670 nm light from LED-pumped solid-state laser (DPSSL-DRIVER II, Cobolt; China) attached to a laser probe (0.4 mm). The distance between the probe and the eye was 7-9 cm. A laser beam was delivered directly to the rats' eyes for 90 seconds using a convex lens (20X) to focus the beam on the retina. The power of the laser was 50 mW/cm^2 (only 2 sessions/1 week, 3 days apart), and the total radiant energy exposure was approximately 9000 mJ/cm^2 (~9.0 J/cm^2) for each eye. The rats were euthanized under anesthesia after 3 days, 1 week, or 2 weeks, and the eyes were removed for analysis.

Extraction of Retinal Protein

The retinas were resected from the posterior chamber of the eye and homogenized in protein lysis buffer (RIPA) with protease inhibitor (Sigma-Aldrich, Inc.; St. Louis, MO, USA). The samples were sonicated for 20

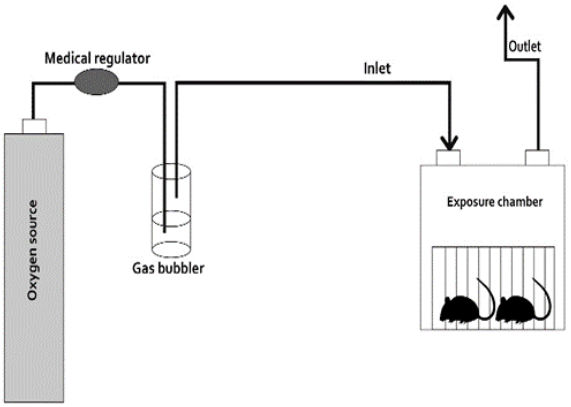

Figure 1. Schematic Presentation for the Hyperoxia Exposure Chamber.

seconds and then centrifuged for 20 minutes at 12 000 rpm. The supernatant was then withdrawn for different measurements.

The total retinal protein content was estimated colorimetrically at 750 nm (Spectrophotometer Evo 600, Thermo Fisher Scientific; Madison, WI, USA).[26] Retinal protein electrophoresis was performed via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), using 3% stacking gel and 12% separating gel. The molecular weights obtained for the retinal proteins were characterized using an automatic scanner (R-112, Beckman Coulter; Brea, CA, USA).[27]

The total antioxidant capacity (TAC) of the retinas was determined using a colorimetric method at 510 nm (Biodiagnostic Co., Egypt), through an enzymatic reaction of the antioxidants in the retina with a pre-defined amount of exogenously provided hydrogen peroxide (H_2O_2).[28] The quantitative determination of H_2O_2 was carried out colorimetrically at 510 nm (Biodiagnostic Co.; Egypt). The H_2O_2 product for the retinal protein was correlated to the total concentrations of oxidative molecules in the sample.[29] Malondialdehyde (MDA) levels were detected at 534 nm (Biodiagnostic Co., Egypt). It depends on thiobarbituric acid interaction with the MDA in the retina to yield thiobarbituric acid reactive substances (TBARS).[30]

Histological Examination

The eyes were removed from the rats, injected with, and soaked in 4% glutaraldehyde in 0.1 M phosphate buffer saline (PBS; pH 7.4). The posterior portion of the eye was dissected into 1 mm^3 sections and then fixed for an additional 8 hours with freshly prepared glutaraldehyde buffered solution (pH 7.4). The sections were washed for 1 hour with PBS at 4°C, immersed in 1.33% osmium tetroxide, and dehydrated in cold ethanol (50%, 70%, 80%, 90%, and 96%). The samples were embedded in freshly prepared Araldite CY212 mixtures. Semi-thin (1 μm) sections were cut using an ultratome (LKB Produkter; Sweden) and were then stained with toluidine blue, after being placed on glass slides for microscopic examination.

Statistical Evaluation

The data were obtained as mean ± SD, and the variations between the data were analyzed using a one-way analysis of variance (ANOVA) test. The Student's *t* test was used to compare the control with different groups. Statistical significance was set at $P < 0.05$.

Results

Total Protein Content

The protein content of the control retina was 15.4 ± 0.7 mg/g tissue (Figure 2). In the hyperoxia groups, this increased to 16.2 ± 0.7 mg/g tissue ($P < 0.05$), 18.6 ± 0.7 mg/g tissue, and 22 ± 0.6 mg/g tissue ($P < 0.001$) after 3 days, 1 week, and 2 weeks respectively, with corresponding changes of 5.2%, 21%, and 43% compared to the control. In the LED laser therapy groups, the protein content showed progressive improvement, with values of 15.3 ± 0.8 mg/g tissue, 16.2 ± 0.4 mg/g tissue, and 15.6 ± 0.8 mg/g tissue after 3 days, 1 week, and 2 weeks respectively, with corresponding changes of about 0.6%, 5.2%, and 1.3%.

SDS-PAGE for Retinal Protein

The control pattern of the retinal protein was separated into 11 fractions with different intensities (optical densities) for evaluation, at molecular weights of 285, 235, 209, 175, 149, 120, 110, 83, 67, 46, and 36 kDa (Figure 3). After 3 days of hyperoxia, the separation pattern revealed the disappearance of the 285 kDa peak and a significant shift in the molecular weight from 235 to 247 kDa with an associated increase in the intensity. The fraction at 175 kDa shifted to 184 kDa, with an associated increase in intensity, and the fraction at 83 kDa shifted slightly to a lower molecular region, with an associated decrease in intensity. The treatment of the retina with LED laser showed the reappearance of the 285 kDa peak, with a small increase in the molecular weight and improvement of most fractions.

After 1 week of hyperoxia, the scanning pattern showed a significant increase in the peak intensities at 285, 235, 209, 175, 149, and 67 kDa, in addition to an apparent decrease in the intensity at 83 kDa (Figure 4). The scanning pattern after 1 week of hyperoxia and treatment with LED laser was characterized by an improvement in the peak intensities in the low, middle, and high molecular weight regions. By extending the hyperoxia to 2 weeks, the scanning pattern showed a noticeable elevation in the intensities of most retinal protein fractions compared with the control group (Figure 5), in addition to the disappearance of the fraction at 235 kDa. In contrast, the scanning pattern of SDS-PAGE after LED laser treatment

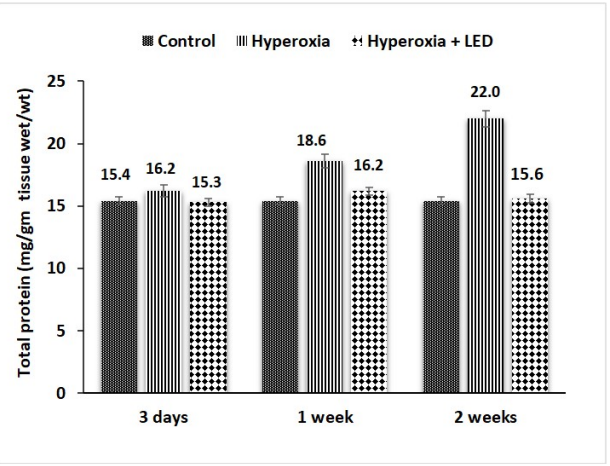

Figure 2. The Total Protein Content of the Retina After Different Periods of Hyperoxia and After LED Laser Treatment Compared With the Control Group.

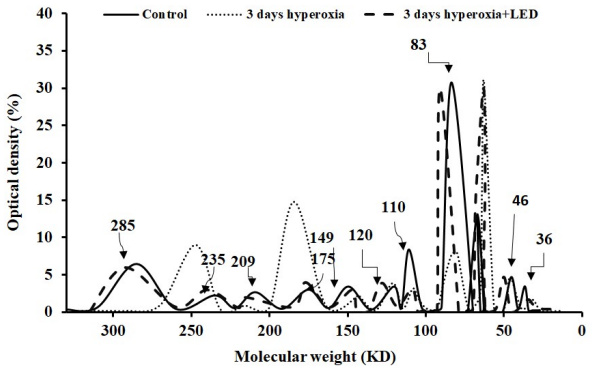

Figure 3. SDS-PAGE Scanning Patterns of Retinal Protein Exposed to Hyperoxia for Three Days and After Treatment With LED Laser Compared With the Control Group.

showed improvement in most of the protein fractions, with a slight shift towards the lower molecular weight region.

Oxidative Stress Determination

The TAC, H_2O_2, and MDA concentrations in the rat retinas exposed to hyperoxia and treated with LED laser are shown in Table 1. The TAC levels significantly increased compared to the control after 3 days and 1 week of exposure to hyperoxia, with a percentage change of 16.8% ($P<0.05$) and 31.8% ($P<0.001$) respectively, followed by a significant reduction after 2 weeks, with a percentage change of 26.2% ($P<0.01$). Moreover, the groups treated with low-level LED laser therapy showed gradual improvement in the TAC level compared to the hyperoxia group, with percentage changes of 10.4% ($P<0.05$), 22.7% ($P<0.01$), and 11.5% ($P<0.05$) after 3 days, 1 week, and 2 weeks respectively (Figure 6).

The level of H_2O_2 significantly increased after 3 days of hyperoxia, with a percentage change of 11.0% ($P<0.05$). By continuing the hyperoxia for one week and two weeks, a highly significant increase in H_2O_2 levels was detected (Figure 7), with percentage changes of 39.0% and 61.0% ($P<0.001$) compared with the control group. Moreover, treatment with LED laser revealed significant improvement in H_2O_2 levels compared with the hyperoxia group. The percentage changes after 3 days, 1 week and 2 weeks were 3.9 % ($P>0.05$), 9.8 % ($P<0.05$) and 15.7 % ($P<0.01$), as compared with the control group.

The MDA level in the control rat retina, a by-product of lipid peroxidation, was 61.3 ± 0.5 nM/g tissue. After 3 days of hyperoxia, there was a noticeable elevation in the MDA level (Figure 8), with a percentage change of approximately 10.1% ($P<0.05$). Moreover, there were significant increases in MDA after 1 and 2 weeks of hyperoxia, with percentage changes of 28.5% ($P<0.01$) and 57.0% ($P<0.001$) respectively. Treatment with 670 nm LED laser therapy after 3 days of hyperoxia revealed a non-significant increase ($P>0.05$) in the MDA level. After 1 and 2 weeks, the hyperoxia-treated groups showed significant improvement based on the period of hyperoxia exposure, with percentage changes of 11.2% ($P<0.05$) and 28.0% ($P<0.01$) respectively.

Histological Examination

Light micrograph of the control rat retina (Figure 9a) showed various retinal layers: retinal pigment epithelium (RPE), photoreceptor layer (PRL), outer and inner segments (OS and IS), outer limiting membrane (OLM), outer nuclear layer (ONL), outer plexiform layer (OPL), inner nuclear layer (INL), inner plexiform layer (IPL), ganglion cell layer (GCL), nerve fiber layer (NFL), and inner limiting membrane (ILM).

In the three-day hyperoxia group (Figure 9b), slight fragmentation of the photoreceptor of the OS (single arrow) and edema of the IPL and GCL with the thickened endothelial lining layer (double arrows) were seen. After one week (Figure 9c), there were fragmented

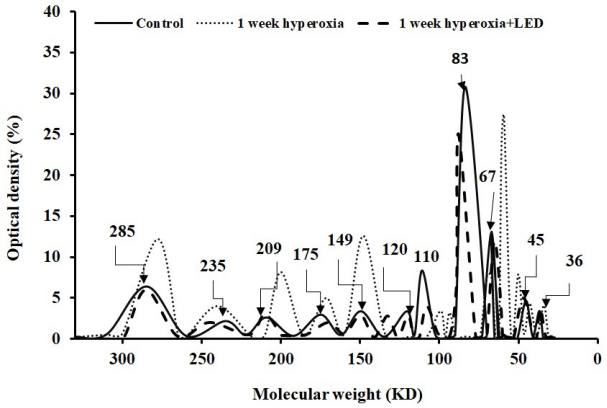

Figure 4. SDS-PAGE Scanning Patterns of Retinal Protein Exposed to Hyperoxia for One Week and After Treatment With LED Laser Compared With the Control Group.

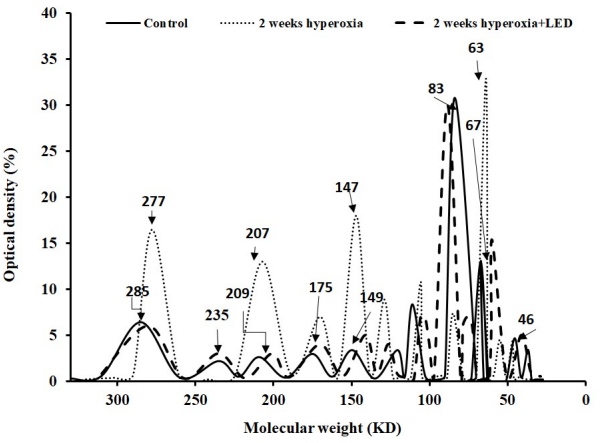

Figure 5. SDS-PAGE Scanning Patterns of Retinal Protein Exposed to Hyperoxia for Two Weeks and After Treatment With LED Laser Compared With the Control Group.

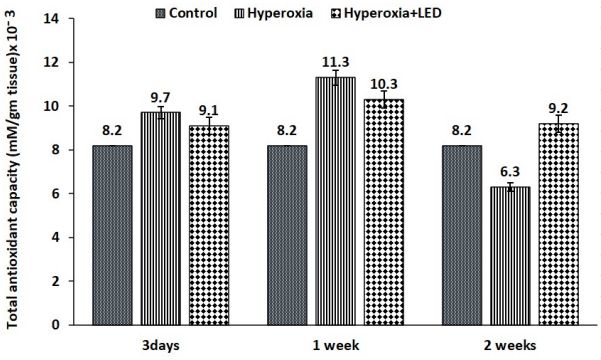

Figure 6. Total Antioxidant Capacity (TAC) of Rat's Retina Exposed to Hyperoxia and Treated With LED Laser Compared With the Control Group.

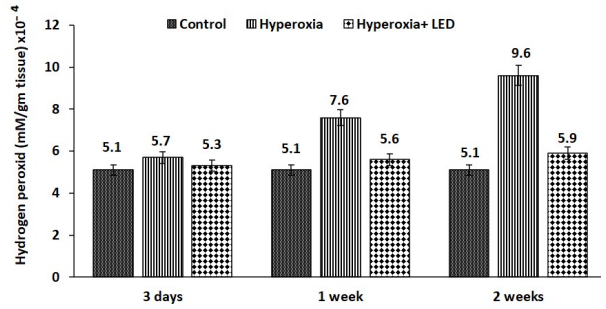

Figure 7. Hydrogen Peroxide (H2O2) Content of Rat's Retina Exposed to Hyperoxia and Treated With LED Laser Compared With the Control Group.

photoreceptors in the OS, intense staining of the ONL and INL, and edema of the IPL. Moreover, two weeks of hyperoxia (Figure 9d) led to the accumulation of numerous dense granules in the pigment epithelial cells (arrow) and complete photoreceptor fragmentation in the OS, and many cells were deeply stained in the ONL and INL. Additionally, apparent neovascularization (double arrows) was detected in both the OPL and the IPL. Furthermore, after 670 nm LED laser therapy, all retinal layers showed well-preserved cytoarchitecture (Figures 9e, 9f, and 9g).

Discussion

In the present study, we evaluated the effectiveness of 670 nm LED laser therapy in treating hyperoxia-induced alterations in retinal proteins and structures. A very high rate of O_2 is consumed by the retina, and free radicals are generally generated in the mitochondria under hypoxic conditions. Under hyperoxic conditions, the number of free radicals' increases, which can disrupt cellular constituents such as proteins, lipids, and nucleic acids, triggering cytotoxic effects.[31]

The results of the present study indicate that hyperoxia causes a significant increase in the protein content of the retina. This increase became more pronounced as the length of time exposed to hyperoxia increased. The increase in protein content was observed throughout the structural changes in the retinal proteins, as seen on SDS-PAGE. Several protein fractions in the low, middle and high molecular weight regions showed an increase in their intensities. The increase in protein content after exposure to hyperoxia (Figure 2) corresponds with increased fractions intensity. Moreover, the alterations in molecular weights may be due to structural degeneration and aggregation of the retinal proteins (Figures 3, 4, and

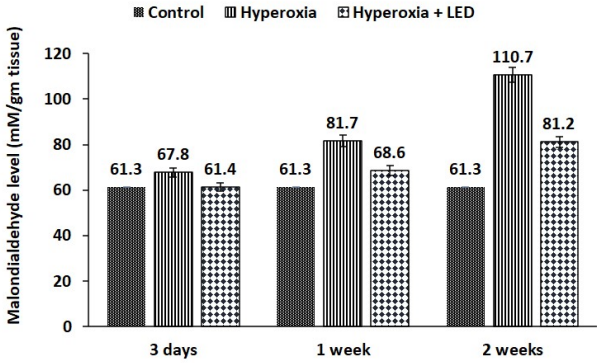

Figure 8. Malondialdehyde (MDA) of Rat's Retina Exposed to Hyperoxia and Treated With LED Laser Compared With the Control Group.

Table 1. TAC, H_2O_2, and MDA of Rat's Retina Exposed to Hyperoxia and Treated With LED Laser Compared With the Control Group

Groups	Periods	TAC (mM/g tissue ×10^{-3})		H_2O_2 (mM/g tissue ×10^{-4})		MDA (nM/g Tissue)	
		Mean± SD	% Change	Mean± SD	% Changes	Mean± SD	% Changes
Control	-	8.2±0.5	-	5.1±0.2	-	61.3±0.5	-
Hyperoxia	3 Days	9.7±0.2	16.8%	5.7±0.3	11.0%	67.8±0.5	10.1%
	1 Week	11.3±0.3	31.8%	7.6±0.1	39.0%	81.7±0.3	28.5%
	2 Weeks	6.3±0.4	26.2%	9.6±0.5	61.0%	110.7±0.4	57.0%
Hyperoxia+ LED laser	3 Days	9.1±0.6	10.4%	5.3±0.6	3.9%	61.4±0.6	0.2%
	1 Week	10.3±0.5	22.7%	5.6±0.4	9.8%	68.6±0.3	11.2%
	2 Weeks	9.2±0.2	11.5%	5.9±0.2	15.7%	81.2 ±0.3	28.0%

Abbreviations: TAC, total antioxidant concentration; H_2O_2, hydrogen peroxide; MDA, malondialdehyde.

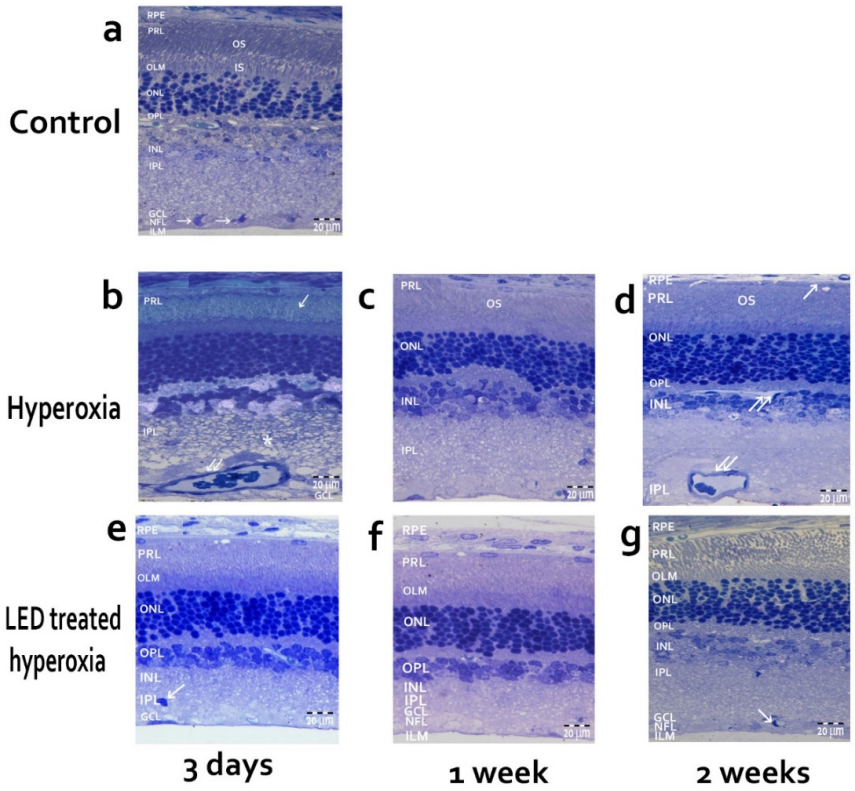

Figure 9. Light Micrograph of the Rat' Retina: The control (**a**); hyperoxia for 3 days (**b**) with slight fragmentation of the photoreceptor layer (single arrow), edema of the inner plexiform layer (IPL) * and thickened of endothelial lining the ganglion cell layer (double arrows); Hyperoxia for one week (**c**) fragmented photoreceptor with deep staining of outer and inner nuclear layers (ONL and INL), and edema of the IPL; Hyperoxia for two weeks (**d**) accumulated granules in the pigment epithelial cells (arrow), complete fragmentation of photoreceptor outer segment (OS), several intensified stained cells of ONL and INL and neovascularization (double arrows) in both the outer plexiform layer (OPL) and IPL; hyperoxia for one day and treated with LED laser (**e**) showing more or less normal structure of retina with intact glial cell (arrow); hyperoxia for one week and treated with LED laser (**f**) unchanged structure; hyperoxia for two weeks and treated with LED laser (**g**) well preserved cytoarchitecture of the retina with intact glial cell (arrow).

5).

Our results provide some reasonable expectations regarding the mechanisms that induce retinal protein changes after hyperoxia. One such mechanism is hyperoxia-induced proliferative retinopathy (HIPR).[6] HIPR causes disruption of retinal vessels, changes in vascular structure, angiogenesis, disorganization of the intra-retinal sheet, retinal detachment, inflammation, persistent hyaloid vasculature, and thinning in the central retina.[6] In addition, hyperoxia-induced factor-1α protein levels, vascular endothelium growth factor, and the protein levels of the nuclear factor (erythroid-derived 2) increased. Intra-retinal fibrinogen, inflammatory lymphocytes and macrophages were also observed.[32] Furthermore, stimulation of retinal angiogenesis by reactive oxygen species (ROS) in the retina, via molecular pathomechanisms and the activation of pro-inflammatory pathways, may lead to the production of tumor necrosis factor and a subsequent generation of inflammatory and angiogenic mediators.[33] Moreover, in the experimental model of O_2-induced retinopathy (OIR), retinal neovascularization was originated between the vascular and avascular retinal boundaries.[34] These new vessels contain different blood components responsible for elevating the protein content and changes in protein composition.

Living organisms have complex antioxidant systems that counteract ROS and limit their harmful effects.[35-37] These antioxidants consist of enzymes, macromolecules, and a collection of small molecules. The complete antioxidant system consists of both endogenous antioxidants and food-derived antioxidants. Retinal lipids consist of different polyunsaturated fatty acids, such as choline phosphoglycerides and arachidonic acids, which are vulnerable to peroxidation.[38]

In the present study, we used the TAC assay to determine the effects of hyperoxia and LED laser therapy. Under hyperoxia, a large influx of ROS is produced due to the oxidation of cell components and lipid peroxidation of cell membranes.[39] Two of the most prominent types of ROS are H_2O_2 and MDA (a lipid peroxide sensitive indicator produced when the free radicals attack the polyunsaturated fatty acids). They are considered important signs of oxidative stress.[38,39]

This is in accordance with the results of the present study, which show a significant increase in H_2O_2 and MDA content after exposure to hyperoxia, the values of which increase with increasing periods of exposure. A disturbance between the antioxidant and oxidant levels occurs because of the generation of ROS after hyperoxia, and the established free radicals may damage cellular molecules.[40,41] Contrarily, LED laser therapy stimulates the antioxidant defense system by elevating TAC and the deterioration of H_2O_2 and MDA, as shown in Table 1.

Free radicals interact with polyunsaturated fatty acids in cell membranes, resulting in lipid peroxidation that alters and breaks down membranous structures.[42] These free radicals assault several cellular organelles, injuring and distorting lipids, proteins, and deoxyribonucleic acid (DNA). Retinal cell membranes with large quantities of photoreceptors are most affected by free radicals[23] which cause damage and degeneration, rendering them inactive.[43]

The results of the present study showed fragmentation of the photoreceptors of the OS and edema of the IPL and GCL with a thickened endothelial lining layer after exposure to hyperoxia for three days (Figure 9b). Moreover, after one week of hyperoxia, there were fragmented photoreceptors in the outer segment (OS), intense staining of the INL and ONL, and edema of the IPL (Figure 9c). Moreover, accumulation of dense granules in the pigment epithelial cells, fragmentation of photoreceptors in the OS, deeply stained cells in the ONL and INL, and neovascularization in the OPL and IPL were observed after two weeks of hyperoxia (Figure 9d). All these changes in the retinal structure after exposure to hyperoxia are in line with previous reports regarding the exposure of rats to hyperoxia, which showed an overgrowth of retinal vessels and several changes in the horizontal cell count, the thickness of OPL, and endothelial lining.[44] Another previous study indicated the prevalence of neovascularization in the OPL, edema of the IPL, and deep staining of the ONL and INL. This growing neovascularization may cause retinal traction, vitreous hemorrhage, and retinal detachment.[6]

In contrast, low-level LED laser therapy after three days, one week, and two weeks of hyperoxia revealed no significant changes in retinal histology compared with the control. The results of the present study suggest that the significant decrease in intracellular production of free radicals that affect polyunsaturated fatty acid content in photoreceptors may be responsible for the disappearance of edema and improvement of retinal layers (Figures 9e, 9f and 9g). Our results are in agreement with previous studies of the mouse retina, which proved the safety of 670 nm light for the treatment of hyperoxia-induced damage, which can reduce stress markers and inflammatory markers in diseased retinas.[14,45] These effects can be referred to as low-level laser interactions with the living system, which regulate the expression of several genes responsible for cell growth and vital cellular functions, such as protein synthesis enzymes, DNA repair proteins, antioxidant enzymes, and protein maintenance.[46]

The precise mechanism of LED laser therapy has not yet been defined. It has been suggested that light photons in the range of 670 nm are absorbed by the primary photo acceptor of 670 nm light, termed cytochrome c oxidase (COX), which is the rate-limiting enzyme in the terminal phosphorylation of the mitochondrial respiratory chain.[46,47] It has been proposed that 670 nm light could increase the activity of COX in retinas and primary neurons. The low-level laser used in the present study facilitates the increase in redox status in mitochondria and elevates ATP synthesis and mitochondrial membrane potential, leading to cytoprotective, antioxidant and antiapoptotic effects, while accelerating cellular repair and healing.[48-52]

Conclusion

Prolonged hyperoxia in experimental rats causes many changes in the retina, characterized by retinal protein changes, the elevation of oxidative stress, and alterations in retinal histological structures. Our findings proposed that applying low-level LED laser therapy using 670 nm light is an effective and easy procedure to reduce retinal inflammation and oxidative stress and to display a more substantial enhancement in the retina from hyperoxia-induced retinal abnormalities. Further, short-term exposure to a 670 nm LED laser may have a therapeutic role in several retinal diseases such as retinal neovascularization, photoreceptor degeneration associated with retinopathies such as DR and proliferative retinopathy.

Ethical Considerations

All experiments were performed in accordance with the directions articulated in the Guide for Care and Use of Laboratory Animals and were permitted by the local experimental ethics committee at the Research Institute of Ophthalmology, Giza, Egypt.

Conflict of Interests

The authors declare that they have no conflict of interest.

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