The combined effect of photobiomodulation therapy and chia seeds supplementation for the treatment of diabetic retinopathy in experimental rats

Salwa Abdelkawi[a], Amal E. Ibrahim[a], Dina F. Ghoneim[b], Aziza A. Hassan[b], Maha S.A. Eldaiem[c]

[a]Biophysics and Laser Science Unit, Vision Science Department, Research Institute of Ophthalmology, Giza, [b]Medical Application of Laser Department, Ophthalmic Unit, National Institute of Laser Enhanced Science, Cairo University, [c]Physics Department, Faculty of Science, Al-Azhar University, Cairo, Egypt

Correspondence to Salwa Abdelkawi, Prof. Dr., Biophysics and Laser Science Unit, Vision Science Department, Research Institute of Ophthalmology, 2 Alahram St., Giza 12511, Egypt. Tel : +201001670590; fax: 35735688; e-mail: saelkawi@yahoo.com

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Background
Photobiomodulation (PBM) plays an essential role in accelerating wound healing and in the recovery of the retinal function. This study aimed to evaluate the efficacy of PBM therapy in combination with chia seeds as an antioxidant in the treatment of experimental diabetic retinopathy (DR).
Materials and methods
A total of 80 Wistar rats were divided into two groups: group 1 control (n=8 rat), and group 2 streptozotocin (STZ)-induced DR group (72 rats), which were injected intraperitoneally with 55 mg/kg of STZ. After 6 weeks, the rats were examined for DR and divided into three subgroups (each 24 rats): (2a) STZ-induced DR group, (2b) STZ-induced DR group exposed to 670-nm diode laser for 6 successive weeks, and (2c) STZ-induced DR group was exposed to 670-nm diode laser and supplemented with 250 mg/kg of chia seeds for 2 weeks before induction of diabetes and continued till the end of the experiment. Electroretinogram (ERG), total protein concentration in retina, and Fourier transform infrared spectrum were assessed after 1, 2, 4, and 6 weeks (six rats each).
Results
DR group showed a progressive decrease ($P<$ 0.05) in a-wave and b-wave of the ERG and retinal protein, in addition to distinct change in the amide I region during 6 weeks, whereas treatment with PBM showed improvement in ERG (a-wave and b-wave, $P<0.05$), protein content ($P<0.05$), and in the amide I region ($P<$ 0.05). However, the group treated with PBM and chia seeds showed better improvement in the a/b ratio of ERG, retinal protein, and in structural component of amide I region compared with the control (α-helix: 37 vs. 34.3%, β-sheet first peak was 12.6 vs. 14.39%, and β-sheet second peak was 20 vs. 20.36%).
Conclusion
Chia seeds with PBM has more beneficial effects in the treatment of DR than by PBM alone.

Keywords:
chia seeds, diabetic retinopathy, electroretinogram, Fourier transform infrared spectroscopy, photobiomodulation, protein concentration

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Introduction

Diabetic retinopathy (DR) is a foremost microvascular complication of diabetes mellitus (DM) as it causes visual impairment and blindness in working-age people worldwide [1]. Microvascular abnormalities can result in increased vascular permeability and capillary nonperfusion [2]. Moreover, the blood-retinal barrier (BRB) breakdown is a hallmark of DR, leading to vascular leakage and the development of retinal edema, which can cause blindness in patients with DR [3–5].

Photobiomodulation (PBM) is the application of low-level light using coherent (lasers) or noncoherent (light-emitting diodes) light source that has a biological effect *in vitro* and *in vivo*. Numerous studies have shown that light in the far-red to near-infrared region of the spectrum (630–1000 nm) can have beneficial effects such as pain relief or wound healing and ulcers, inhibit the development of tissue pathology, lead to improvement in Parkinson's disease, and show amelioration of experimental autoimmune encephalomyelitis [4–8]. Recently PBM has risen to the consideration of the ophthalmology community as a predictable novel approach to treat many retinal diseases including retinal and optic nerve toxicity, optic nerve degeneration, photoreceptor degeneration,

diabetic macular edema, age-related macular degeneration, retinopathy of prematurity, and DR [8–10].

Salvia hispanica L., the common name being chia seeds, is an annual herbaceous plant corresponding to the Lamiaceae or Labiatae family [11]. Chia seeds are an excellent source of essential minerals, necessary for strong and healthy bones. They are also high in vitamin B, exhibit a very favorable Omega-3 (alpha-linolenic acid) to Omega-6 ratio of 3–1, and have very high levels of essential fatty acids and antioxidants which protect the body from free radicals [12]. Chia is recommended for ocular health; it is very beneficial for tear production, in lens transparency and elasticity, in the treatment of patients with macular degeneration, in dry eyes, helping to minimize DR, and reducing markers for inflammation by more than 30% [13].

Previous therapeutic approaches, such as upright glycemic control, high-energy laser photocoagulation, or intravitreal injections of antivascular endothelial growth factor therapies or triamcinolone, are invasive, damaging, or require direct involvement by healthcare professionals, and not all patients respond to these approaches, so new therapeutic approaches are needed. Therefore, this study aims to evaluate the effects of PBM therapy alone or combined with chia seeds supplementation in the treatment of DR in experimental rats by measuring retinal protein concentration, a-wave and b-wave amplitudes by electroretinogram (ERG), and retinal protein secondary structure in the amide I region by Fourier transformation infrared spectroscopy (FTIR) analysis.

Materials and methods

Materials

Streptozotocin (STZ) ($C_8H_{15}N_3O_7$) was purchased from Sigma Aldrich (St. Louis, Missouri, USA) and was used in 0.1 mol/l freshly prepared citrate buffer (pH=4.4) to induce experimental diabetes.

Chia seeds (Planton Company, New Delhi, India) were minced and used as an antioxidant. Low-level diode laser was generated from a diode-pumped solid-state laser (Cobolt; DPSSL-DRIVER II, Solna, Sweden).

ERG was recorded for the rats in all groups using a computerized system (EREV 99 apparatus; Lace Eletronica Co., Pisa, Italy) that can be used for advanced ocular electrophysiology.

Experimental animals

A total of 80 female Wistar rats weighing 200±20 g were involved in this study. The rats were provided by the animal house of Research Institute of Ophthalmology Giza, Egypt. The rats were maintained in a standard 12-h light–dark cycle with free access to water and a balanced diet at a temperature of 30±2°C and 50% humidity. All rats' eyes were examined by slit-lamp biomicroscope to indicate no signs of edema or intraocular inflammation.

Ethical approval

All the experiments were done in compliance with the Public Health Guide for the Care and Use of Laboratory Animals and by the protocols approved by the local experimental ethics in ophthalmic and vision research.

Induction of diabetic retinopathy

Rats were injected intraperitoneally with a single dose of 55 mg/kg of STZ. The blood glucose level of rats was measured by using an electronic blood glucose meter (Accu- Check, Roche Diabetes Care, Inc., Indianapolis; USA). The blood glucose level was displayed in units of mg/dl. Rats with blood glucose levels more than 280±50 mg/dl are included in the study. The diabetic group was followed up for 6 weeks until the establishment of DR and examined for the detection of abnormal blood vessels formation, vessels congestion, and blood vessel leakage.

Study design

The rats were divided into the following groups:

Group 1: control group which did not receive any treatment (n=8 rat).
Group 2a: STZ-induced DR group (n=24 rats), which did not receive any treatment.
Group 2b: STZ-induced DR group (n=24 rats) was exposed to 670 low-level diode laser for 6 weeks.
Group 2c: STZ-induced DR group (n=24 rats), in which every rat was exposed to 670-nm low-level diode laser and administrated daily with 250 mg/kg of chia seeds flour for 2 weeks before induction of diabetes and continued till the end of the estimated periods.

ERG, total protein concentration in retina and FTIR were assessed after 1, 2, 4, and 6 weeks.

Tissue sampling

At the end of each estimated periods (1, 2, 4, and 6 weeks), six rats of each group were subjected to ERG measurements and then decapitated; the eyes were enucleated, in 0.9% saline, and the retinas were carefully removed from the posterior chamber of the

eye for total protein measurements and FTIR spectroscopy.

Methods

Low-level diode laser application

Low-level diode laser was calibrated to deliver 670-nm light at a power of 5 mW/cm^2, for 90 s, using laser prop and convex lens for beam focusing. The distance between the laser prop and rat eye was ~7–9 cm. The weekly radiant exposure energy was 900 mJ/cm^2 or ~1 J/cm^2 for each eye.

Electroretinogram measurements

The rats were anesthetized by xylazine (21 mg/kg of body weight) and ketamine hydrochloride (45 mg/kg) administrated intramuscularly. The pupil of the eye was dilated with topical 1% Mydriacyl (Alcon Eye Care UK Limited, Frimly, Surrey, UK). Extracellular ERG was recorded by using a wick electrode as an active electrode situated on the cornea; the other electrode was placed on the skin of the lower eyelid as a reference one. The resulted electrophysiological signals were collected and analyzed by data studio 1.9.8 software (PASCO, Roseville, California, USA).

Total soluble protein concentration

Retinae samples were added to 1 ml ice-cold PBS (pH 7.9) and centrifuged at 10 000 rpm (Awel centrifuge MF 20; Awel International, Blain, France) for 10 min. Retinal total soluble protein concentration was determined using a kit of Biodiagnostics Inc. (Cairo, Egypt) and spectrophotometer type 'Thermo Evolution 300 UV-Visible spectrophotometer (Thermo Electron Scientific Instruments LLC, Madison, WI USA)' according to the method of Lowry *et al.* [14].

Fourier transformation infrared spectroscopy

Retinae from each group were separated, weighed, lyophilized, and mixed with KBr powder (95 mg KBr: 5 mg sample) to prepare the KBr disks. FTIR spectra were measured using 'Thermo Nicolet iS5 FTIR spectrometer (Thermo Electron Scientific Instruments LLC, Madison, WI USA)' in the range of 4000–1000/cm at room temperature with an effective resolution of 2/cm. A total of 100 sample interferograms were recorded for each spectrum. The spectrometer is operated under a continuous dry nitrogen gas purge to eliminate interference from atmospheric carbon dioxide and water vapor. The data were baseline corrected and smoothed to remove the noise before Fourier transformation. The average of three spectra for each group was obtained using Origin Pro 9 software (OriginLab Corporation; Northampton, MA 01060, USA).

Statistical evaluation

The result was specified as mean±SD, and the variations between different groups were analyzed using a commercially available software package (SPSS-11 for Windows; SPSS Inc., Chicago, Illinois, USA). The result was considered significant at *P* value less than 0.05.

Results

Electroretinogram measurements

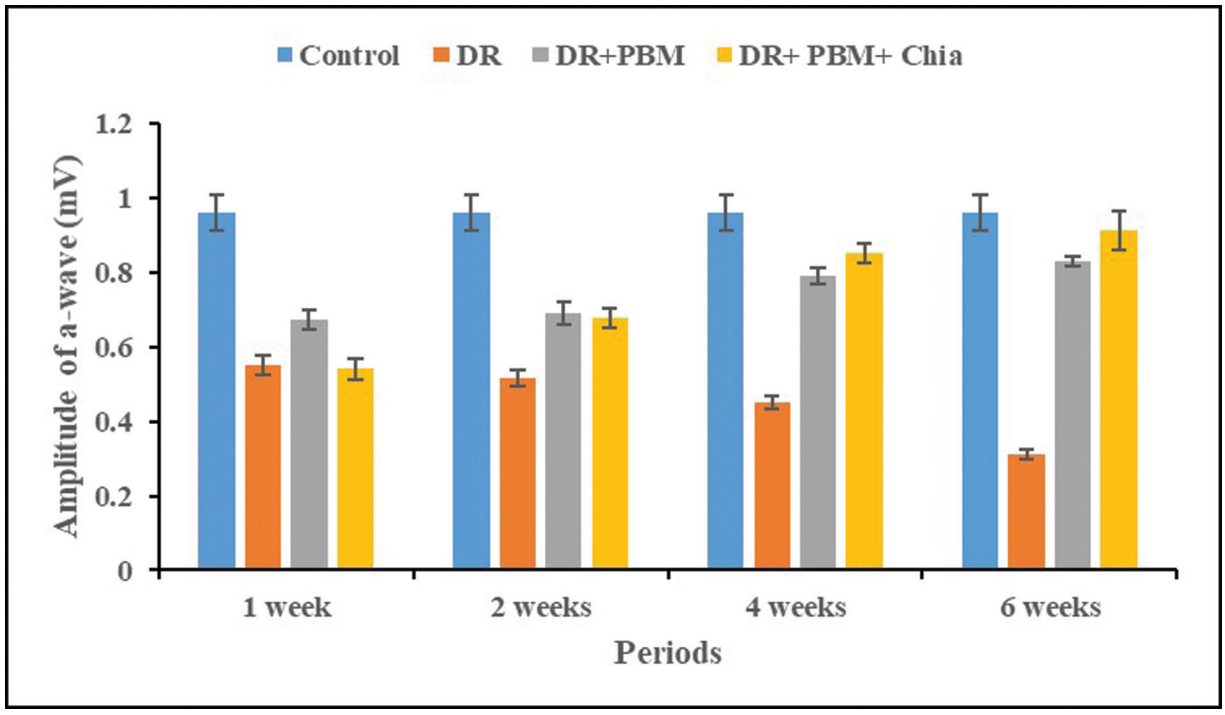
The recorded ERG amplitudes for both a-wave and b-wave for control, DR, DR+PBM, and DR+PBM+chia seeds groups are shown in Table 1. After induction of DR and as the period of diabetes was extended, the amplitudes

Table 1 Amplitude of a-wave (mV), amplitude of b-wave, b/a ratio, and total protein of the retina for control, diabetic retinopathy, diabetic retinopathy+photobiomodulation, and diabetic retinopathy+photobiomodulation+chia seeds groups after 1, 2, 4, and 6 weeks

Groups	Periods	a-wave (mV)	b-wave (mV)	b/a ratio	Total protein (mg/g tissue wet weight)
Control group 1	–	0.96±0.05	1.77±0.05	1.85±0.09	86±2
STZ induced DR group 2					
Group 2a (DR)	1 week	0.55±0.03[a]	1.22±0.06[a]	2.20±0.11[a]	203±2[a]
	2 weeks	0.52±0.03[a]	1.12±0.05[a]	2.17±0.09[a]	221±3[a]
	4 weeks	0.45±0.02[a]	1.06±0.05[a]	2.35±0.11[a]	270±2[a]
	6 weeks	0.31±0.02[a]	0.90±0.05[a]	2.90±0.14[a]	323±3[a]
Group 2b (DR+PBM)	1 week	0.67±0.03[a]	1.46±0.07[a]	2.17±0.07[a]	195±4[a]
	2 weeks	0.69±0.03[a]	1.43±0.07[a]	2.07±0.05[a]	174±4[a]
	4 weeks	0.79±0.04[a]	1.45±0.07[a]	1.839±0.08	134±2[a]
	6 weeks	0.83±0.04[a]	1.53±0.07[a]	1.836±0.08	93±2
Group 2c (DR+PBM+chia seeds)	1 week	0.54±0.03[a]	1.53±0.08[a]	2.83±0.08[a]	184±4[a]
	2 weeks	0.68±0.03[a]	1.60±0.08[a]	2.36±0.07[a]	163±3[a]
	4 weeks	0.85±0.04[a]	1.63±0.07[a]	1.90±0.081	101±2[a]
	6 weeks	0.91±0.05	1.68±0.07	1.84±0.05	83±2

All values are expressed as mean±SD. DR, diabetic retinopathy; PBM, photobiomodulation; STZ, streptozotocin. [a]$P<0.05$, statistical different than control group.

Figure 1



Amplitude of a-wave (mV) of the retina for control, DR (diabetic retinopathy), DR+PBM (photobiomodulation), and DR+PBM+chia seeds groups after 1, 2, 4, and 6 weeks.

of a-wave and b-wave were significantly decreased after 1, 2, 4, and 6 weeks ($P<0.05$), correspondingly. Moreover, these retinal changes induced by the effect of diabetes were enhanced after treatment with PBM therapy ($P<0.05$) alone and by treatment with PBM plus chia seeds ($P<0.05$) after 1, 2, 4, and 6 weeks, correspondingly, as shown in Figs 1 and 2. Moreover, the b/a ratio in the retina was increased in the DR group ($P<0.05$) after 1, 2, 4, and 6 weeks as listed in Table 1. The b/a ratio in the other two groups (PBM and PBM+chia seeds) showed nonsignificant changes after the fourth and sixth weeks, and their values were approximately similar to the control value, as shown in Fig. 3.

Total protein concentration

The total soluble protein concentration of the control sample (86±2 mg/g tissue wet weight) of rat's retina was significantly increased in the DR group after 1, 2, 4, and 6 weeks ($P<0.05$), correspondingly (Table 1). On the contrary, treatment with PBM alone or combined with chia seeds showed progressive improvement in retinal protein ($P<0.05$) after 1, 2, 4, and 6 weeks correspondingly with respect to the control group.

Fourier transformation infrared spectroscopy for the retina (amide ? region)

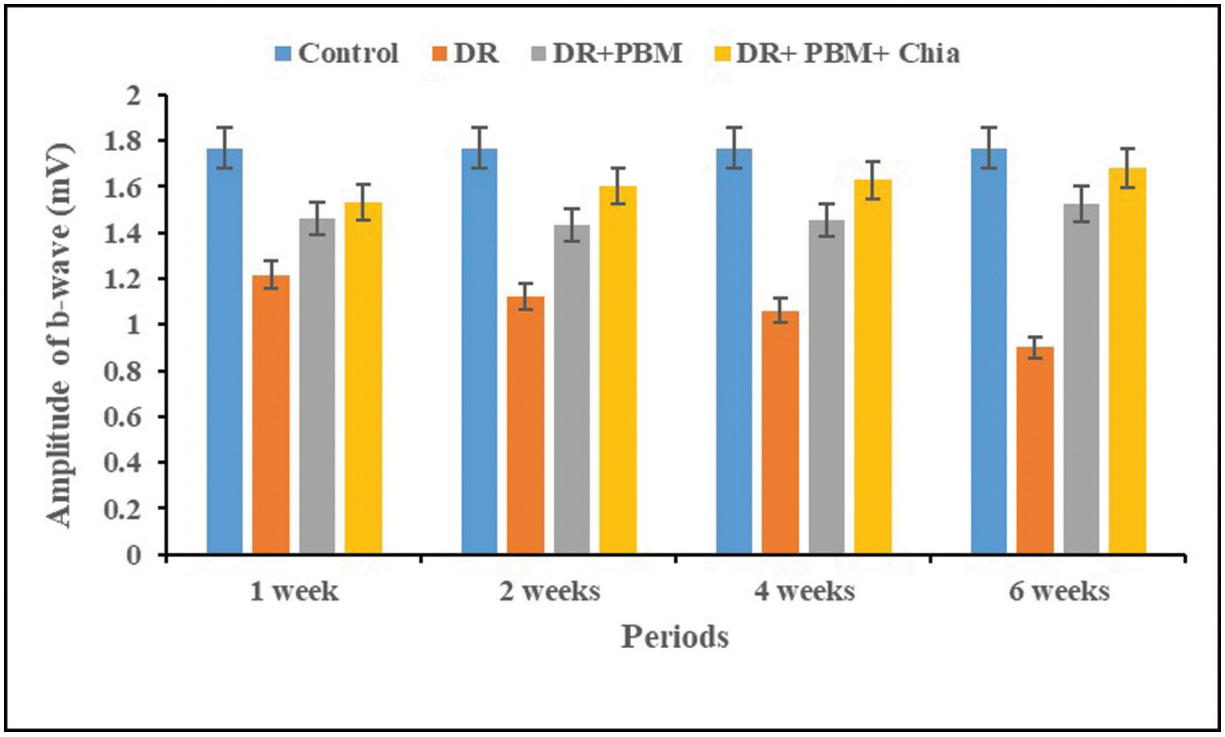
The FTIR results were analyzed for amide I spectral region (1700–1600/cm) after 1, 2, 4, and 6 weeks. The curve enhancement procedure resolves the contour of the control amide I band to five structural components that are centered at 1679±3 and 1666±3/cm for β-turn structure, 1651±1/cm for α-helix, and 1637±2 and 1623±3/cm for β-sheet (Table 2).

In the DR group, after 1 week, the structural components were characterized by the following: (a) the second band of β-turn at 1666±3/cm was restricted, (b) decrease in the area percentage of α-helix (P? 0.05), (c) formation of amide I random coil at 1647±4/cm, and (d) changes in the area percentage and bandwidths of β-sheet components ($P<0.05$).

On the contrary, the structural components of amide I for PBM-treated group after 1 week were characterized by (a) a significant decrease in bandwidth of β-turn band at 1679±3/cm and in the area percentage ($P<0.05$); (b) the second band of β-turn was renewed by some increasing in wave number, bandwidth, and area percentage; and (c) increase in the bandwidth and area percentage of the second band of β-sheet ascribed at 1623±3/cm ($P<0.05$), in addition to restriction of amide I random coil band.

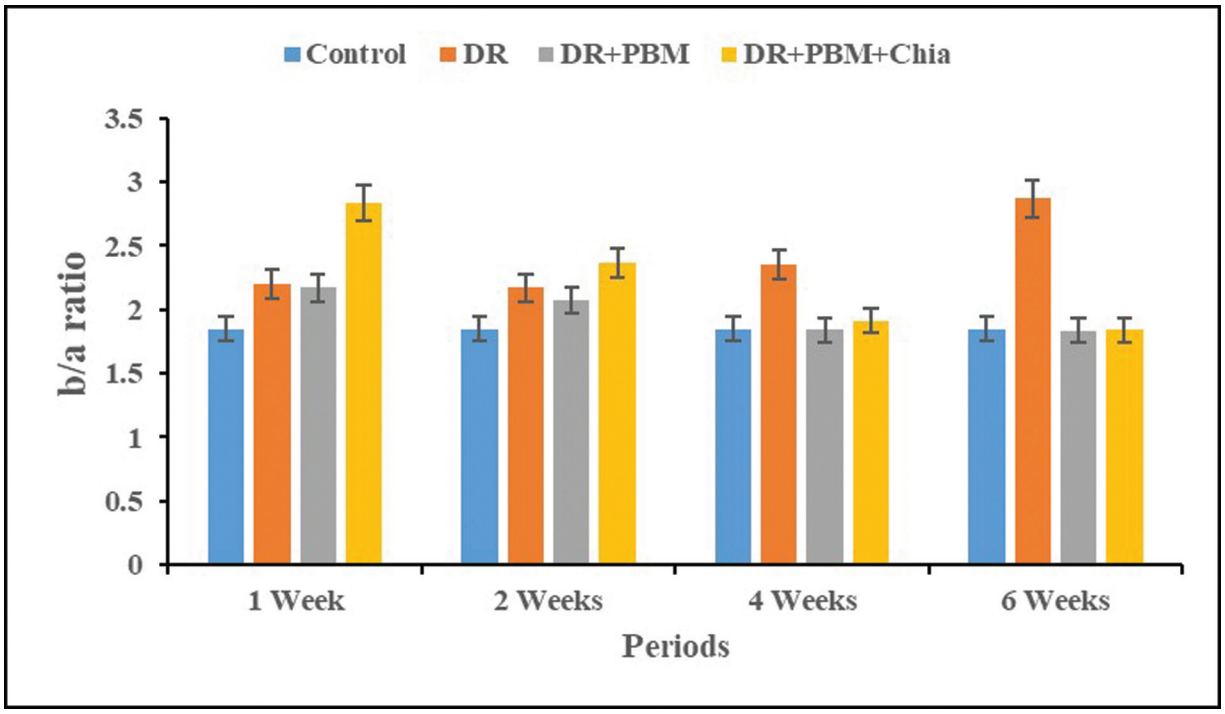
The DR rats treated with PBM combined with chia seeds were characterized by the following: (a) the α-helix band at 1651±1/cm showed a significant

Figure 2



Amplitude of b-wave (mV) of the retina for control, DR (diabetic retinopathy), DR+PBM (photobiomodulation), and DR+PBM+chia seeds groups after 1, 2, 4, and 6 weeks.

Figure 3



The ratio between b-wave to a-wave of the retina for control, DR (diabetic retinopathy), DR+PBM (photobiomodulation), and DR+PBM+chia seeds groups after 1, 2, 4, and 6 weeks.

Table 2 The structural components of amide I region (1700–1600/cm) for all studied groups after 1 week

Amide I components after 1 week	Group 1 (control)	STZ induced DR group 2		
		Group 2a (DR)	Group 2b (DR+PBM)	Group 2c (DR+PBM+chia seeds)
β-Turn				
First peak	1679±3	1670±3	1683±1	1686±5
	24±4	16±5[a]	8±3[a]	10±1[a]
	19.4%	16.5%	1.4%[a]	10.5%
Second peak	1666±3	–	1671±5	1674±1
	14±4	–	20±5[a]	13±2
	11.58%	–	19.8%	12.3%
α-Helix	1651±1	1655±6	1652±2	1653±1
	19±11	11±5[a]	20±7	33±3[a]
	34.3%	23.47%	34%	40%
Amide I random coil	–	1647±4	–	–
	–	9±3	–	–
	–	18.78%	–	–
β-Sheet				
First peak	1637±2	1639±3	1638±3	1637±1
	14±5	7±2[a]	15±5	4±2[a]
	14.39%	9%[a]	12.72%	11.11%
Second peak	1623±3	1627±4	1626±2	1627±1
	20±4	18±3	26±3[a]	26±1[a]
	20.36%	32.17%	31.72%	25.67%

The first line represents the wave number (/cm). The second line represents the bandwidth (/cm). The third line represents the area percentage under peaks. DR, diabetic retinopathy; PBM, photobiomodulation; STZ, streptozotocin. [a]$P<0.05$, statistical different than control group.

Table 3 The structural components of amide I region (1700–1600/cm) for all studied groups after 2 weeks

Amide I components after 2 weeks	Group 1 (control)	STZ induced DR group 2		
		Group 2a (DR)	Group 2b (DR+PBM)	Group 2c (DR+PBM+chia seeds)
β-Turn				
First peak	1679±3	1672±1	1685±3	1684±1
	24±4	13±2[a]	16±2[a]	6±1[a]
	19.37%	13.7%	7.79%	12.5%
Second peak	1666±3	1663±.5	1668±4	1668±3
	14±4	3±1[a]	21±13[a]	21±5[a]
	11.58%	2.65%	13.86%	22.9%
α-Helix	1651±1	1651±1	1654±2	1651±3
	19±11	24±3[a]	16±9	16±6
	34.3%	48.96	29.74%	15.33%
β-Sheet				
First peak	1637±3	1637±1	1638±2	1636±2
	14±5	5±1[a]	27±8[a]	16±1
	14.39%	5.9%	30.7%	33.7%
Second peak	1623±3	1626±1	1619±6	1622±4
	20±4	20±4	18±9	15±4[a]
	20.36%	28.7%	11.32%	12.25%
Third peak	–	–	1608±2	1608±4
	–	–	7±2	12±7
	–	–	6.6%	2.42%

The first line represents the wave number (/cm). The second line represents the band width (/cm). The third line represents the area percentage under peaks. DR, diabetic retinopathy, PBM, photobiomodulation; STZ, streptozotocin. [a]$P<0.05$, statistical different than control group.

increase in area percentage and bandwidth ($P<0.05$), and (b) a significant decrease in the bandwidth and in area percentage ($P< 0.05$) of the first peak of β-sheet at 1637±2/cm.

Analysis of amide I bands after 2 weeks (Table 3) revealed that the wave number for all groups showed nonsignificant changes, and the most noticeable changes were in the bandwidths and the area

Table 4 The structural components of amide I region (1700–1600/cm) for all studied groups after 4 weeks

Amide I components after 4 weeks	Group 1 (control)	STZ induced DR group 2		
		Group 2a (DR)	Group 2b (DR+PBM)	Group 2c (DR+PBM+chia seeds)
β-Turn				
First peak	1679±3	1681±1	1684±1	1685±1
	24±4	16±1[a]	17±1[a]	6±1[a]
	19.37%	17.3%	10.93%	12.91%
Second peak	1666±3	1662±1	1670±2	1673±1
	14±4	4±1[a]	16±4	15±4
	11.58%	4.13%	12.36%	11.11%
α-Helix	1651±1	1651±1	1656±4	1651±4
	19±3	24±3	21±3	18±3
	34.3%	43%	28.49%	33.63%
β-Sheet				
First peak	1637±3	1636±1	1636±6	1637±1
	14±5	5±1[a]	25±2[a]	4±1[a]
	14.39%	6.9%	30.66%	21%
Second peak	1623±3	1623±3	1618±4	1624±2
	20±4	26±3[a]	17±5	21±6
	20.36%	28.67%	10.64%	21.3%
Third peak	–	–	1608±1	1608±1
	–	–	7±1	5±1
	–	–	7%	5%

The first line represents the wave number (/cm). The second line represents the band width (/cm). The third line represents the area percentage under peaks. DR, diabetic retinopathy; PBM, photobiomodulation; STZ, streptozotocin. [a]$P<0.05$, statistical different than control group.

percentage of most the structural components. Moreover, one additional band corresponding to β-sheet appeared in both PBM group and PBM +chia seeds at 1608±1/cm with low area percentage of 6.6 and 2.42%, whereas in DR group, this band was missed.

Analysis of amide I bands after 4 weeks (Table 4) revealed the following: (a) the first peak of β-turn at 1679±3/cm in all studied groups showed a significant decrease in their bandwidths and area percentage ($P< 0.05$), (b) the area percentage and the bandwidths for the β-turn second peak component at 1666±3/cm was improved in both DR+PBM and DR+PBM+chia seeds-treated groups, (c) the band at 1651±1/cm corresponding α-helix and the second peak of β-sheet at 1623±3/cm were significantly improved after treatment with PBM+chia seeds; and (d) finally, one additional band corresponding to β-sheet appeared in DR+PBM and DR+PBM+chia seeds-treated groups.

The structural components for amide I region after 6 weeks (Table 5) were characterized by the following: (a) the β-turn first peak component at 1679±3/cm was still affected in all studied group whereas the second peak at 1666±3/cm was improved in bandwidths and area percentage after treatment with PBM alone and with PBM+chia seeds; (b) in DR group, the band at 1651±1/cm corresponding to α-helix showed significant increase ($P<0.05$) in its bandwidth and area percentage in addition to significantly decreased ($P<0.05$) in their bandwidths and area percentage of the two β-sheet peaks and then showed better improvement in PBM+chia seeds than in PBM alone; and (c) one additional band, corresponding to β-sheet, was appeared at 1611±2/cm in the DR group and restricted in the other treated groups.

Discussion

In DM, chronic hyperglycemia induces oxidative stress and increase the concentration of oxygenated free radicals in the retinas of diabetic animals. Moreover, this rise is directly related to the increase in the rate of membrane lipid peroxidation as a chain reaction [15]. Moreover, the retina, which is a tissue very rich in polyunsaturated fatty acids, is particularly sensitive to the reactivity of oxygenated free radicals [15,16]. Once generated, free radicals can attack various molecule types, thereby resulting in obstruction of their activity. If these damaging free radicals are not readily neutralized, they damage macromolecules – proteins, lipids, and DNA, and affect the retinal cells and consequently their function [17].

Photoreceptors cells, where there is a significant collaboration of cell membranes, are quite weak to

Table 5 The structural components of amide I region (1700–1600/cm) for all studied groups after 6 weeks

Amide I components after 6 weeks	Group 1 (control)	STZ induced DR group 2		
		Group 2a (DR)	Group 2b (DR+PBM)	Group 2c (DR+PBM+chia seeds)
β-Turn				
First peak	1679±3	1683±1	1680±1	1680±1
	24±4	23±1	14±1[a]	11±1[a]
	19.37%	15.12%	10.76%	7.5%
Second peak	1666±3	1668±1	1670±1	1671±1
	14±4	8±1[a]	12±1	13±2
	11.58%	5%	12.1%	10.64%
α-Helix	1651±1	1654±2	1649±1	1650±2
	19±5	33±8[a]	31±3[a]	21±3
	34.3%	55%	39%	37%
β-Sheet				
First peak	1637±3	1636±1	1636±1	1636±1
	14±5	7±1[a]	15±1	15±1
	14.39%	6.2%	12.1%	12.6%
Second peak	1623±3	1625±1	1627±3	1626±2
	20±4	15±2[a]	16±6[a]	19±12
	20.36%	16.1%	26%	20%
Third peak	–	1611±2	–	–
	–	13±5	–	–
	–	3%	–	–

The first line represents the wave number (/cm). The second line represents the band width (/cm). The third line represents the area percentage under peaks. DR, diabetic retinopathy; PBM, photobiomodulation; STZ, streptozotocin. [a]$P<0.05$, statistical different than control group.

free radicals. The attack of free radicals on polyunsaturated fatty acids results in lipid peroxidation that breaks down membranous structures and function [17]. It was believed that the a-wave begins from photoreceptors (rods and cones), and the decline that has been seen in a-wave amplitude values in the DR group during the follow-up periods (Table 1 and Fig. 1), suggests functional variances in the retina [18].

On the contrary, the b-wave is thought to be a delicate index of retinal ischemia and reflects mainly the activity of ON-center bipolar cells and/or Muller cells (middle retina). In this study, the progressive decreases in the b-wave amplitude of the DR group (Fig. 2) may be revealed to the severity of the retinal ischemia [18]. Consequently, changes in a-wave and b-wave in the DR group leads to changes in b/a ratio (Fig. 3), which is believed to be a positive marker of retinal ischemia and useful in evaluating the loss of retinal function [19,20].

In this study, it is noticed that there is a distinct improvement in ERG after treatment by PBM. This result is well matched with the result obtained by previous success of PBM therapy with 670-nm light in obstruction of the diabetes-induced production of the oxidative stress in rats and human patients with diabetic macular edema, amelioration of the light damage-induced changes in Muller cells, significant drop in ganglion cell death as well as a 50% improvement of the photopic b-wave amplitude [9,11,21]. Additionally, more improvements in a-wave and b-wave were noticed in the group supplemented with chia seeds than in PBM therapy, suggesting the introduction of the scavenging effect and its ability to overcome the accumulation of the free radicals [22].

The total soluble protein content in the DR group (Table 1) showed a progressive increase after 1, 2, 4, and 6 weeks concerning the control, respectively. This increase may be owing to increased leakage of macromolecules, predominantly albumin, from the blood and accumulate in interstitial tissue space through retinal capillaries [23]. Previous reports discussed the mechanisms of BRB breakdown in DR, as many proinflammatory factors are up-regulated in the retina and vitreous humor, including TNF-a, IL-6, and IL-1b and are associated with vascular hyperpermeability. Moreover, some inflammatory factors and cell surfaces adhesion molecules are increased. These events finally lead to retinal vascular inflammation, capillary occlusion, and endothelial cell death and contribute to BRB breakdown associated with the initiation and progression of diabetic microangiopathy [8,24–26].

Otherwise, this study shows gradient improvement in protein content after PBM therapy after 1, 2, 4, and 6 weeks, correspondingly. Furthermore, supplementation with chia seeds before (2 weeks) and during PBM therapy (6 weeks) produces better improvement in the content of retinal protein than PBM therapy alone. This improvement highlights the beneficial effects of this low-level laser and chia seeds in reducing the effects of DR to a good bit.

Infrared spectroscopy is employed to investigate the functions and structure of tissues and to highlight the change in biochemical mechanisms under some pathological conditions. The amide I band arises mainly from absorption of the C═O stretching vibration and CN stretching vibration. Hence, the amide I band is signifying a conformational change in α-helixes [27]. The present results of FTIR analysis indicate that induction of DR has different effects on the amide I region. After 1 week of DR, the α-helix area percentage is decreased from 34.3 to 23.47% (Table 2) with the formation of amide I random coil at 1647±4/cm. The decrease in α-helix and the conversion of the helix to random coil might be a transitional state, progressively increasing β-sheet structural content, which in turn induces the disordered structure of the helical structure, causing protein insolubility, aggregation, less folding, and retinal geometry disorders [28]. On the contrary, the structural components of amide I for both PBM-treated and PBM+chia-treated groups after 1 week were characterized by the reformation of the second band of β-sheet and restriction of amide I random coil band, which formed in DR at 1647±4/cm.

Moreover, after 2 weeks, PBM therapy alone or with chia seeds significantly improve most of the amide components (Table 3). These improvements may be produced because of low-level laser action that utilizes a nonthermal process involving endogenous chromophores producing photophysical and photochemical effects [29]. As it is well known that numerous signaling pathways are triggered after absorption of light photons by reactive oxygen species (ROS), NO, cyclic AMP, and Ca^{2+} leading to initiation of transcription factors [30].

In this regard, the analysis of amide I bands after 4 weeks of treatment with PBM alone or associated with chia seeds (Table 4) revealed improvement in the area percentage and the bandwidths for all amide I region. After 2 and 4 weeks, new band corresponding to β-sheet at 1608/cm was formed in both treated groups and may be attributed to the photochemical effect of low-level laser with the retinal tissue as it was missed in DR group.

By extending the DR periods till the sixth week (Table 5), the bandwidth and area percentage of α-helix at 1651±1/cm showed significant increase associated with a decrease in area percentage. The two peaks of β-turns indicate the conversion of the helix to random coil and leads to increase in the conformation, misfolding, and aggregation of β-sheet structural progressively. β-Turns have a vital role in connecting the other protein secondary structure (α-helix and β-sheet) and help the protein to maintain its globular structure. Moreover, this increase in α-helix and the decrease in β-sheet structural components showed definite improvement after PBM+chia seeds treatment. The disappearance of the new band at 1611±2/cm, corresponding to β-sheet by PBM therapy alone or with chia seeds, is a positive step, but the affected β-turn at 1679±3/cm may require increasing laser sessions and chia seed doses.

The action of PBM could be summarized as follows: when the light enters the cells' mitochondria and is absorbed by the chromophores, including cytochrome *c* oxidase (CCO), it increases its activity. CCO is responsible for the reduction of oxygen to water. Moreover, nitric oxide (NO), ROS, and ATP are the foremost molecules affected in the mitochondria when they absorb the photons delivered during PBM. The NO created in the mitochondria could hinder breathing by obligating CCO and competitively replacing oxygen, especially in stressed or hypoxic cells. Light absorption displaces or photo-dissociates the NO and thus allowed the CCO to recover and cellular respiration to resume. When NO breaks down, the mitochondrial membrane potential is elevated, more oxygen is consumed in glucose metabolization, and more ATP is produced, accelerating the healing process. Moreover, the burst of ROS created in the mitochondria by light photons may trigger some mitochondrial signaling pathways leading to cytoprotective, antioxidant, and antiapoptotic effects in the cells and affecting cellular repair and healing [10,20]. Furthermore, administration with chia seeds produced more improvements in the measured ERG, protein content, and retinal protein secondary structure. These improvements may be attributed to the biological effects of chia seed in the enhancement of endogenous antioxidant ability, prevention of structural abnormalities, reduction of inflammation, and reduction of oxidative stress owing to its high concentration of antioxidants [31,32].

Conclusion

The conclusion that is well supported from this investigation is that PBM could improve functional changes in retinal cells and photoreceptors, protein insolubility, and aggregation corresponding to DR. Moreover, treatment modes of PBM and chia seeds at the onset of the disease may have more beneficial and useful effects because it has a high antioxidant capacity, able to remove ROS and reduce oxidative stress.

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Conflicts of interest

There are no conflicts of interest.

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