Photobiomodulation therapy for diabetic macular edema: Fourier transform infrared spectroscopy study

Salwa A. Abdelkawi[a], Aziza A. El Saeid[b], Ahlam M. El-Rashedi[b], Maha S. Abd-Eldaiem[a]

[a]Biophysics and Laser Science Unit, Research Institute of Ophthalmology, Giza, [b]Physics Department, Faculty of Science, AL Azhar University (Girls), Cairo, Egypt

Correspondence to Salwa A. Abdelkawi, PhD, Biophysics and Laser Science Unit, Vision Sciences Department, Research Institute of Ophthalmology, 2 Elahram Street, Giza, 12511, Egypt. Tel: +20 100 167 0590; fax: 5735688; e-mail: saelkawi@yahoo.com

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Background/Aim
Photobiomodulation (PBM) is the application of low-level light that has a beneficial biological effect, such as to relieve pain, to heal existing tissue damage, and to inhibit the development of tissue pathology. The aim of the current study is to evaluate the effect of PBM therapy on diabetic macular edema (DME) in albino rats as assessed by Fourier transform infrared spectrum.
Materials and methods
Twenty-five albino rats with the same sex (200±20 g) were involved in this study. Diabetes was induced in albino rats after intraperitoneal injection of 55 mg/kg streptozotocin. The experimental animals were divided into three main groups: (i) control group, (ii) DME group that did not received any treatment, and (iii) DME group that was exposed to two sessions/week of 660-nm low-level laser source for a period of 2 weeks. The rat's eye received a power of 5 mW/cm^2 for 90 s, with a total energy of 450 mJ, in each session. Fourier transform infrared spectrum analysis was applied after 2 weeks for comparison between the diabetic and PBM-treated groups.
Results
The results confirmed that DME was associated with changes in the retina structure that appeared after receiving a single dose of streptozotocin 55 mg/kg. These changes obviously appeared in the NH–OH as shown in $_{str}$O–H ($P<0.05$), $_{str}$O–H$_{sym}$ ($P<0.01$), C–H$_{ring}$ ($P<0.01$), CH stretching, fingerprint, and amide I regions. Treatment with PBM significantly improved most of the amide I components except the first peak of β-turn and formation of new bands corresponding to β-sheet.
Conclusion
The treatment with PBM by using low-level diode laser was associated with different beneficial effects on the retina constituents, as showed by the obvious improvement in the retinal protein secondary structure using Fourier transform infrared analysis. More PBM sessions and long-term follow-up are needed for use of the PBM therapy as a treatment method.

Keywords:
diabetic macular edema, Fourier transform infrared, photobiomodulation, retina

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Introduction

Diabetic macular edema (DME) is the most common cause of vision loss in the working age population, 20–74 years old. DME occurs because of an increase of water in the retinal tissue, subsequent by an increase in its thickness. This increase in water content of the retinal tissue may be initially intracellular (cytotoxic edema) or extracellular (vasogenic edema) [1]. It is mainly due to sustained hyperglycemia causing a breakdown in the blood–retinal barrier (BRB). This breakdown of vessel walls leads to accumulation of fluids in extracellular space, and the ultimate formation of DME [2].

Photobiomodulation (PBM) is the application of low-level light that has a biological effect. Numerous studies have shown that light in the far-red to near-infrared region of the spectrum (630–1000 nm) can have beneficial effects *in vitro* and *in vivo* to heal existing tissue damage and to inhibit the development of tissue pathology [3–6].

Retina is ~0.5 mm thick with an optic nerve at the center. There are slightly oval-shaped, blood vessel-free reddish spots (the fovea) at the center of the area known as the macula. Photoreceptors (the rods and cones) are present at the exterior of retina, and the

absorption of photons by the visual pigment of the photoreceptors is converted to an electrical signal that can stimulate all the neurons of the retina. Central retina close to the fovea is much thicker than peripheral retina. This is owing to the increased stuffing density of photoreceptors, specially the cones. In the past 10 years, there has been a cumulative interest in the use of 670-nm red light to manage retinal injuries. Early reports verified the ability of 670-nm red light to diminish damaging effects of methanol intoxication in rat retinas. More recent studies have shown that 670-nm light treatment provides significant protection to photoreceptors in retinas exposed to damaging levels of light [7]. This study aimed to explore the efficacy of PBM therapy in treatment of DME as assessed by Fourier transform infrared spectroscopy (FTIR).

Materials and methods

Twenty-five female albino rats were involved. The rat's ages ranged from 8 to 12 weeks, and their mean weight was 200±20 g. The rats were supplied 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 balanced diet at a temperature of 30±2°C and 50% humidity. All rats' eyes were examined by slit lamp biomicroscope before induction of diabetes. The results exhibited no signs of edema or intraocular inflammation in all eyes. The rats were divided into three groups: (i) control group (shame control) which did not receive any treatment (n=5 rat); (ii) 2-week diabetic group (n=10), which was intraperitoneal injected with 55 mg/kg of streptozotocin in 0.1 mol/l freshly prepared citrate buffer pH=4.4; and (iii) diabetic group (n=10) treated with PBM for 2 weeks. Fasting blood glucose levels were monitored after 72 h of streptozotocin injection, and animals with blood glucose levels of more than 400 mg/dl were selected. The diabetic rats were followed up for 8 weeks using slit lamp examination till the establishment of macular edema and then treated with PBM therapy.

Photobiomodulation protocol

Low-level diode laser was generated and calibrated to deliver 660-nm light at a power of 5 mW/cm^2, for 90 s (two sessions/week), using a system consisting of 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 thus 900 mJ/cm^2 or (~1 J/cm^2) for each eye. The rats were exposed to this radiation for 2 weeks. This protocol has been documented and selected to decrease retinal toxicity and light damage [8,9].

Extraction of the retina

At the end of the estimated period, the albino rats were decapitated; the eyes were enucleated and then the retinas were carefully removed from the posterior chamber of the eye. Extraction of retinal cells was carried out and directly prepared for FTIR.

Fourier transformer infrared spectroscopy

Samples of retinal cells were freeze dried and then mixed with KBr powder (98 mg KBr: 2 mg of lyophilized retina) to prepare the KBr disks for FTIR measurements. FTIR spectra were measured in the range of 4000–1000/cm at room temperature using ThermoNicolet iS5 FTIR spectrometer (Thermo Fisher Scientific Inc., Madison, Wisconsin, USA) with effective resolution of 2/cm. Hundred sample interferograms were recorded for each spectrum to enhance the apparent resolution of a spectrum or decrease the width of all lines contributing to the investigated spectral range. The spectrometer is operated under a continuous dry nitrogen gas purge to get rid of interference from atmospheric carbon dioxide and water vapor. The data were baseline corrected and smoothened by Savitzky–Golay to remove the noise before Fourier transformation. The obtained group spectrum was normalized and analyzed for the following spectral regions: (i) NH–OH region at 3700–3000/cm; (ii) CH stretching region at 3000–2800/cm; and (iii) the fingerprint region at 1800–1000/cm, which includes the amide I band (1800–1600/cm). The average of the individual spectrum for each group was obtained using Origin Pro 9 software (OriginLab Corporation, Northampton, MA 01060, USA).

Statistical evaluation

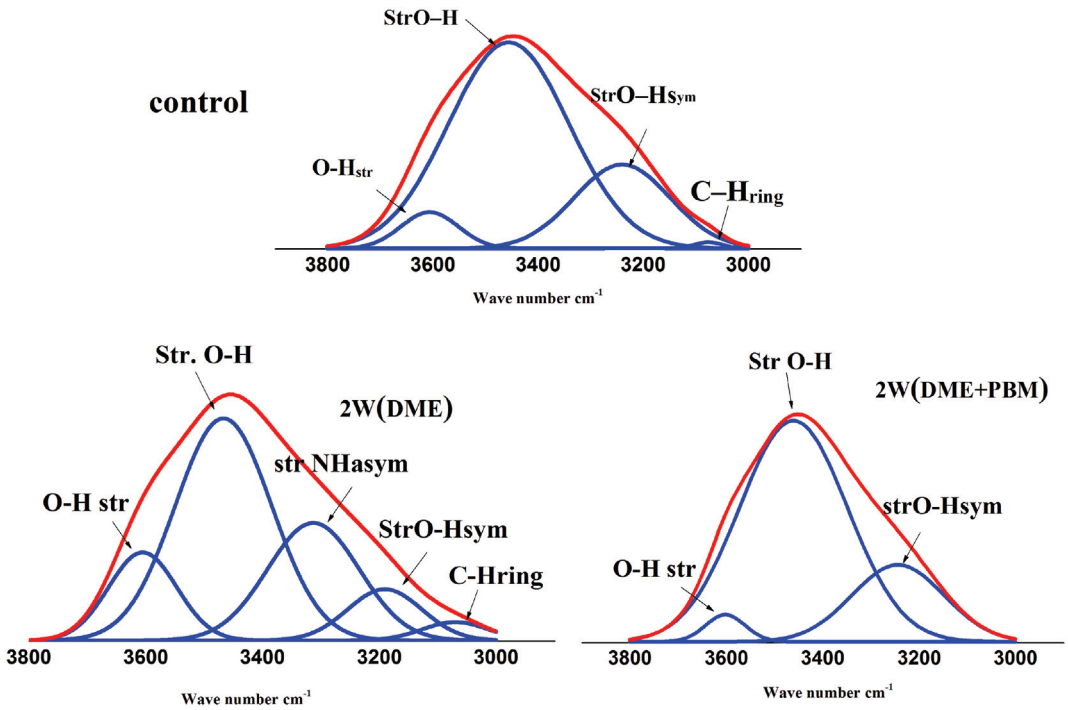
Data were expressed as mean±SD. The analysis of variance procedure was used for comparison between different groups, where a commercially available software package (SPSS-11, for windows; SPSS Inc., Chicago, USA) was used. The result was considered significance at P less than 0.05.

Results

NH–OH region

Figure 1 showed the IR frequency range at 3800–3000/cm corresponding to stretching NH–OH region. Table 1 showed the wave numbers and bandwidths of the deconvoluted peaks for control, non-PBM-treated diabetic, and diabetic group exposed to PBM therapy after 2 weeks. By applying the curve enhancement procedure, the control retina revealed

Figure 1



FTIR of the retina for control, DME, and DME+PBM in the NH–OH region (3800–3000/cm) after 2 weeks. DME, diabetic macular edema; FTIR, Fourier transform infrared spectroscopy; PBM, photobiomodulation.

Table 1 Wave number and bandwidth and the different component of the NH–OH region (3800–3000/cm) for all studied groups

Components	Control	2-W DME	2-W DME+PBM
$_{str}$O–H	3606±4	3605±7	3602±8
	128±6	137±8*	97±6*
$_{str}$O–H	3455±9	3476±2*	3461±5
	269±5	194±2*	264±4
$_{Str}$NH$_{asym}$	–	3313±4	–
	–	185±5	–
$_{str}$O–H$_{sym}$	3240±8	3190±9*	3243±7
	214±2	149±3*	225±2*
C–H$_{ring}$	3076±7	3070±5	–
	65±3	133±2*	–

The first line in each cell indicates the vibrational frequency, whereas second line reflects the bandwidth. DME, diabetic macular edema; PBM, photobiomodulation; w, weeks. *Statistically significant.

four characteristic bands appeared at 3606±4, 3455±9, 3240±8, and 3076±7/cm corresponding to O–H$_{str}$, $_{str}$O–H, $_{str}$O–H$_{sym}$, and C–H$_{ring}$, respectively [10]. For the non-PBM-treated DME group, the band at 3455/cm attributed to $_{str}$O–H was shifted to 3476±2/cm and its bandwidth was decreased (P<0.05). In addition, the band corresponding to $_{str}$O–H$_{sym}$ was decreased in both wave number and bandwidth (P<0.01). Moreover, the band of C–H$_{ring}$ at 3076/cm increased in bandwidth (P<0.01) with formation of new band at 3313±4/cm corresponding to $_{Str}$NH$_{asym}$.

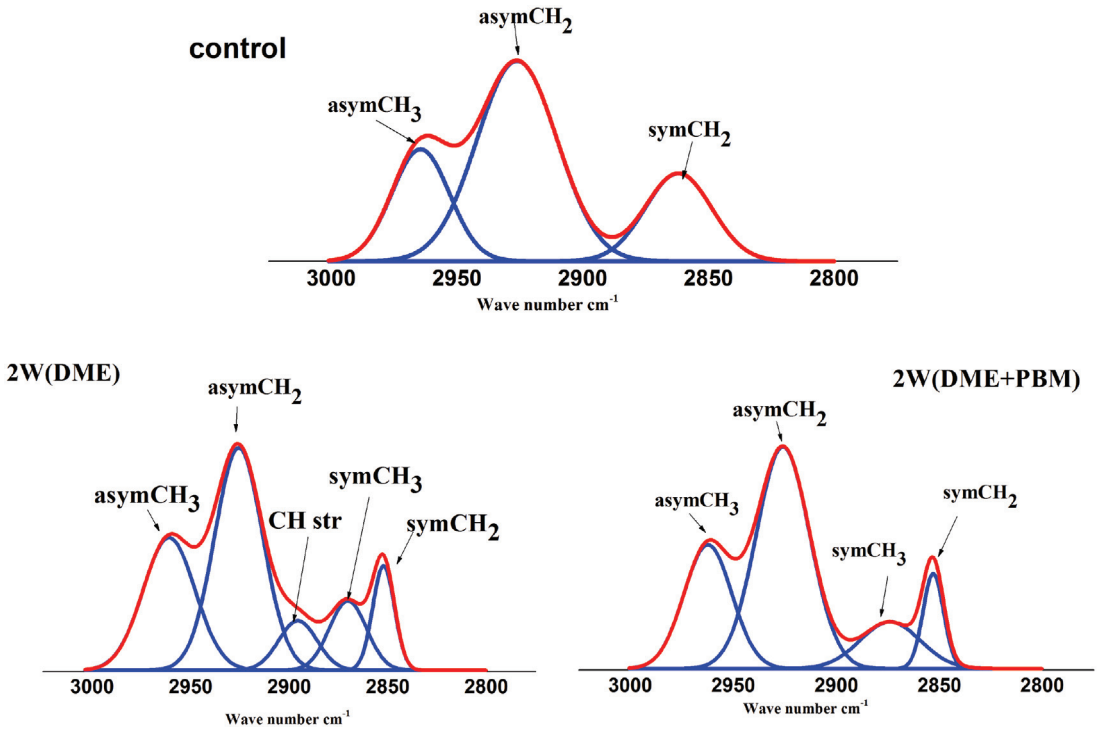
In PBM group, the bands of $_{str}$O–H at 3455±9/cm and $_{str}$O–H$_{sym}$ were obviously improved in their vibrational frequencies and bandwidths. Moreover, the bandwidth of the first $_{str}$O–H was significantly decrease (P<0.05) and the band corresponding to the C–H$_{ring}$ band was missing.

CH stretching region

Figure 2 showed the CH stretching region at frequency range of 3000–2800/cm for the retina of the control, non-PBM-treated diabetic, and diabetic group treated with PBM after 2 weeks. The profile of the control group revealed the presence of three peaks centered at 2963±2, 2926±3, and 2860±2/cm corresponding to CH_3 asymmetric, CH_2 asymmetric, and CH_2 symmetric vibration modes (Table 2), respectively [11].

In DME group, the bands corresponding to CH_3 asymmetric, CH_2 asymmetric, and CH_2 symmetric showed nonsignificant changes in their wave numbers and bandwidth except the bandwidth of CH_2 symmetric, which showed significant decrease (P<0.05). Moreover, two new bands at 2895±1 and 2870±1/cm corresponding to CH_{stre} and $_{sym}CH_3$ appeared, indicating an environmental change. The PBM group showed significant decrease in the bandwidth of CH_2 symmetric (P<0.05) and appearance of new band at 2874±1/cm corresponding to CH_3 symmetric vibration.

Figure 2



FTIR of the retina for control, DME, and DME+PBM in the CH stretching region (3000–2800/cm) after 2weeks. DME, diabetic macular edema; FTIR, Fourier transform infrared spectroscopy; PBM, photobiomodulation.

Table 2 Wave numbers and bandwidths of CH stretching region for control, diabetic macular edema, and PBM-treated diabetic macular edema groups after 2 weeks

Components	Control	2-W DME	2-W DME+PBM
CH_3 asymmetric	2963±2	2961±2	2962±3
	25±2	31±5	27±1
CH_2 asymmetric	2926±3	2925±1	2925±1
	36±1	28±1	30±5
CH_{stre}	–	2895±1	–
	–	22±7	–
CH_3 symmetric	–	2870±1	2874±1
	–	22±3	34±3
CH_2 symmetric	2860±2	2852±2	2853±1
	35±4	12±3*	11±5*

The first line in each cell indicates the vibrational frequency, whereas the second line reflects the bandwidth. DME, diabetic macular edema; PBM, photobiomodulation; w, weeks. *Statistically significant.

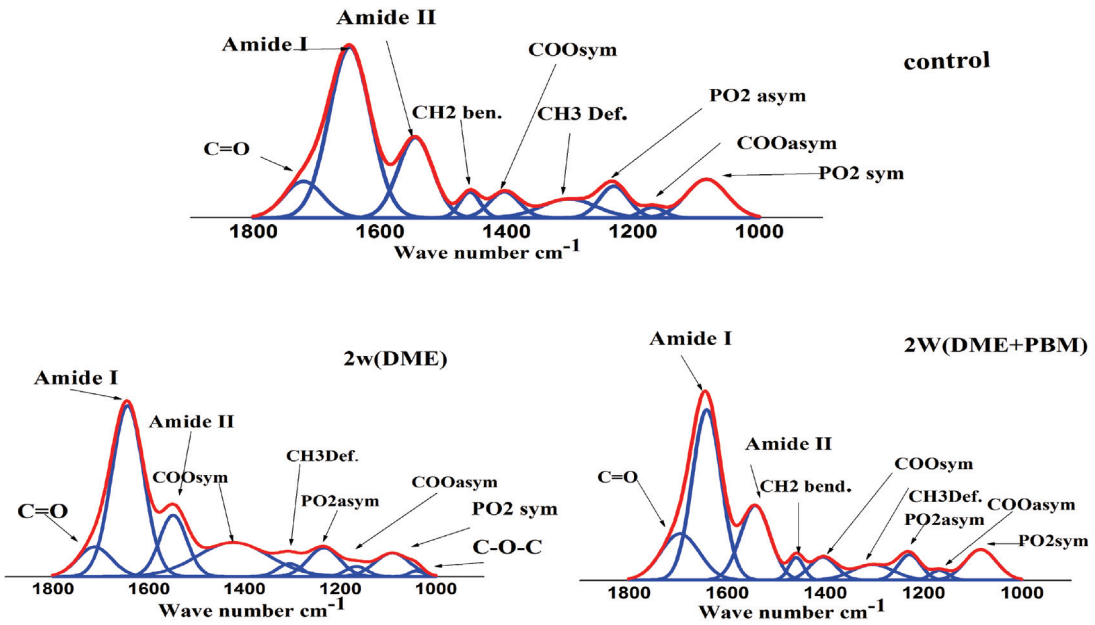
Fingerprint region

Figure 3 showed the fingerprint region (1800–1000/cm) for the control, non-PBM-treated diabetic, and diabetic group treated for 2 weeks by PBM therapy. The control group revealed the presence of 9 bands: (i) C=O stretching vibrations mode of carboxylic acid of the amino acid at 1720±1/cm, (ii) amide I at 1648±1/cm, (iii) the amide II (NH bending and CH stretching) at 1544±5/cm, (iv) CH_2 bending at 1458±5/cm, (v) COO_{sym} at 1403±5/cm, (vi) CH_3 deformation at 1301±2/cm, (vii) $_{str}PO_{2asym}$ at 1230±5/cm, (viii) COO_{asym} at 1230±1, and (ix) $_{sym}PO_2$ at 1083±1/cm [7,12].

As shown in Table 3, in the non-PBM-treated DME group, the amid I and amide II located at 1648±1 and 1544±5/cm, respectively, were shifted to 1644±1 and to 1549±1/cm, respectively, and their bandwidths could be considered with no changes. Although the control and diabetic groups were characterized by the same number of peaks (nine peaks), the curve enhancement procedure used to resolve the overlapped peaks indicated disappearance of CH_2 bending mode and formation of additional new band at 1041/cm, which may be assigned to C–O–C group. The band corresponding to COO_{sym} at 1403±5/cm was characterized by increased band position and bandwidths. CH_3 deformation at 1301±2/cm and $_{str}PO_{2asym}$ at 1230±5/cm were significantly changed in their bandwidths ($P<0.05$ and <0.01, respectively), whereas the band corresponding to COO_{asym} showed nonsignificant change in its bandwidth ($P>0.05$).

After treatment of the retinal macular edema for two weeks with PBM therapy, the absorption band that ascribed to C=O stretching vibration mode of carboxylic acid of the amino acid and the amide I was significantly shifted to lower wave number ($P<0.05$). The amide II, CH_2 bending, COO_{sym}, CH_3 deformation, and $_{Str}PO_{2asym}$ have nonsignificant change in their vibrational frequencies and in their bandwidths.

Figure 3



FTIR of rat's retina for control (nine peaks), DME, and DME after 2 weeks of PBM therapy, in the fingerprint region (1800–1000/cm). DME, diabetic macular edema; FTIR, Fourier transform infrared spectroscopy.

Table 3 Wave number and bandwidth of fingerprint region for control, DME, and DME group exposed to photobiomodulation therapy after 2 weeks

Peaks	Control	2-W DME	2-W DME+PBM
Ester	1720±1	1713±2	1696±2*
C=O	73±1	82±2*	91±2*
Amide I	1648±1	1644±1	1641±1*
	76±0.5	78±1	69±1*
Amide II	1544±5	1549±1	1543±3
	65±0.5	66±5	70±5
CH_2 bending	1458±5	–	1459±1
	37±1	–	35±1
COO_{sym}	1403±5	1423±5*	1405±5
	54±1	176±3*	62±2
CH_3 deform	1301±2	1306±2	1304±2
	101±5	58±3*	98±7
$_{Str}PO_{2asym}$	1230±5	1234±1	1229±5
	53±1	76±4*	53±2
COO_{asym}	1168±1	1166±2	1168±1
	42±2	52±4	43±2
$_{sym}PO_2$	1083±1	1091±1*	1084±1
	75±5	84±4*	75±0.5
C–O–C	–	1041±1	–
	–	33±4	–

First line in each cell indicates the vibrational frequency, while second line reflects the bandwidth. DME, diabetic macular edema; PBM, photobiomodulation; w, weeks. *Statistically significant.

Amide I region

Figure 4 showed the analysis of the amide I band after 2 weeks by using the curve enhancement procedure. The data revealed that the control amide I band can be resolved into five structural components: (i) two bands located at 1979±3 and 1666±3/cm for β-turn, (ii) one band located at 1651±1/cm for α-helix, and (iii) two bands located at 1637±3 and1623±3/cm for β-sheet, respectively [8,13,14].

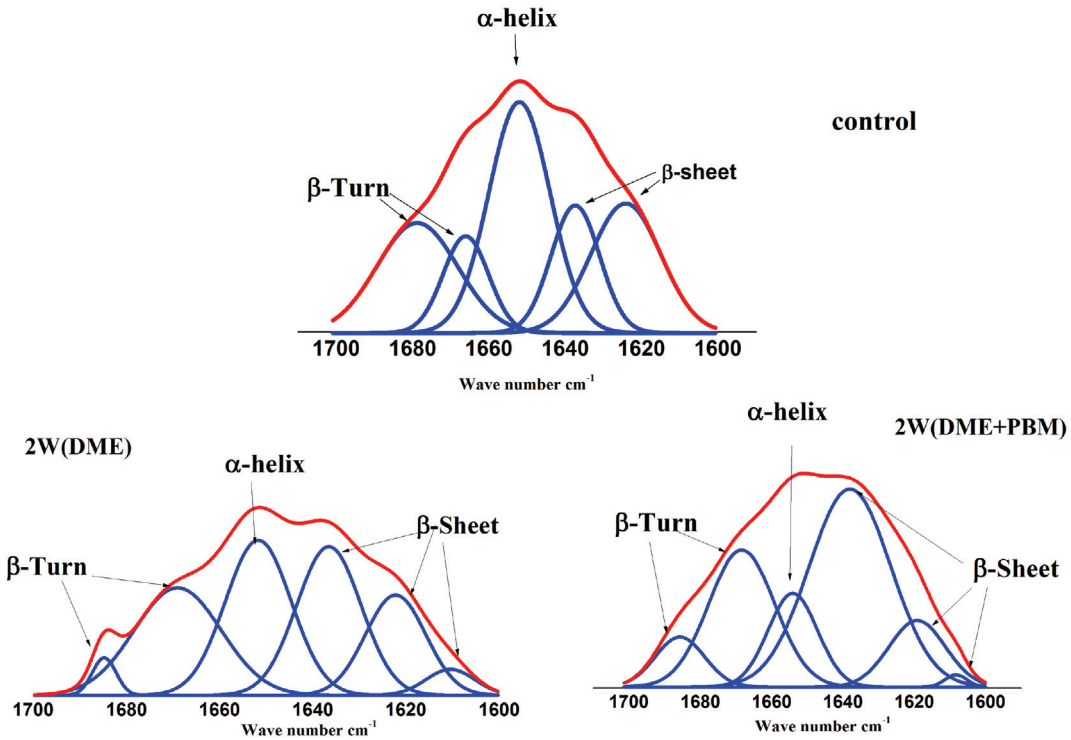
Analysis of amide I bands revealed the following: (i) the β-turn (first peak component at 1679/cm) showed significant decrease in their bandwidths in both non-PBM-treated DME and PBM-treated DME groups ($P<0.01$), (ii) the second peak corresponding to β-turn showed significant change in bandwidths of both non-PBM-treated DME and PBM-treated DME group ($P<0.05$), (iii) the band corresponding α-helix showed nonsignificant change ($P>0.05$), (iv) the band width of the first peak of β-sheet was increased in diabetic group and improved after PBM therapy, and (v) finally, one additional band corresponding to β-sheet appeared in both non-PBM-treated diabetic group and PBM-treated diabetic group at 1608 and 1610/cm, respectively.

Discussion

FTIR is appropriate as an investigation to detect the different secondary structures of proteins and polypeptides [12].

In the present study, all the NH–OH region components changed in DME, as shown in $_{str}$O–H ($P<0.05$), $_{str}$O–H_{sym} ($P<0.01$), and C–H_{ring} ($P<0.01$), along with the formation of a new band corresponding to $_{str}NH_{asym}$. After treatment with PBM, the bands of $_{str}$O–H and $_{str}$O–H_{sym} were obviously improved in their vibrational frequencies

Figure 4



FTIR of rat's retina in the amide I (1700–1600 cm) region for control, DME and DME group exposed to photobiomodulation therapy after 2 weeks. DME, diabetic macular edema.

and bandwidths and the band corresponding to the C–H_{ring} band was missed. In the NH–OH region, the NH bond exists in several membrane constituents that contain protein and lipid. Therefore, low-level laser exposure may lead to changes in membrane structure of retinal cells. Moreover, the change in the vibrational frequencies of $_{str}$O–H and $_{str}$O–H_{sym} indicated that the hydrogen bond has been destructed and/or weakened [15,16].

In non-PBM-treated diabetic group, the bandwidth of CH_2 symmetric at 2860/cm was decreased ($P<0.05$) in addition to the formation of two new bands at 2895±1 and 2870±1/cm corresponding to CH_{str} and $_{sym}CH_3$, respectively, indicating an environmental change leading to disorder in the lipid hydrocarbon chains. On the contrary, the appearance of new band at 2874 ±1/cm corresponding to CH_3 symmetric vibration in the PBM-treated group, indicating change in end methyl group of the hydrocarbon chain, may be due to laser exposure.

By analysis of the fingerprint region (Table 3), it is found that the DME induced obvious changes in the vibrational frequencies and bandwidths of some estimated components. The shift of the C=O to 1713±2/cm, and increase in its bandwidth may be connected with destruction of old H-bond and formation of new one [10]. Additionally, disappearance of CH_2 bending mode can be attributed to the retina cell membrane disorder, and the formation of additional new band at 1041/cm may be assigned to C–O–C group [16]. The change in bands corresponding to COO_{sym}, CH_3 deformation, and C=C stretching mode could be attributed to modification and redistribution of the intensities of different components after establishment of macular edema. The change occurring in the symmetric stretching vibration of $_{sym}PO_2$ at 1083/cm was characterized by increased band position, and the bandwidth seems to be connected with spatial changes in the position of the phosphate groups in the protein helix [10]. The treatment of DME with PBM therapy induced improvement in most bands of the fingerprint region except the absorption band of 1720/cm which ascribed to C=O stretching vibrations mode of carboxylic acid of the amino acid, and the amide I appeared at 1648/cm. The remained structural changes of the C=O and amide I after PBM therapy may be related to a tendency to remain after treatment, so they have irreversible nature or more sessions of low-level therapy (PBM) are required.The secondary structure of the proteins depended on the amide I mode in the 1800–1600/cm spectral region. The results presented in Fig. 4 and Table 4 showed that the protein secondary structure was affected in both

Table 4 Wave numbers and bandwidth of amide I (1700–1600/cm) region for control, DME, and DME group exposed to photobiomodulation therapy after 2 weeks

Peaks	Control	2-W DME	2-W DME+PBM
β-Turn	1679±3	1685±3	1684±1
	24±4	16±2*	6±1*
β-Turn	1666±3	1668±4	1668±3
	14±4	21±3*	21±5*
Helix-α	1651±1	1654±2	1651±3
	19±11	16±9	16±6
β-Sheet	1637±3	1638±2	1636±2
	14±5	27±8*	16±12
β-Sheet	1623±3	1619±6	1622±4
	20±4	18±9	15±4
β-Sheet	–	1608±1	1610±9
	–	7±2	12±7

The first line in each cell indicates the vibrational frequency, whereas the second line reflects the bandwidth. DME, diabetic macular edema; PBM, photobiomodulation; w, weeks. *Statistically significant.

DME and after PBM therapy where there are changes in the β-sheet and β-turn bandwidths. Treatment with PBM therapy significantly improved the first peak of β-sheet, with formation of new bands corresponding to β-sheet appeared in both non-PBM-treated diabetic group and PBM-treated diabetic group at 1610 and 1608/cm, respectively. Furthermore, the increase in β-sheet structure leads to the splitting or formation of new band of β-sheet in the DME, increasing the disorder of the helical structure, and development of disordered chains aggregates [17]. The formation of this β-sheet structure remains irreversible even after treatment with PBM, and all bandwidths showed significant changes assumed to be due to vibrational motion.

Conclusion

The results obtained in this work indicate that DME induced changes in the NH–OH and the CH stretching region components even after treatment with PBM. Therefore, low-level laser exposure may lead to changes in membrane structure of retinal cells and destructed and/or weakened hydrogen bond. DME induced obvious changes in the vibrational frequencies and bandwidths in the fingerprint region. Additionally, retina cell membrane disorder presents modification and redistribution of the intensities of different components after establishment of macular edema and spatial changes in the position of the phosphate groups in the protein helix. PBM therapy induced improvement in most bands of the fingerprint region, except C=O stretching vibrations and the amide I. The last finding in this study concluded that DME induced progressive changes on the secondary structure of proteins in the amide I spectral region where there were changes in the β-sheet and β-turn. Treatment with PBM significantly improved most of the amide I components except the first beak of β-turn and new bands corresponding to β-sheet. Alternatively, it is not clear if they have irreversible nature or more PBM sessions are needed.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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