

Research Article

Ameliorative Effect of Grape Seed Proanthocyanidin Extract on Argon-Laser Induced Retinal Damage

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Abstract. Photocoagulation is routinely used as a major treatment method for many retinal disorders. The present study was conducted to investigate the protective effect of Grape seed extract (GSE) on reactive oxygen species produced during argon laser photocoagulation and also to explore the benefit of retinal photocoagulation over two session's argon laser compared to single session. Forty two pigmented rabbits weighted 2–2.5 Kg were used in this study. The animals were classified into three groups. The left eye for each animal was photocoagulated with 200 mW, 400 mW and 400 mw fractionated dose (FD) argon laser with and without GSE supplementation respectively. After 24 hours the retina was separated carefully, malondialdehyde level (MDA), total antioxidant capacity (TAC) and Fourier transform infrared spectroscopy (FT-IR) for retinal tissue were detected. FT-IR findings showed a positive result for GSE supplementation in reducing laser effects on the retinal tissue. Also, GSE supplementation improved the level of TAC in rabbit's retina exposed to argon laser with a concurrent decrease in MDA level. In conclusion, GSE has an extremely beneficial role in overcoming the resultant adverse biological effects of argon laser photocoagulation on retinal tissues due to its potent antioxidant properties. Results also revealed that retinal photocoagulation over two sessions was more protective for retinal tissue than single session argon laser.

Keywords: Grape Seed Extract, Proanthocyanidin, Photocoagulation, Argon-Laser, Antioxidant, Reactive Oxygen Species.

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1. Introduction

Photocoagulation is used as a major treatment method for many retinal disorders, but it causes visible laser scars that can enlarge postoperatively and leads to many complications such as choroidal neovascularization and subretinal fibrosis Birnbach et al., 1994 [4]. Laser-induced injuries mainly include necrosis in the area caused by laser irradiation and apoptosis in the adjacent area due to a thermal effect. Many visual problems appear because injuries can occur in the adjacent normal tissue, such as retinal scarring, visual field defects and night vision loss Rui et al., 2014 [18]. Many researches are focused on reducing the absorbed laser energy such that it can be specifically confined to the retinal pigment epithelium and thus collateral retinal damage reduced or avoided Luttrull and Dorin 2012 [13].



Grape seed proanthocyanidins extract (GSPE) has been well-validated for its effect as an antioxidant, and it has been used as a therapy in substantial diseases of different organs and tissues. Its activity is mediated by considerable array of functional ingredients with a phenolic nature such as monomeric flavanols; dimeric-, trimeric- and polymeric procyanidins; and phenolic acids Cheah et al., 2009 [6]. These compounds are characterized by their capacity to scavenge singlet oxygen and peroxy radicals via the reduction of multiple O–H bonds Duthie and Morrice, 2012 [9]. The capability of phenolic compounds as free radical scavengers was verified to highly exceed those of vitamins E and C (the major recognized antioxidants in biological systems) El-Ashmawy et al., 2006 [10].

For our best knowledge, information regarding the protective effect of GSPE on retinal tissue during argon laser photocoagulation is rare. Therefore, the present study was aimed to evaluate the protective role of GSPE on reactive oxygen species (ROS) produced due to argon laser photocoagulation. Retinal photocoagulation with argon laser over two sessions was also tested.

2. Material and Methods

2.1. Animals

Forty two healthy mature Chinchilla rabbits of both sexes, weighing 2–2.5 kg were randomly selected from the animal house at the Research Institute of Ophthalmology, Giza, Egypt. All animals were housed in specially designed cages with good ventilation, illumination, and in a central temperature of 20–25°C, they were fed adequate standard diet. The animals were handled according to the Association for Research in Vision and Ophthalmology (ARVO) statements for the use of animals in research. They were classified into three groups I, II, and III as follows:

Group I: consists of 14 rabbits subdivided into two subgroups (7 rabbits each). First sub-group was supplemented with 10 mg/Kg body weight of GSPE daily for one week then the left eye's retina was photocoagulated with 200 mW argon laser. GSPE supplementation was continued till decapitation. The left eyes from the second subgroup was photocoagulated with 200 mW argon laser without GSPE supplementation. The right eyes from the two subgroups were kept as control. All rabbits were decapitated after 24 hours of laser exposure.

Group II: consists of 14 rabbits, and were treated as the previous group, but were photocoagulated with 400 mW argon laser.

Group III (fractionated dose–FD): consists of 14 rabbits subdivided into two subgroups (7 rabbits each). One of the two subgroups was supplemented with 10 mg/Kg body weight GSPE. The retinas of left eye from the two subgroups (with or without GSPE supplementation) were photocoagulated with 400 mW argon laser in two weekly settings (200 mW in each setting). GSPE supplementations were continued till decapitation and all the rabbits of the FD group were decapitated after 24 hours from the second setting.

2.2. GSPE Supplementation

The rabbits were daily supplemented with 10 mg/Kg Grape seed Extract {Arab Co. for Pharm. and Medicinal Plants (MEPACO) Egypt}. The supplementation was given orally by stomach

Table 1: Argon laser parameters.

Peak Power [W]	0.2 W	0.4 W
Pulse energy [J]	0.02 J	0.04 J
Wavelength [nm]	514/532 nm	514/532 nm
Delivered Spot size diameter [cm]	0.04 cm	0.04 cm
Treatment Pulse duration [sec]	0.1 s	0.1 s
Intensity [Watts/cm ²]	79.6 W/cm ²	159.23 W/cm ²
Fluence [Joules/cm ²]	7.961 J/cm ²	15.923 J/cm ²

tube 7 days before retinal photocoagulation with Argon laser. The daily GSPE supplementations were continued till decapitation. The rabbit groups exposed to argon laser without GSPE supplementation were daily supplemented orally with saline solution in order to account for the stress caused by the daily injection.

2.3. Argon Laser Photocoagulation

The left eyes, in all groups underwent retinal photocoagulation using NIDEK-OT-45C green laser photocoagulator. The rabbits received Atropine eye drops 15 minutes before the treatment, to dilate the pupil of the eye. The laser used in the present study was a conventional continuous wave (CW) Argon laser with a wavelengths in the green region of the visible spectrum (514/532 nm), with a 400 μ m diameter spot size, and a total beam exposure duration of 100 ms. Argon laser was used at the same power setting used with patients. As shown in (Table 1); the setting was used at the 200 mW, and 400 mW, that produces light laser burns, and burns of commensurate grade. Approximately 50 laser spots were applied randomly to each retina around the optic nerve head, taking care to avoid the macula region and major blood vessels; all spots were applied in the posterior hemisphere of the eye.

After 24 hours of argon laser photocoagulation, rabbits were decapitated; the eyes were enucleated and then opened carefully by corneal section through the ora serrata. The cornea and lens were removed and the retinas were carefully separated. The following measurements were carried out on retinal tissues from both right eyes (control) and left eyes (treated).

2.4. Antioxidants

2.4.1. Lipid Peroxidation (Malondialdehyde)

Quantitative determination of malondialdehyde (MDA) for rabbit's retina was carried out calorimetrically using kit purchased from Biodiagnostic Co., Egypt, according to the method described by Martínez et al., (2002) [15].

Table 2: MDA level for all the studied groups ($\mu\text{M/g}$ wet wt.).

Group	Without GSPE			With GSPE		
	Mean \pm SD	<i>P</i> value	% Change	Mean \pm SD	<i>P</i> value	% Change
Control	13.64 \pm 0.05	—	—	13.64 \pm 0.05	—	—
200 mW	14.85 \pm 0.98	NS	8.87 %	14.08 \pm 0.88	NS	3.23 %
400 mW	19.59 \pm 0.95	VHS	43.62 %	18.28 \pm 0.34	VHS	34.02 %
400 mW (FD)	17.64 \pm 0.37	HS	29.33 %	16.39 \pm 0.61	HS	20.16 %

(NS) $P > 0.05$, (S) $P > 0.005$, (HS) $P > 0.01$, (VHS) $P > 0.001$

2.4.2. Total Antioxidant Capacity (TAC)

Calorimetric method was performed for determination of the TAC of rabbit's retina using kit purchased from Biodiagnostic Co., Egypt, according to the method described by Koracevic et al., (2001).

2.4.3. Fourier Transform Infrared Spectroscopy (FT-IR)

Retinas of each groups were lyophilized, weighed, and then mixed with KBr powder (98 mg KBr: 2 mg retina) for FTIR analysis. FTIR spectra were measured using Nicolet 6700 spectrometer (Thermo Electron, Madison WI, USA), with effective resolution of 2 cm^{-1} . Hundred sample interferograms were recorded for each spectrum. The spectrometer was operated under a continuous dry nitrogen gas purge to remove interference from atmospheric carbon dioxide and water. The data 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 8 software.

3. Results

In the current study, the effect of GSPE as a powerful antioxidants, on argon laser photocoagulation to rabbit retina was estimated. The measurements were carried out on control rabbits, rabbits supplemented with GSPE for one week before argon laser photocoagulation, and rabbits subjected to argon laser photocoagulation without GSPE supplementation.

3.1. Lipid Peroxidation (Malondialdehyde)

MDA level of retina from control rabbit was $13.64 \pm 0.048\ \mu\text{M/L}$ (Table 2). Upon exposure of rabbit's eye to 200 mW and 400 mW argon lasers, the MDA levels increased by increasing laser dose. Supplementation of rabbits with GSPE leads to decrease of the MDA level. When large dose of argon laser (400 mW) was fractionated into two settings (200 mW every week), the MDA level was improved as shown in Figure (1).

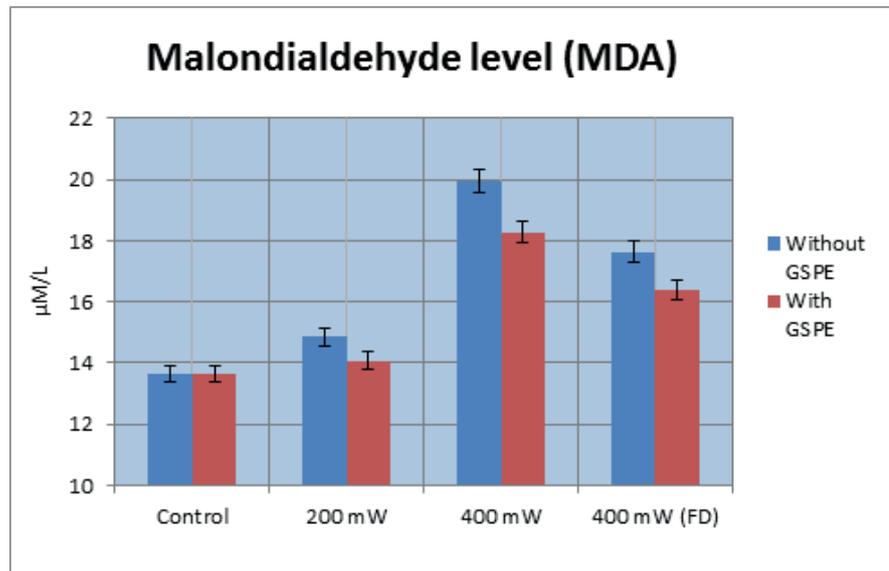


Figure 1: MDA levels for all the studied groups (μM/L).

3.2. Total Antioxidant Capacity (TAC)

TAC summarizes the overall activity of antioxidants and antioxidant enzymes. The TAC of retina from control rabbit was 3.64 ± 0.311 mM/L (Table 3). When rabbits were supplemented with GSPE for one week before photocoagulation of retina with 200 mW and 400 mW argon lasers, the total antioxidant capacity decreased to 2.73 ± 0.382 and 1.99 ± 0.39 mM/L respectively. Supplementation of rabbits with GSPE improved the total antioxidant capacity of retina. When large dose of argon laser (400 mW) was fractionated into two settings (200 mW every week), the total antioxidant capacity improved as compared to 400 mW in one setting as illustrated in Figure (2).

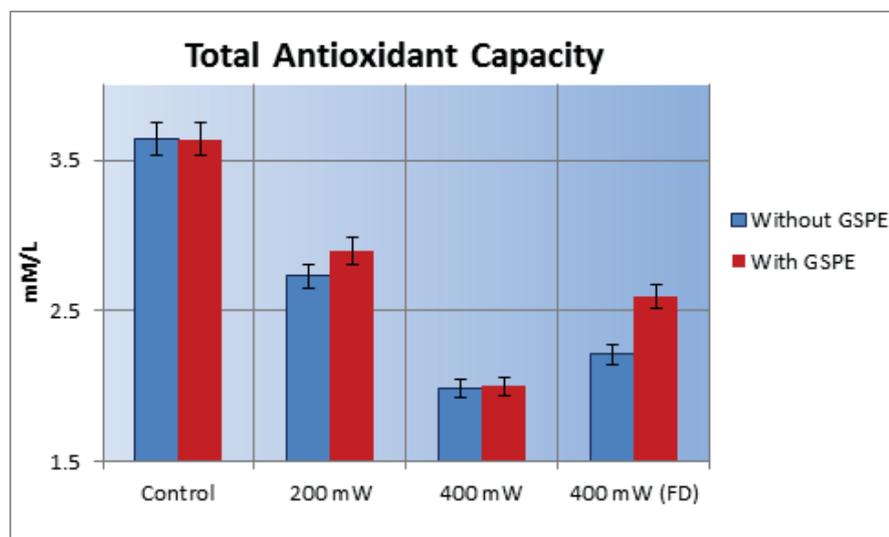


Figure 2: Total antioxidant capacity (TAC) for all the studied groups (mM/L).

Table 3: Total antioxidant capacity (TAC) for all the studied groups (mM/g wet wt.).

Group	Without GSPE			With GSPE		
	Mean \pm SD	<i>P</i> value	%Change	Mean \pm SD	<i>P</i> value	%Change
Control	3.64 \pm 0.31	—	—	3.64 \pm 0.31	—	—
200 mW	2.73 \pm 0.38	NS	-25.00 %	2.96 \pm 0.04	NS	-18.68 %
400 mW	1.99 \pm 0.39	VHS	-45.33 %	2.09 \pm 0.33	VHS	-42.58 %
400 mW(FD)	2.21 \pm 0.21	HS	-39.28 %	2.67 \pm 0.301	HS	-26.64 %

(NS) $P > 0.05$, (S) $P > 0.005$, (HS) $P > 0.01$, (VHS) $P > 0.00$

3.3. FTIR of Retina

The spectral analysis were performed in three distinct frequency ranges namely NH–OH region (3700–3000 cm^{-1}), stretching region (3000–2800 cm^{-1}) and finger print region (1800–900 cm^{-1}) as previously described by Akkas et al., 2007 and Cakmak et al., 2006 [1, 5]. Figure (3) illustrates the NH–OH region (3700–3000 cm^{-1}) for retina from control rabbits, retina photocoagulated with 200 mW and 400 mW argon laser and retina photocoagulated with fractionated dose (FD) argon laser with and without GSPE supplementation respectively. The main band of control rabbit was resolved into four structural components centered at 3505 cm^{-1} ($_{\text{str}}\text{OH}$), 3353 cm^{-1} ($_{\text{str}}\text{NH}_{\text{sym}}$), 3250 cm^{-1} ($_{\text{str}}\text{OH}_{\text{sym}}$) and 3055 cm^{-1} (CH_{ring}) as described by Dovbeshko et al., 2000 [8]. When rabbit's retinas were photocoagulated with argon laser without GSE supplementation, there was change in all band position and $_{\text{str}}\text{OH}$ band was splitted into two bands. Moreover, after photocoagulation with 400 mW, $_{\text{str}}\text{NH}_{\text{sym}}$ band was detected in IR pattern

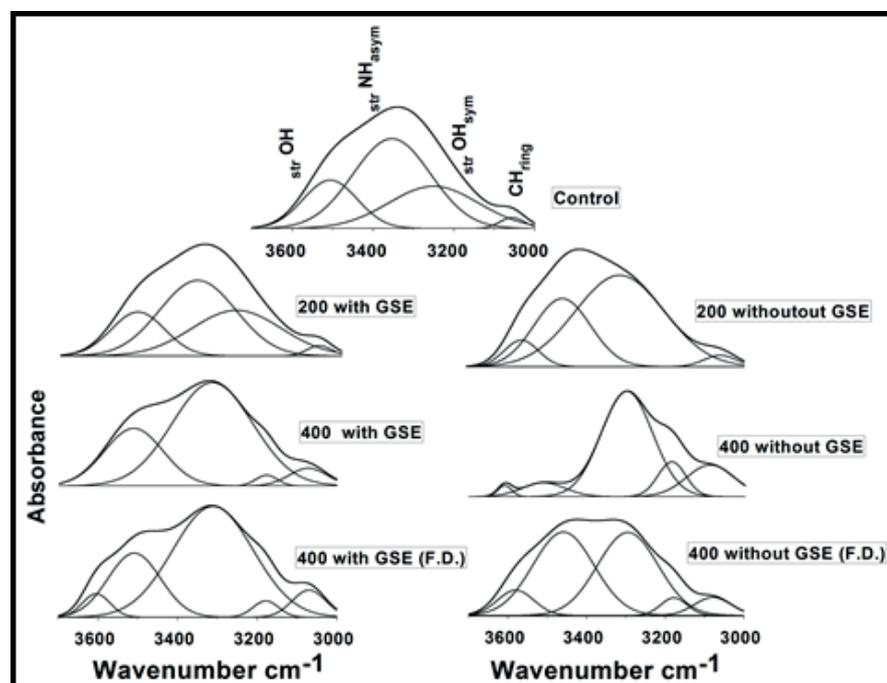
**Figure 3:** NH-OH region (3700–3000 cm^{-1}) for all the studied groups.

Table 4: NH–OH region (3700–3000 cm^{-1}) of photocoagulated retina from for all the studied groups.

Groups	Peaks assignment				
	str^{OH}	$\text{str}^{\text{NH}}_{\text{asym}}$	$\text{str}^{\text{OH}}_{\text{sym}}$	$\text{str}^{\text{NH}}_{\text{sym}}$	CH_{ring}
Control	3505 \pm 3.42	3353 \pm 2.09	3250 \pm 1.84	—	3055 \pm 2.73
	33.75	48.23	55.25	—	15.25
200 mW with GSPE	3505 \pm 0.98	3357 \pm 0.89	3261 \pm 1.25	—	3055 \pm 3.04
	33.25	47.58	55.16	—	15.50
200 mW without GSPE	3560 \pm 2045	3313 \pm 1.02	—	—	3060 \pm 1.62
	3457 \pm 1.47 21.57 33.81	47.43	—	—	17.75
400 mW with GSPE	3512 \pm 3.05	3314 \pm 3.25	—	3177 \pm 1.25	3072 \pm 1.44
	46.04	50.15	—	13.50	25.52
400 mW without GSPE	3608 \pm 3.15	—	3299 \pm 1.92	3186 \pm 1.12	3088 \pm 2.76
	3510 \pm 1.64 10.09 27.29	—	32.25	17.56	30.25
400 mW with GSPE (FD)	3604 \pm 1.65	3311 \pm 1.48	—	3178 \pm 3.28	3069 \pm 1.57
	3508 \pm 1.87 11.61 33.19	50.26	—	16.79	21.09
400 mW without GSPE(FD)	3580 \pm 1.45	—	3295 \pm 2.18	3177 \pm 2.36	3078 \pm 1.98
	3458 \pm 3.65 23.75 38.06	—	39.43	16.75	23.25

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

(Table 4). When rabbits supplemented with GSPE one week before laser photocoagulation with 200 mW, there was no significant change in IR pattern.

The CH stretching region (3000–2800 cm^{-1}) Figure (4) and (Table 5) for retina from control rabbits was resolved into 4 bands at 2961 cm^{-1} , 2924 cm^{-1} , 2882 cm^{-1} , and 2852 cm^{-1} which corresponds to CH_3 asymmetric, CH_2 asymmetric, CH_3 symmetric and CH_2 asymmetric respectively; Severcan et al., (2000) [24]. The main change in CH stretching region was obvious in rabbit's retina photocoagulated with 400 mW argon laser (one setting) where $\text{CH}_{2\text{asym}}$ band was splitted into 2 bands. Moreover, $\text{CH}_{2\text{sym}}$ in retina of rabbit photocoagulated without GSPE supplementation was also splitted into 2 bands. The IR pattern of rabbit's retina supplemented with GSPE then photocoagulation with 400 mW argon laser in two setting (FD) mimiced the control.

The Finger print region Figure (5) of normal rabbit retina was characterized by eight absorption bands centered at 1740 cm^{-1} (Ester), 1657 cm^{-1} (Amide I), 1541 cm^{-1} (Amide II), 1454 cm^{-1} (CH_2 bend), 1396 cm^{-1} ($\text{str}^{\text{Coo}}_{\text{sym}}$), 1316 cm^{-1} ($\text{CH}_{3\text{def}}$), 1234 cm^{-1} ($\text{str}^{\text{PO}}_{2\text{asym}}$) and 1074 cm^{-1} ($\text{str}^{\text{PO}}_{2\text{sym}}$). After photocoagulation with 400 mW argon laser, there was change in position of amide I and amide II bands, $\text{CH}_{3\text{def}}$ band disappeared and $\text{PO}_{2\text{sym}}$ band was splitted

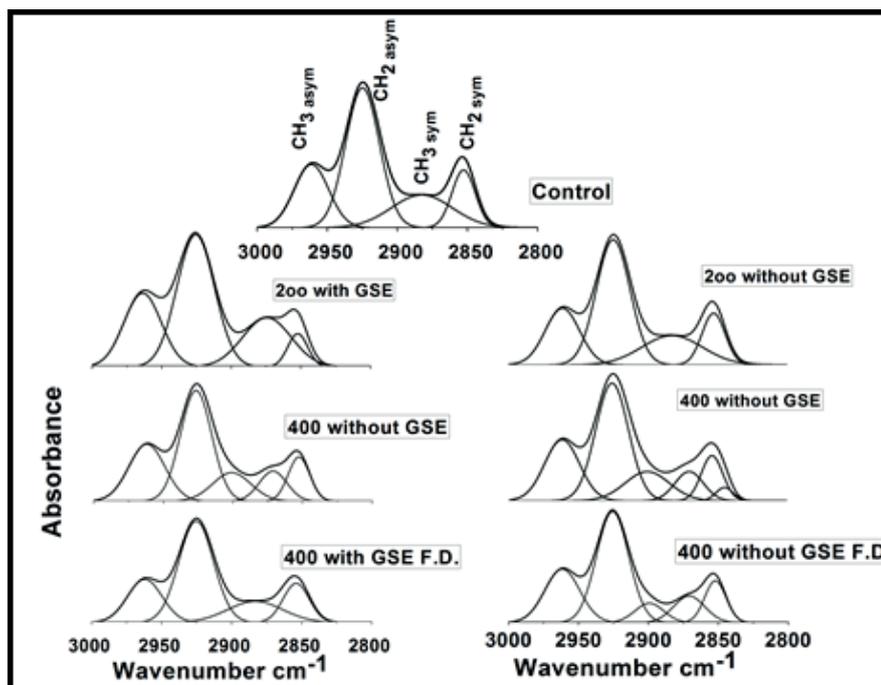


Figure 4: CH stretching region (3000–2800 cm^{-1}) for all the studied groups.

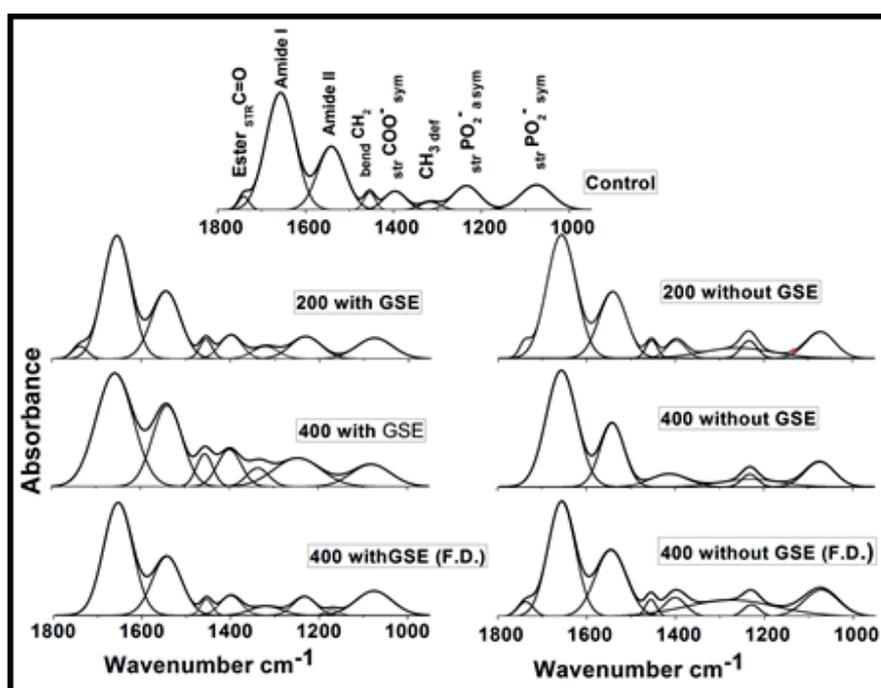


Figure 5: Finger print region (1800–1000 cm^{-1}) for all the studied groups.

into 2 bands. Supplementation of GSPE improved FTIR spectra in different grades. The highly significant improvement appeared in case of photocoagulation of retina with 200 mW argon laser (Table 6).

Table 5: CH stretching region (3000–2800 cm^{-1}) of photocoagulated retina for all the studied groups.

Groups	Peaks assignment			
	$\text{CH}_{3\text{asym}}$	$\text{CH}_{2\text{asym}}$	$\text{CH}_{3\text{sym}}$	$\text{CH}_{2\text{sym}}$
Control	2961 ± 3.64	2924 ± 1.37	2882 ± 3.04	2852 ± 2.18
	24.49	23.99	34.29	16.45
200 mW with GSPE	2961 ± 1.05	2924 ± 2.47	2883 ± 1.28	2852 ± 1.36
	26.66	26.87	34.76	14.22
200 mW without GSPE	2961 ± 3.78	2925 ± 3.18	2874 ± 0.95	2853 ± 2.19
	24.49	23.99	16.45	16.45
400 mW with GSPE	2961 ± 4.04	2926 ± 1.25	2871 ± 1.34	2853 ± 1.27
	25.87	2901 ± 2.68	19.90	15.32
		22.31 27.61		
400 mW without GSPE	2961 ± 1.25	2925 ± 2.41	2870 ± 1.05	2853 ± 1.47
	24.94	2900 ± 3.57	20.16	2844 ± 2.35
		23.44 31.77		15.26 11.96
400 mW with GSPE (FD)	2961 ± 1.92	2925 ± 3.18	2883 ± 2.51	2853 ± 1.66
	24.59	24.67	39.53	18.52
400 mW without GSPE (FD)	2961 ± 2.16	2925 ± 2.54	2870 ± 2.47	2852 ± 2.14
	24.86	2900 ± 1.26	22.16	15.07
		22.87 20.49		

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

4. Discussion

Nowadays, there is considerable interest in the potential health benefits of natural remedies such as medicinal plants and their extracts. One of these natural extracts, is grape seed proanthocyanidin extract (GSPE) which has been reported to possess a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress Bagchi et al. 2000 [2]. Studies on the mechanisms of laser induced-retinal damage showed that the damage was mainly due to thermal effects, which could produce oxidative stress, induce free radical generation and cause the formation of lipid peroxide. Malondialdehyde, which is one of the end-products of polyunsaturated fatty acid peroxidation and is a good indicator of the degree of lipid peroxidation, is believed to originate under stress conditions and has high capability of reaction with multiple biomolecules such as proteins or DNA that leads to the formation of adducts Zarkovic et al., 2013 [23]. The recorded increase in MDA level in retinal tissue after treatment with argon laser indicated that laser cause oxidative stress to retinal tissue and this stress increased by increasing argon laser dose. MDA increase after photocoagulation of rabbit retina is accompanied with a decrease in total antioxidant capacity. TAC determines the capacity of neutralization of free radicals, and it is a sensitive and reproducible marker to detect changes

Table 6: Wavenumber and bandwidth of finger print region for all the studied groups.

Group	Peaks								
	Ester C=O	Amid I str C=O	Amid II N=H band	CH ₂ band	COO _{sym} ⁻	CH ₃ _{def}	str PO ₂ _{sym}	str COO _{sym} ⁻	str PO ₂ _{sym}
Control	1740	1657	1541	1454	1396	1316	1234	—	1074
	23.93	66.08	62.55	25.19	49.34	45.70	60.61	—	72.50
200 mW with GSPE	1737	1657	1541	1454	1398	1321	1233	—	1076
	33.26	62.15	64.52	27.74	49.27	56.88	68.84	—	80.50
200 mW without GSPE	—	1657	1541	1453	1398	—	1258 1233	—	1072
	—	65.97	62.22	25.65	42.79	—	155.06 36.46	—	65.06
400 mW with GSPE	—	1657	1539	1455	1399	1335	1246	—	1080
	—	83.23	67.01	36.86	52.74	49.19	105.64	—	89.00
400 mW without GSPE	—	1655	1541	—	1414	—	1231 1228	—	1073
	—	68.73	54.02	—	81.05	—	34.95 131.50	—	66.02
400 mW with GSPE (FD)	—	1653	1543	1454	1399	1319	1232	1196	1076
	—	65.76	65.55	25.92	48.03	69.25	50.93	30.97	81.85
400 mW without GSPE (FD)	—	1656	1545	1455	—	—	1275 1229	—	1071
	—	61.12	69.66	24.48	—	—	202.93 35.57	—	76.46

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

in oxidative status, which often cannot be determined by measuring the antioxidants separately Saenz-de-Viteri et al., 2014 [19].

The present results revealed that GSPE supplementation improved the level of TAC in rabbit's retina exposed to argon laser with a concurrent decrease in MDA level. So, GSPE is a potent antioxidant and acts as a free radical scavenger and can protect from oxidative stress caused to retinal tissue after photocoagulation with argon laser. Wang et al. 2008 [22] reported that GSPE has the ability to interfere directly with free radicals and restore the balance of oxidants/antioxidants. This may give reason for the observed decrease in MDA and increase in TAC. Additionally, GSPE contains flavonoids, which are involved in elimination of oxidative stress in vitro and in vivo by increasing the endogenous antioxidant condition Martinez-Florez et al. 2002 [15]. The present results also revealed that photocoagulation of retina with high dose (400 mW) in two sessions was more protective for retinal tissue than single session argon laser. Recovery may occur to retinal tissue after photocoagulation with low power argon laser (200 mW) every week.

The FTIR results indicated that photocoagulation of retina with argon laser leads *led* to changes in NH-OH region ($3700\text{--}3000\text{ cm}^{-1}$). The NH bond exists in several membrane constituents that contain protein and lipid. Therefore, photocoagulation with argon laser may lead to changes in membrane structure of retinal cells. When rabbit's retinas were photocoagulated with argon laser of power 200 mW with GSPE supplementation, there were no significant change in IR patterns; this indicated that GSPE supplementation shows some protective effect for the retinal tissue at this region. Kalt et al., 2010 [12] demonstrated that anthocyanins and other flavonoids modulate visual pigment function.

The main change in CH stretching region was obvious in rabbit's retina photocoagulated with 400 mW argon laser. This region is used to estimate lipid level in biological tissues. It gives valuable information about the state order of hydrocarbon tails in lipids because they are good monitors of the changes in acyl chain Toyran et al., 2008 [21]. The split in CH_2 stretching bands after exposure of rabbit retina to high power of argon laser revealed that laser may enhance lipid peroxidation process. This peroxidation was detected by the present increase in MDA (Table 2) which is a by-product of lipid peroxidation. After supplementation with GSPE, some sort of repair was recorded. GSPE extract has a positive influence on the permeability and tendency to hemorrhage of retinal vessels Thomas, 1999 [20]. Flavonoids have multiple properties that are potentially of benefit for the prevention and treatment of ocular diseases, particularly those that involve the loss of nerve cells Maher and Hanneken, 2005 [14]. The obtained results agreed with Baudouin et al., 1999 [3], and Fernandez-Robredo et al., 2005 [11] results, who showed that antioxidant treatment could improve the progression of some retinal disorder.

Changes in band position and band width in the fingerprint region ($1800\text{--}1000\text{ cm}^{-1}$) of retina were recorded after photocoagulation with argon laser. The peaks observed at 1657 cm^{-1} and at 1543 cm^{-1} correspond to amide I and amide II vibration of structural proteins respectively. The amide I absorption is mainly associated with the $\text{C}=\text{O}$ stretching vibration of the protein amide. The amide II absorption arises from amide N-H bending vibration coupled to C-N stretching vibration mode of the protein backbone Dogan et al., 2007 [7]. The change in peak position of amide bands after argon laser photocoagulation may reflect alterations in the composition of protein secondary structure. The vibrational motion around the ester $\text{C}=\text{O}$ (due to lipid) was detected in control and after photocoagulation of rabbits retina with 200 mW and GSPE supplementation only and were not detected in other studied groups. This may reflect the changes in lipids of rabbit retina. In non-supplemented GSPE groups, $\text{CH}_{3\text{def}}$ band was disappeared and $\text{PO}_{2\text{sym}}$ band was splitted into two bands. The band at 1076 cm^{-1} represents PO_2 symmetric stretching modes of phosphodiester group in nucleic acids Cakmak et al., 2006 [5]. Supplementation of GSPE improved FTIR spectra at different grades. The highly significant improvement appeared in case of photocoagulation of retina with 200 mW argon laser. GSPE has received much attention due to its numerous biological activities and antioxidant effects Bagchi, et al., 2000 [2]. This protective flavonoid has the ability to function within cell membranes. Proanthocyanins are useful to support vision health and regenerate reduced glutathione from oxidized glutathione and treat macular degeneration and diabetic retinopathy Pileggi, 2004 [16]. Also, the obtained results agreed with Robredo et al., 2005 [17] who showed that antioxidant treatment could improve the progression of some retinal disorder.

In conclusion, oxidative damage induced by Argon laser photocoagulation can damage the tissue of retina. Retina is extremely rich in polyunsaturated lipids; this makes it particularly

sensitive to reactive oxygen species (ROS). Grape seed extract GSPE supplementation improved the level of TAC in rabbit retina exposed to argon laser in concurrent with a decrease in MDA level and improved FTIR spectra. So, GSPE is a potent antioxidants and acts as a free radical scavenger and can improve the progression of some retinal disorder. Retinal photocoagulation over two sessions was more protective for retinal tissue than single session argon laser.

Competing Interests

The authors declare no competing interests.

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