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Helium-Neon Laser Effects on Human Whole Blood by Spectroscopy *In vitro* Study

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Low-power helium-neon laser recently has been used numerously in medical applications. FTIR and UV-Vis spectroscopic technique is employed to study the spectral differences in the serum of whole blood samples.
Aims: To study (He-Ne) laser (λ= 632 nm, power=2 mW) effect on human whole blood, after irradiated to different times from 10 min to 50 min.
Study Design: Human Whole Blood Irradiated to (He-Ne) laser (λ= 632 nm, power=2 mW).
Place and Duration of Study: Institute of Laser, Sudan University of science and technology (SUST), Soba Hospital, Khartoum- Sudan, February 2018.
Methodology: Blood samples were collected from healthy volunteers; blood sample exposed to (H-N) laser and control compared; UV-Vis spectrophotometer and FTIR were used to study the effect of laser radiation.
Results: Absorption spectrum and FTIR spectra of whole blood are compared before and after He-

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Ne laser radiation shows, a significant decrease in intensity. FTIR spectrum of non exposed blood showed the peaks due to O-H (free group), C=O (amide I group), N=O (nitro group), and C-H (aromatic group). N-H (Amino acid (amide II) Laser radiation changes in transmittance in FTIR spectra for C=O group and O-H, N=O, the percentage of transmittance were increased. The most effects are found when whole blood irradiated to He-Ne laser radiation for 10 and 20 min and transmittance decreases for C-H, and N-H, due to denaturation of the protein.

Conclusion: Photodegradation of blood components due to absorption of laser radiation causes changes in the structure and conformational changes in the polypeptide and decrease intensity.

Keywords: Laser; blood; UV-Vis; FTIR; spectroscopic.

1. INTRODUCTION

Low-intensity helium-neon laser has been used extensively in medical applications. Interaction of lasers with biological materials such as blood, skin, and tissues is important to be understood. The study of blood change by spectroscopic techniques can be used for understanding the biological nature of the disease, and also for the diagnosis of the disease [1,2].

Photobiomodulations involves exposing tissues to low-level light. This type of therapy called Low-level laser therapy (LLLT), also known as cold laser therapy as the power densities used produces no heating effect on the tissues. LLLT has a photochemical effect which means the light is absorbed and cause a chemical change [3,4,5].

FTIR and UV-Vis spectroscopic technique are employed to study the spectral differences in the serum of normal blood samples [2], blood samples were irradiated in that study by He-Ne laser (Wavelength λ = 632.8 nm, Power = 3 mW). The FTIR spectra for irradiated blood samples showed significant changes [1]. He Ne laser (λ= 632 nm, power=2 mW) was used to irradiate human red blood cells and investigated by absorption spectrum, FTIR and fluorescence spectra of RBC. The absorption spectrum of RBC after exposure to He-Ne laser shows a significant decrease in absorbance. The FTIR spectrum of irradiated RBC clearly showed changes in transmittance [6]. Some rheological factors of the human blood, such as complete blood count (CBC) parameters and blood sedimentation rate (BSR) affected by low-level laser radiation (LLLR) laser blood biostimulation investigated the effect of LLLT on rheological parameters of human blood, they noticed a change in both viscosity and size of erythrocytes [7,8]. Human blood exposed to low-intensity He–Ne-laser radiation causes clearly defined changes in the IR and visible absorption spectra of the blood and erythrocytes. These spectral changes arise as a result of partial photodissociation of haemoglobin–ligand [9].

This paper investigates the effect of He-Ne laser (Wavelength λ = 632.8 nm, Power = 2mW) with different exposure times using UV-Vis spectrophotometer and FTIR spectrometer.

2. MATERIALS AND METHODS

2.1 Samples Collection

Blood samples were taken from healthy volunteers; 3 ml of each volunteer by medical standard laboratory conditions and blood samples were saved in a tube to prevent from coagulation to (EDTA) and each sample was divided into two samples one sample was control and other exposed to the helium-neon laser with different exposure times.

2.2 Laser Irradiated

Samples were exposed to a Helium-Neon laser beam, operating in continuous wave mode, as a radiation source (632.8 nm, 2 mW), for (10, 20, 30, 40 and 50) minutes The distance between the laser source and the samples was set to be 10 cm and the diameter of a laser spot was chosen to be 1.5 cm. To studied the effect of laser radiation were used UV-Vis spectrophotometer (Jasco-670) and Fourier Transform Infra Red Spectra (FTIR) were obtained used FTIR spectrophotometer (Shimadzu) for control, and He-Ne laser irradiated blood serum samples.

FTIR spectra of sera samples were recorded in the frequency range 4000 – 450 cm-1 on using Shimadzu at Central Laboratory University of Khartoum. IR transparent Thallium Bromide material without the serum was scanned as the background for each spectrum and 16 scans were co-added at a spectral resolution of 1 cm-1.

FTIR spectra were obtained by spreading a small volume of serum on a Thallium Bromide plate (IR transparent material) and allowed to dry for few minutes to remove the water bands. To minimize problems from avoidable baseline shifts, the spectra were baseline corrected and normalized.

UV -Vis spectra. Blood were diluted with normal saline and placed in Kartell disposable polystyrene cuvette of 10 mm path length. The cuvette is placed in Jasco-670) UV -Vis spectrophotometer for analysis the spectra were scanned in the region between 300 nm to 800 nm using Jasco-670) at Laser institute laboratory, SUST, Khartoum.

3. RESULTS AND DISCUSSION

3.1 UV-Vis Spectra

Fig. 1 shows the spectrum of non- irradiated blood sample (control). This spectrum referred to non- irradiated blood sample which specified by peaks at (576.0, 542.0, 416.0 and 340.0) nm with intensities 0.793, 0.755, 2.604 and 1.253 respectively.

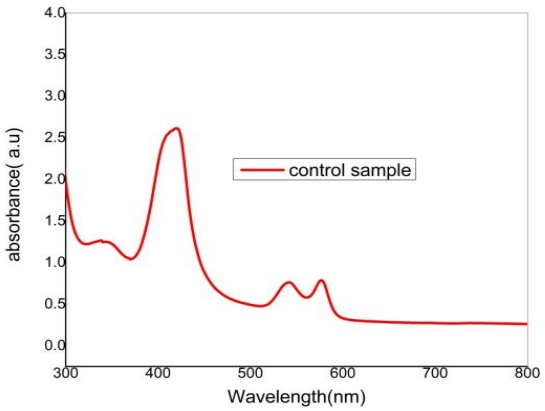

Fig. 1. Spectrum of non- irradiated blood sample (control)

The absorption spectra of the whole blood recorded in the range of 300–800 nm Fig. 2. Contain absorption bands with λ_{max}= 340, 416 nm, a doublet band with λ_{max} = 542 and 576 nm. We investigated only those changes in the absorption spectra of the whole blood exposed to the (He-Ne laser) radiation that was detected for all of the samples studied.

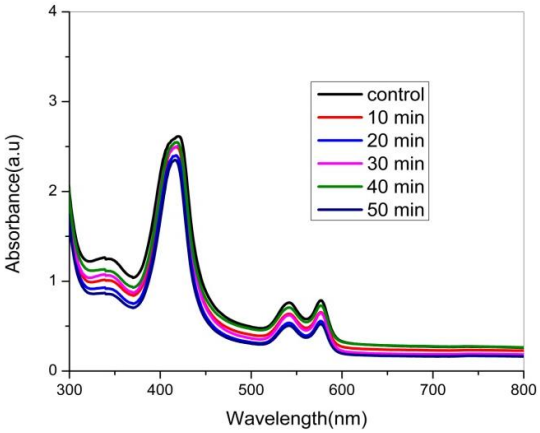

Fig. 2. Relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 2 mW

Different serum samples are analyzed quantitatively by calculating the intensities among the absorption peaks which is show decrease intensity, all irradiated serum sample less than control serum sample. These results indicate to that there is photodegradation happened to the blood components. Laser radiation interacts with blood at the molecular level. Hemoglobin is a blood photoreceptor that selectively absorbed.

Absorption intensity slightly decreases for all peaks at, due to increasing ligand electronegativity [9].

In the UV-visible absorption spectrum of the irradiated blood, (Fig. 2 and Table 1) the most intense absorption band at 416 nm, the light with this wavelength that strikes these biological tissues will be highly absorbed. This phenomenon is the key for the desired effect on the tissues [10].

Table 1. The intensity of normal and irradiated samples

Wavelength (nm)	Absorbance (a.u.)					
	Control	10 min	20 min	30 min	40 min	50 min
340	1.253	1.01	0.933	1.065	1.12	0.868
416	2.604	2.49	2.391	2.501	2.538	2.347
542	0.755	0.633	0.536	0.614	0.699	0.492
576	0.793	0.653	0.547	0.633	0.718	0.525

Fig. 2 compared the light absorption at 340 nm, 414 nm, 542 nm and 576 nm for different irradiation time. The minimum light absorption occurred at 50 minutes of irradiation with the fewer intensities recorded. The concentration of absorbing centers is decreasing. This fluctuation of light absorption is known as biphasic response. The mechanism of LLLT at cellular level has been associated with the absorption of monochromatic visible and near infrared radiation. Effective tissue penetration is maximized at specific optical window [11].

3.2 FTIR Spectra

An FTIR spectrum of whole blood in vitro without laser radiation is shown in Fig. 3. Table 2 shows the groups OH, C=O, N=O, C-O and C-H in the

Table 2. FTIR spectral data (wave number, function group and transmission) for normal blood control

FTIR spectral data for normal blood (control)			
Sr. no.	**Wave number 1/cm**	**Group**	**% T**
1	3444.63	O-H	0.48
2	1650.95	C=O	1.19
3	1548.73	N=O	6.36
4	1452.30	C-H	14.26
5	1317.29	N-H	15.3
6	1168.78	C-O	17.12

Table 3. FTIR spectral data (wave number, function group and transmission) for irradiated blood sample blood control

FTIR spectrum of blood irradiated with he-ne laser for duration 10, 20, 30,40 and 50 min				
Sr. no	**Irradiated time (minute)**	**Wave number 1/CM**	**Group**	**T%**
1	10	3396.77	O-H	0.77
2		1650.96	C=O	1.78
3		1545.10	N=O	4.49
4		1450.73	C-H	15.20
5		1312.59	N-H	16.12
6		1161.74	C-O	18.70
7	20	3442.45	O-H	0.65
8		1651.63	C=O	1.68
9		1545.10	N=O	4.68
10		1451.01	C-H	11.43
11		1312.59	N-H	12.58
12		1161.74	C-O	13.76
13	30	3410.57	O-H	4.92
14		1651.63	C=O	6.50
15		1551.23	N=O	12.82
16		1451.01	C-H	22.14
17		1312.59	C-H / N-H	24.29
18		1167.96	C-O	26.31
19	40	3304.04	O-H	12.12
20		1645.41	C=O	13.21
21		1545.10	N=O	16.46
22		1447.23	C-H	25.43
23		1312.59	N-H	27.11
24		1161.74	C-O	28.50
25	50	3442.45	O-H	2.49
26		1651.63	N-H	6.46
27		1545.10	N=O	12.54
28		1451.01	C-H	22.28
29		1318.81	N-H	23.44
30		1167.96	C-O	25.59

region between the wave number 4000 cm^{-1} to 500 cm^{-1}. The most intense absorption band in proteins is the amide I peak, which is observed at 1650.95 cm^{-1}. Amide I is mainly associated with C=O symmetric stretching and or C-O stretching vibrations. There are another very strong prominent amide absorptions one at 1545 cm^{-1} due to strong N-H in-plane bending and termed as an Amide II band. The strong characteristic band at 3295 cm^{-1} due to N-H symmetric stretching confirmed the existence of amino acid group [2] The medium band at 2873 cm^{-1} due to C-H asymmetric and symmetric stretching of CH3 group established the presence of lipids and the medium bands at 2854 cm^{-1} due to C-H symmetric stretching of CH2 group established the presence of lipids, fatty acids [12,13,14,15]. The FTIR spectra of blood showed clear bands at1080, and 12451 cm^{-1}, are composed of mononuclear cells containing nucleic acids such as DNA and RNA. The nucleic acid components found in WBCs [9]. The bands at 1170 cm^{-1} is associated with triglycerides of human blood. The band at 2936 cm^{-1} is related to platelets due to -C-H symmetric stretching of –CH2 [16,17].

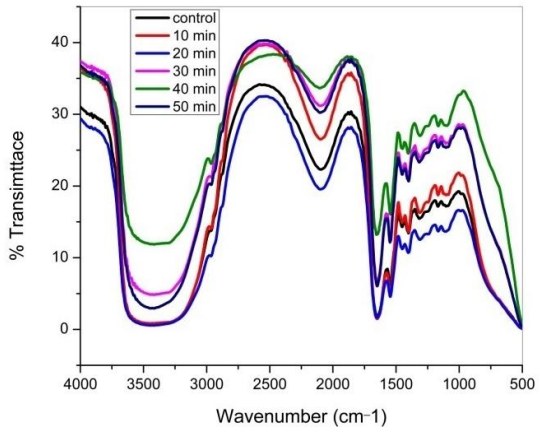

Fig. 3. FTIR spectra of irradiated blood by He-Ne laser for (0, 10, 20, 30, 40 and 50) minutes

The whole blood sample is irradiated to He-Ne laser radiation for 10, 20, 30, and 40min. and 50 min duration respectively, Fig. 3. Table 3 shows the groups associated with spectral peaks whole sample irradiated to He-Ne laser radiation for 10 min duration shows an increase in transmittance for all groups except for C-H decreases due to the denaturation of the protein. FTIR spectra of whole blood irradiated with He-Ne laser for 20 minute show decreases in transmission for group, C-H, and N-H, to denaturation of protein i.e. it breaks the polypeptide bonds due to conformational changes of proteins, but in 30, 40, and 50 minutes show an increase in transmittance for all groups is observed the separate chromophore and auxochrome groups (C=O, N=O, C-H, N-H, C-O, O-H) show also significant changes and indicates a significant inecreasing in their concentration. Laser irradiation of blood causes changes in absorption band in stretching and bending Vibrations of peptide group.

4. CONCLUSION

This work had shown that laser radiation interacts with blood at the molecular level. Hemoglobin is a blood photoreceptor that selectively absorbed He-Ne Laser beam with output power 2 mW, (632.8 nm). The absorption of laser beam by blood leads to partial photodissociation. The results showed a decrease in intensity, all irradiated serum sample intensity was less than control serum sample; this result indicates that there is photodegradation happened to the blood components, this causes changes in the structure and conformational changes in the polypeptide of N-H and CO and COO– groups in the regions 1500–1700 and 3000–3500 cm–1 of the IR spectrum. Sample irradiated for 30, 40, and 50 minutes show an increase in transmittance for all groups is observed the separate chromophore and auxochrome groups (C=O, N=O, C-H, N-H, C-O, O-H) show also significant changes and indicates a significant inecreasing in their concentration.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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