

***In vitro* UV-Visible and FTIR Spectroscopy Study of Low Power He-Ne Laser Irradiation on Human Blood**

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Authors' contributions

This work was carried out in collaboration between all authors. Author MAH designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author AASM managed the analyses of the study. Author MDA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Laser irradiation has biostimulating effect in various cell types low power laser radiation is used clinically for skin and vascular disorders.

Aims: To investigate the effect of (He-Ne) laser ($\lambda=632\text{nm}$, power=1mW) 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($\lambda=632\text{nm}$, power=1mW).

Place and Duration of Study: Soba Hospital, Khartoum- Sudan, Institute of Laser, Sudan University of science and technology (SUST), February 2018.

Methodology: Blood samples were taken from healthy volunteers; blood sample irradiated to (He-Ne) laser and control compared; FTIR and UV-Vis spectrophotometer were used to study laser radiation effect

Results: FTIR spectra and UV-vis absorption spectra of blood samples are compared before and

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after irradiated to He-Ne laser shows increases absorption for 10, and 40 min but it decreases as the exposure time at 30,20 and 50 minutes. This fluctuation of light absorption is known as a biphasic response.

FTIR spectra 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)). For all exposure time He-Ne laser ($\lambda = 632\text{nm}$, power= 1mW) irradiation, showed significant changes. Increased in transmittance at different exposure time for all groups (C=O, O-H, N=O, C-O & C-H, N-H) and indicates significant decreasing in their concentration.

Conclusion: The laser radiation causes changes in the structure and conformational changes in the polypeptide and absorption of blood samples decreased due to increasing ligand electronegativity.

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

1. INTRODUCTION

Laser is a device that generates coherent radiation which is used in medical applications such as cauterizing corrective eye surgery, and a source of heat for cutting. Nevertheless, there is much application of He-Ne laser in the medical field; for instance, Blood cell analysis (cytometry), for the diagnostic and treatment. Helium-Neon laser is a type of small gas laser with the typical operational wavelength of 632.8 nm in the red color of the visible spectrum [1]. The biostimulation effect of low-power laser irradiation has been studied and noticed for about two decades. Some progress was achieved in treating various pathologic processes [2,3,4]. Intravenous low power laser irradiation has been applied clinically to treat various diseases, and the results have been encouraging as to the research data in the literature so far, the bio-effects of laser stimulation are evident; however, the mechanism of interaction is not fully understood [5]. Photobiomodulation 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 causes a chemical change [6,7].

Spectroscopic technique such as FTIR and UV-Vis absorption was used to study the spectral differences in the serum of normal blood samples [8]. Blood samples were exposed to He-Ne laser (Wavelength $\lambda = 632.8\text{ nm}$, Power = 3mW). The FTIR spectra for FTIR spectra of irradiated blood samples. Showed significant changes [9]. He Ne laser ($\lambda = 632\text{nm}$, power= 2mW) was used to irradiation human red blood cells absorption spectrum, FTIR and fluorescence spectra of RBC The absorption spectrum of RBC after irradiated to He-Ne laser showed a significant decrease in absorbance. The FTIR spectrum of irradiated

RBC clearly shows changes in transmittance [10]. Human blood exposed to low-intensity He-Ne-laser radiation caused clearly defined changes in the IR and visible absorption spectra of the blood and erythrocytes [11].

Human blood irradiated to yellow laser power density 450mW/cm^2 showed a significant difference in the absorption of light with varying laser irradiation time [12].

Low power violet laser power 10 mW was used to irradiation on human blood and were investigated the effect laser on Some rheological factors of the human blood, such as complete blood count (CBC) parameters and blood sedimentation rate (BSR), study showed decreasing in The RBCs volume and ESR [13]. Human blood was exposed to He Ne laser ($\lambda = 632\text{nm}$, power= 30mW) to investigate the effect of He Ne laser on viscosity and erythrocytes deformability and blood sedimentation rate (BSR),the study noticed a change in both viscosity and size of erythrocytes [14]. This paper study the effect of He-Ne laser (Wavelength $\lambda = 632.8\text{ nm}$, Power = 1mW on human whole blood with different exposure time using UV-Vis spectrophotometer and FTIR spectrometer.

2. MATERIAL AND METHODS

2.1 Samples Collection

Blood samples were collected 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 Ethylenediaminetetraacetic acid (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

Whole blood Samples were irradiated to a Helium-Neon laser beam, continuous operating wave mode, as a radiation source (632.8 nm, 1 maw), for exposure time (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. Fourier Transform Infra Red Spectra (FTIR) and UV-Vis spectrophotometer (Jasco-670) were used to studied the effect of laser radiation

on human blood and were obtained FTIR and UV-Vis spectrum for He-Ne laser irradiated blood serum samples and non irradiated.

3. RESULTS AND DISCUSSION

3.1 FTIR Spectra Result

In the FTIR spectra of the whole blood ,without irradiation to laser presented Fig. 1 Table 1 shows the groups O-H, C=O, N=O, C – H, N-H, and C–O in the region between the wave

Table 1. Show the FTIR spectral data (wave number, function group and transmission) for blood sample control

FTIR spectral data for normal blood (control)				
Sr. No	Wave number cm^{-1}	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 2. Show the FTIR spectral data (wave number, function group and transmission) for irradiated blood sample power 1mW

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 CM^{-1}	Group	T%
1	10	3306.77	O-H	24.79
2		1650.96	C=O	25.63
3		1545.10	N=O	30.12
4		1451.73	C-H	38.87
5		1315.56	N-H	41.11
6		1161.74	C-O	43.24
7	20	3416.04	O-H	24.69
8		1651.63	C=O	26.81
9		1545.10	N=O	30.61
10		1451.01	C-H	38.74
11		1312.59	N-H	40.46
12		1161.74	C-O	42.05
13	30	3442.45	O-H	24.04
14		1651.63	C=O	26.02
15		1545.23	N=O	31.31
16		1451.01	C-H	39.80
17		1312.59	N-H	41.92
18		1167.96	C-O	42.31
19	40	3348.63	O-H	11.90
20		1651.41	C=O	14.21
21		1545.10	N=O	20.40
22		1444.23	C-H	30.48
23		1325.03	N-H	31.94
24		1167.96	C-O	33.94
25	50	33040.4	O-H	22.22
26		1651.63	C=O	24.03
27		1545.10	N=O	27.07
28		1457.01	C-H	34.36
29		1312.59	N-H	35.33
30		1167.96	C-O	36.19

numbers 4000 to 500 cm^{-1} In the spectral region 2800–3700 cm^{-1} , the band with $\lambda_{\text{max}} = 3444.63 \text{ cm}^{-1}$ is O–H bond peak. Amide-I is mainly associated with C–O, C = O, and C–H stretching vibrations and also related to the backbone conformation. The wave numbers 1650.95 cm^{-1} , 1548.73 cm^{-1} 1452.30 cm^{-1} indicate C = O, N=O and C - H peaks respectively. The absorption peak in the 1317.29 cm^{-1} and 1168.78 cm^{-1} arises due to the N-H stretching vibrations of the proteins methylene group of the proteins, and gives rise to the existence of glucose due to C-O symmetric stretching, The prominent absorption peak 3444.63 cm^{-1} is due to the N – H stretching mode (amide - A) of proteins. The most intense absorption band in proteins is the amide I peak, which is observed at 1650.95 $1/\text{cm}$. 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 $1/\text{cm}$ due to strong N-H in-plane bending and termed as an Amide II band [15,16]. The whole blood sample is irradiated to He-Ne laser radiation for 10,20, 30 ,40min. and 50 min duration respectively, Fig. 2 and Table 2. Shows the groups associated with spectral peaks whole sample irradiated to He-Ne laser radiation for 10, 20 , 30 40, and 50 minute duration shows an increase in transmittance for all groups. For all groups is observed, that was the separate chromospheres and auxochrome groups (C=O, N=O, C-H, N-H, C-O, O-H) show also significant changes and indicates a significant increasing in their concentration. Laser irradiation of blood

causes changes in absorption band in stretching and bending Vibrations of peptide group.

3.2 UV-vis Spectra Results

The absorption spectra of the whole blood in the range 300–800 nm Fig. 3. Contain absorption bands with $\lambda_{\text{max}} = 340, 416 \text{ nm}$, , $\lambda_{\text{max}} = 542$ 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 the samples studied Fig. 4. The UV-vis absorption increases for 10 and 40min but it decreases as the exposure time at 50 and it continues to decrease at 30,20 minutes. due to increasing ligand electronegativity [17]. And concentration of absorbing centers is decreasing. This fluctuation of light absorption is known as a biphasic response. The mechanism of LLLT at the cellular level has been associated with the absorption of monochromatic visible and near infrared radiation. Effective tissue penetration is maximized at a specific optical window [18,19, 20]. Laser effect on biological tissues, Bio stimulated it; Fluctuation of light absorption illustrates the biphasic dose-response curve. When the blood sample is irradiated, the enzymatic activity of the membrane sodium (Na^+) and potassium (K^+) ion pump changes in dose and fluence-dependent manner. Consequently, the biological function of the cells is stimulated and increases the light absorption. But a further increase of irradiation time inhibits the enzymatic activities due to the suppression of the Na^+ and K^+ [21].

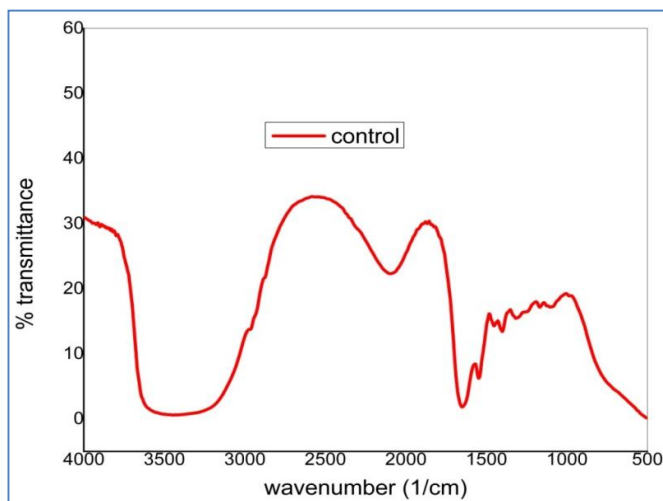


Fig. 1. FTIR spectrum for normal blood samples (control)

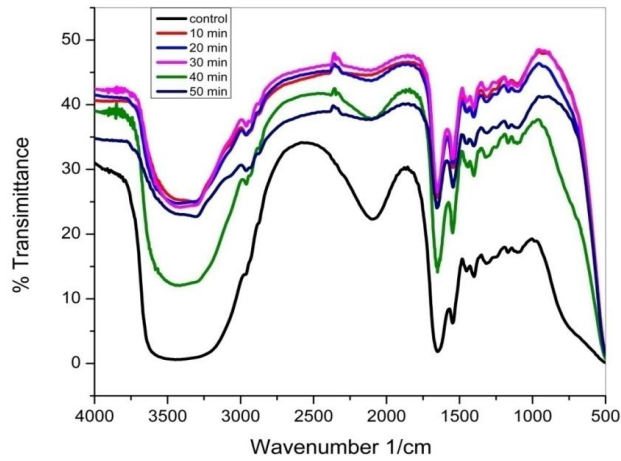


Fig. 2. FTIR spectrum for before and after irradiated blood to He-Ne laser from 10,20,30,40 and 50 minute

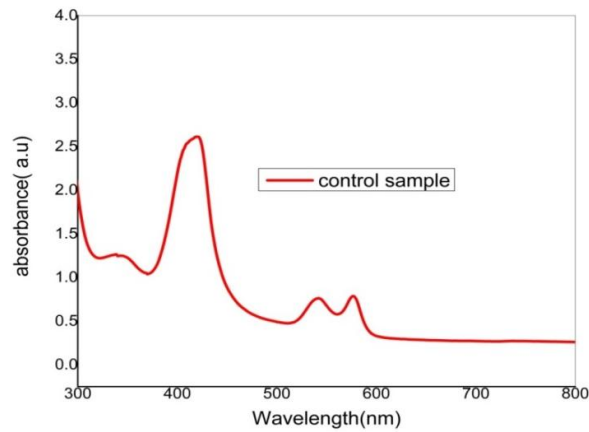


Fig. 3. The relation between Absorbance (a) and wavelength (λ) for whole blood before irradiated to (He-Ne) laser power 1 mW

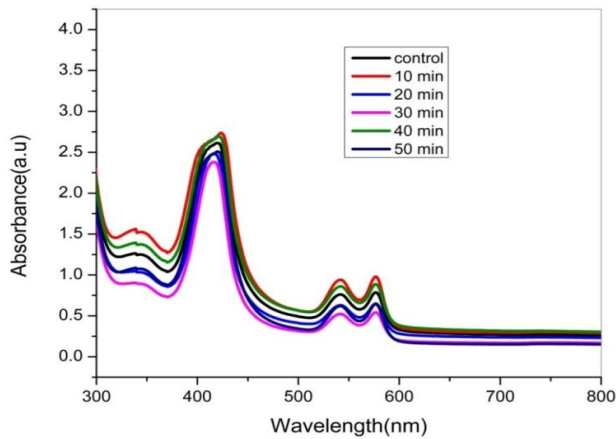


Fig. 4. The relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 1 mW at difference exposure time 10,20,30,40 and 50 minute

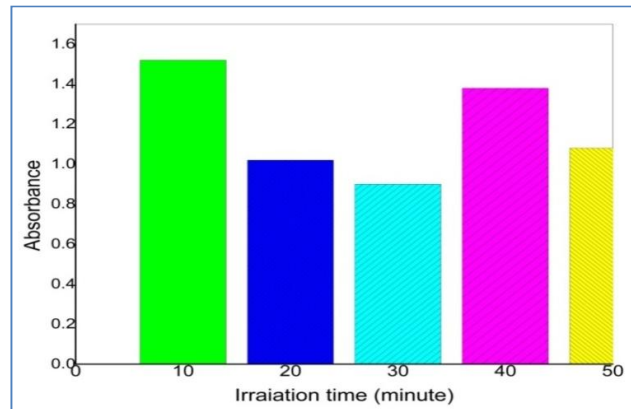


Fig. 5. Absorption of light at 340nm with different irradiation time

4. CONCLUSION

For all exposure time He-Ne laser ($\lambda = 632\text{nm}$, power=1mW) irradiation exposure to whole blood, the transmittance of C=O, O-H, N=O, C-O & C-H, N-H group transmittance increases show also significant changes and indicates a significant decreasing in their concentration.

In general peptide group's bands show more changes. The secondary structures of blood proteins undergo conformational changes. The light absorption increases for 10, and 40 min but it decreases as the exposure time at 30 (Fig. 5), 20, and 50 minutes. This fluctuation of light absorption is known as biphasic response.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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