Original Research Article

Helium Neon Laser Effects on Human Whole Blood by Spectroscopy in Vitro Study

.

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= 632nm, power=2mW) 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= 632nm, power=2mW)

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 are compared before and after He-Ne laser radiation shows, a significant decrease in intensity. FTIR spectrum of non exposed blood show 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, percentage of transmittance increases .the most effect 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 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 tntensity.

Keywords: Laser, blood, UV-Vis, FTIR, spectroscopic

1. INTRODUCTION

Low-intensity helium neon laser has been used extensively in medical applications lately. Interaction of lasers with biological materials such as blood, skin, and tissues is an 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 affect which means the light is absorbed and cause a chemical change. [3,4,5]

FTIR and UV-Vis spectroscopic technique is employed to study the spectral differences in the serum of normal blood samples[2],Blood samples were irradiated by He-Ne laser (Wavelength λ = 632.8 nm, Power = 3mW). The FTIR spectra for FTIR spectra of irradiated blood samples. Show significant changes.[1] He Ne laser (λ= 632nm, power=2mW) is used to irradiate human Red blood cells 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 show changes in transmittance, [6] some rheological factors of the human blood, such as complete blood count (CBC) parameters and blood sedimentation rate (BSR) effected by low-level laser radiation (LLLR) laser blood biostimulations investigated the effect of LLLT on rheological parameters of human blood, they noticed a change in both viscosity and size of erythrocytes, [7].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 photo dissociation of hemoglobin–ligand[8]

this paper investigate the effect of He-Ne laser (Wavelength λ = 632.8 nm, Power = 2mWith different exposure time using UV-Vis spectrophotometer and FTIR spectrometer.

2. MATERIAL 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 tube to prevent from coagulation to (EDTA) and each sample was divided into two samples one sample was control and other exposed to helium-neon laser with different exposure times.

2.2 laser irradiated

Samples were exposed to a Helium-Neon laser beam, operating in continuous wave mod, 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 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 (shimadzo) for control, and He-Ne laser irradiated blood serum samples.

3. RESULTS AND DISCUSSION

3.1 UV-vis spectra

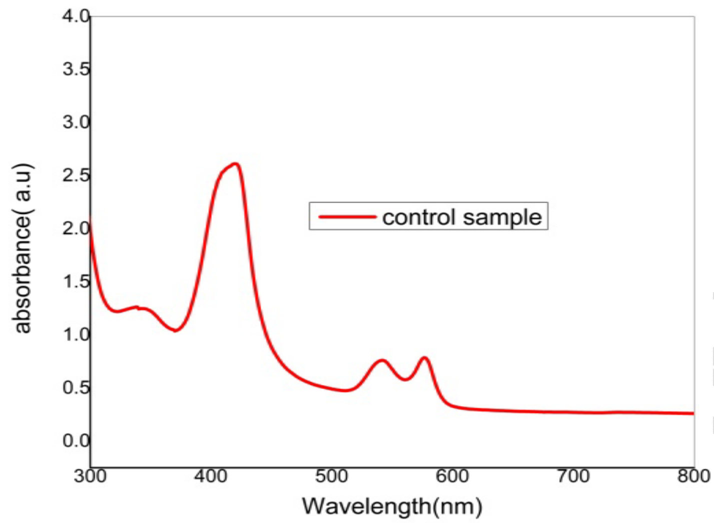

Figure .1 Spectrum of non- irradiated blood sample (control).

Figure .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.

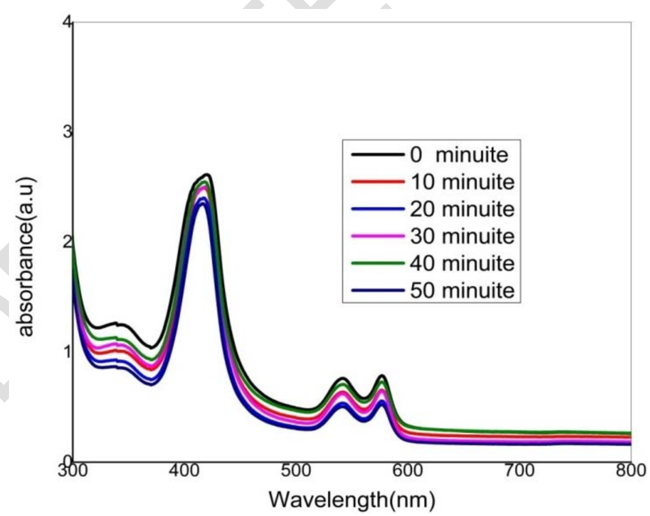

Figure 2. Relation between Absorbance (a) and wavelength (λ) for whole blood before and after irradiated to (He-Ne) laser power 2 mW

The absorption spectra of the whole blood recorded in the range of 300–800 nm Figure 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 were detected for all of the samples studied.

Table.1 The intensity of normal and irradiated samples

Wave number 1/cm	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

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 photo degradation happened to the blood components.

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

In the UV-visible absorption spectrum of the irradiated blood, (figure.2 and table1) the most intense absorption band at 416 nm, the light with this wavelength that strikes this biological tissues will be highly absorbed. This phenomenon is the key for the desired effect on the tissues [10]. Figure 2 compared the light absorption at 340nm, 414nm, 542nm and 576nm for different irradiation time. The minimum light absorption occurred at 50 minutes of irradiation with the less intensities recorded.

3.2 FTIR spectra

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		3304.04	O-H	12.12
20		1645.41	C=O	13.21
21	40	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		3442.45	O-H	2.49
26		1651.63	N-H	6.46
27	50	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

An FTIR spectrum of whole blood in vitro without laser radiation is shown in (Figure) 3. Table2. Shows the groups OH, C=O, N=O, C-O and C-H in the region between the wave number 4000 1/cm to 500 1/cm. The most intense absorption band in proteins is the amide I peak, which is observed at 1650.95 1/cm. Amide I is mainly associated with C=O symmetric stretching and or C-O stretching vibrations. There are anther very strong prominent amide absorptions one at at 1545 1/ cm due to strong N-H in plane bending and termed as an Amide II band. The strong characteristic band at 3295 1/cm due to N-H symmetric stretching confirmed the existence of amino acid group [2] The medium band at 2873 1/cm due to C-H asymmetric and symmetric stretching of CH3 group established the presence of lipids and the medium bands at 2854 1/ cm due to C-H symmetric stretching of CH2 group established the presence of lipids , fatty acids[11,12,13,14].The FTIR spectra of blood showed clear bands at1080, and 12451/ cm, are composed of mononuclear cells containing nucleic acids such as DNA and RNA. The nucleic acid components found in WBCs.[9]

Whole blood sample is irradiated to He-Ne laser radiation for 10 , 20 ,30 ,40min. and 50 min duration respectively, figure (4 to 8) table 3 . Shows the groups associated with spectral peaks whole sample irradiated to

He-Ne laser radiation for 10 min duration shows increase in transmittance for all groups except for C-H dicreases due to denaturation of protein .

FTIR spectra of whole blood irradiated with He-Ne laser for 20 minuite 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 ,50 minutes show increase in transmittance for all groups for all groups is observed. Laser irradiation of blood causes changes in absorption band in stretching and bending Vibrations of peptide group.

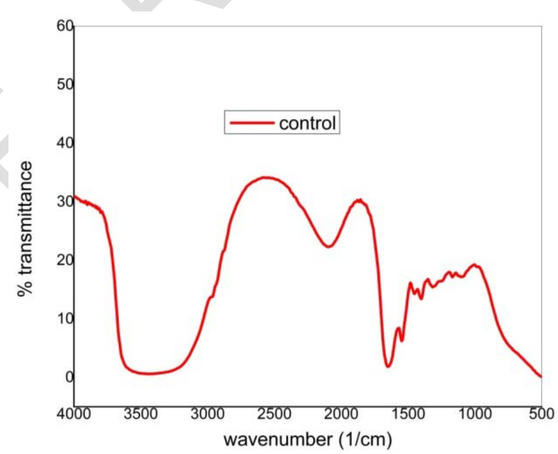

Figure (3), FTIR spectrum for normal blood samples (control)

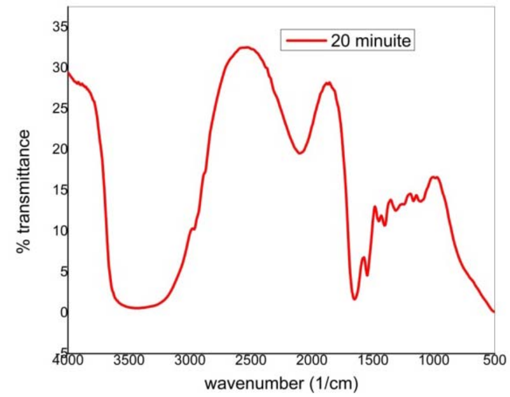

Figure (4), FTIR spectrum for irradiated blood to He-Ne laser (10 min)

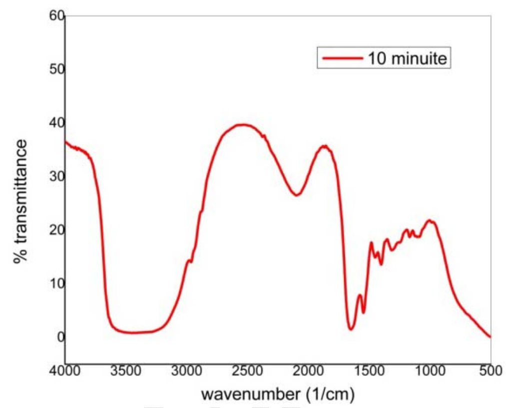

Figure (5), FTIR spectrum for irradiated blood to He-Ne laser (20 min)

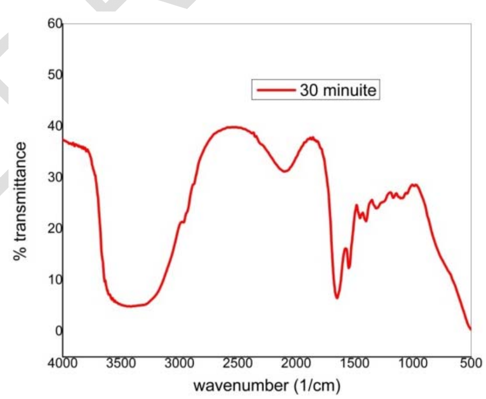

Figure (6), FTIR spectrum for irradiated blood to He-Ne laser (30 min)

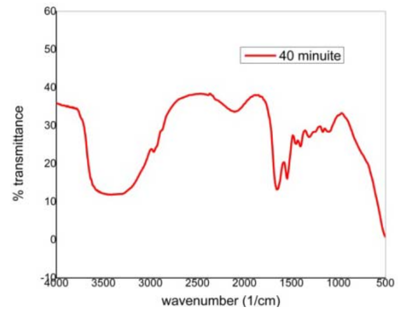

Figure (7), FTIR spectrum for irradiated blood to He-Ne laser (40 min)

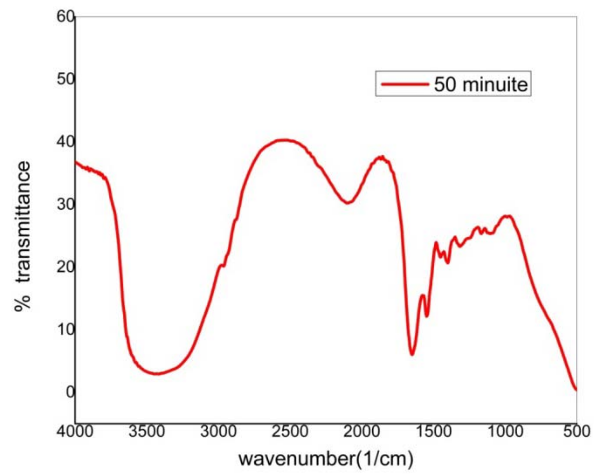

Figure (8), FTIR spectrum for irradiated blood to He-Ne laser (50 min)

4. CONCLUSION

It has been shown that laser radiation effect on blood at the molecular level. Hemoglobin is a blood photoacceptor that selectively absorbs He-Ne Laser radiation power 2mW, (632.8 nm). The absorption of laser radiation by blood leads to partial photodissociation. show decrease intensity, all irradiated serum sample less than control serum sample This result indicate to 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.

REFERENCES

1- Ghadage, V. H., G. R. Kulkarni, and B. N. Zaware. "He-Ne laser irradiation of blood in vitro and FTIR spectral analysis." *Int J Chem Phys Sci* 4 (2015): 148-153

2- Kanagathara, N., M. Thirunavukkarasu, E. C. Jeyanthi, and P. Shenbagarajan. "FTIR and UV-Visible spectral study on normal blood samples." *IJPBS* 1, no. 2 (2011): 74-81.

3- Fuad, Siti Sakinah Mohd, N. Suardi, and I. S. Mustafa. "In Vitro UV-Visible Spectroscopy Study of Yellow Laser Irradiation on Human Blood." In *Journal of Physics: Conference Series*, vol. 995, no. 1, p. 012053. IOP Publishing, 2018

.

4-Csele, M., (2004). Fundamentals of Light Sources and Lasers Fundamentals of Light, Hoboken, New Jersey, USA: Wiely

.

5-Dais, J., (2009). Low Level Laser Therapy Position Paper for the CMTBC : An Examination of the Safety, Effectiveness and Usageof Low Level Laser Therapy for the Treatment ofMusculoskeletal Conditions

6 - V. H. Ghadage, and G. R. Kulkarni," Effects of He-Ne laser irradiation on red blood cells in vitro", Optical Interactions with Tissue and Cells XXII, Proc. of SPIE, BIOS, San Francisco, California, USA, vol. 7897, pp. 789701 - 789710, 2011.

7- Mi, Xian-Qiang, Ji-Yao Chen, Zi-Jun Liang, and Lu-Wei Zhou. "In vitro effects of helium-neon laser irradiation on human blood: blood viscosity and deformability of erythrocytes." *Photomedicine and Laser Therapy* 22, no. 6 (2004): 477-482.

8- Zalesskaya, G. A., and I. I. Kalosha. "Effect of in vivo irradiation of blood by low-intensity emission from a He–Ne laser on molecular components of blood." *Journal of Applied Spectroscopy* 76, no. 5 (2009): 720.

9- Mordehai, Jacov, Jagannathan Ramesh, Mahmoud Huleihel, Zahavi Cohen, Oleg Kleiner, Marina Talyshinsky, Vitaly Erukhimovitch et al. "Studies on acute human infections using FTIR microspectroscopy and cluster analysis." *Biopolymers: Original Research on Biomolecules* 73, no. 4 (2004): 494-502.

10- Chung, Hoon, Tianhong Dai, Sulbha K. Sharma, Ying-Ying Huang, James D. Carroll, and Michael R. Hamblin. "The nuts and bolts of low-level laser (light) therapy." *Annals of biomedical engineering* 40, no. 2 (2012): 516-533.

11- Rathore, Shikha, and Basharath Ali. "Effect of He-Ne Laser Radiation on Viscometric Behavior of Human Blood." *Journal of Chemical, Biological and Physical Sciences (JCBPS)* 3, no. 3 (2013): 2124.

12- Gunasekaran, S., and D. Uthra. "FTIR and UV-Visible spectral study on normal and jaundice blood samples." *Asian Journal of Chemistry* 20, no. 7 (2008): 5695.

13- Fabian & Schultz 2000 etal., Yu & Irudayaraj 2005, IR band assignment of biological molecules

,

14- J. J. Anders, R. C. Borke, and S. K. Woolery, “Low power laser irradiation alters the rate of regeneration of the rat facial nerve,” Lasers Surg. Med., vol. 13, pp. 72 - 82, 1993