Sudan University of Science and Technology

College Graduate Studies

Investigation of the Effects of Diode Laser (532 nm) on Human Whole Blood by UV-Vis Spectroscopy and CBC in-vitro Study

التحقق من تأثيرات ليزر الثنائي (٥٣٢ نانومتر) على الدم البشري الكامل بواسطة التحليل الطيفي للأشعة فوق البنفسجية والمرئية وجهاز تحليل الدم في المختبر

A Dissertation Submitted in Partial Fulfilment for the Requirement of a Master Degree (M. Sc) in Solid State Physics

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2020

الايـة

بسم الله الرحمن الرحيم

قال تعالي:

﴿الْحَمْدُ لِلَّهِ الَّذِي لَهُ مَا فِي السَّمَاوَاتِ وَمَا فِي الْأَرْضِ وَلَهُ الْحَمْدُ فِي الْآخِرَةِ وَهُوَ الْحَكِيمُ الْخَبِيرُ﴾

صدق الله العظيم

سورة سبأ الآية(1)

Acknowledgement:

First and foremost, praises and thanks to the God, the Almighty, for His showers of blessings throughout my research work to complete the research successfully.

I would like to express my grateful appreciation to **Dr Ali Abdel Rahman Saeed Marouf**for his valuable and constructive suggestions during the planning and development of this research work. His willingness to give his time so generously has been very much appreciated.

I am extremely grateful to my parents for their love caring and sacrifices for educating and preparing me for my future. I am very much thankful to my brothers, sisters for their love, and continuing support to complete this research work.

I would like to say thanks to my friends and research colleagues, also I express my special thanks to my friend Almigdad Abdualghani for his genuine support throughout this research work.

Finally, my thanks go to all the people who have supported me to complete the research work directly or indirectly.

Abstract

Lasers are widely used in the modern medicine as important parts of different types of equipment. Laser beams of different powers, wavelengths and optical properties are used in surgery, cosmetology, therapy, diagnostics and number of other medical areas. The present study aims to evaluate the effects of diode laser with wavelength 532 nm on blood optical absorbance property and complete blood count (CBC). The irradiated and non irradiated whole blood, plasma and blood cells samples were characterized by UV–Vis spectroscopy and CBC measurements. The irradiated blood cells samples reveals degradation in the beak of 541.15 nm in their absorbance spectra, the irradiated plasma samples reveals degradation of long-term condition cells (LTCs) with λ_{max}= 414.32 nm in their absorbance spectra. While the results of the effect of laser irradiation on CBC showed that the best laser dose has a positive effect on almost all blood cells and their indicators are approximately 30 minutes as white blood cells, red blood cells and haemoglobin and mean cell volume.

المستخلص

تستخدم الليزرات بشكل واسع في الطب الحديث كجزء مهم من أنواع مختلفة من الأجهزة. شعاع الليزر بقدرات مختلفة وأطوال موجية مختلفة وخصائص بصرية يستخدم في الجراحة والتجميل والعلاج والتشخيص و في عدد من المجالات الطبية. تهدف هذه الدراسة إلى تقييم تأثيرات ليزر الثنائي بطول موجي ٥٣٢ نانومتر على (CBC) العداد الكلي للدم .تم فحص عينات الدم الكامل والبلازما وكريات الدم المشععة وغير المشععة عن طريق مطيافية الأشعة فوق البنفسجية والمرئية وقياسات (CBC) العداد الكلي للدم .أظهرت أطياف امتصاصعينات خلايا الدم المشععة تفكك للقمة عند 541.15 نانومتر، أظهرتأطياف امتصاصعينات البلازما المشععة تفكك خلايا حالة الفترة الطويلة (LTCs) بطول موجي أقصى λ_{max}= 414.32. في حين أظهرت نتائج تأثير التشعيع بالليزر على (CBC) العداد الكلي للدم وجد أن أفضل جرعة ليزر لهاتأثير إيجابي على خلايا الدم ومكوناته (خلايا الدم البيضاء وخلايا الدم الحمراء والهيموقلوبين ومتوسط حجم الخلية) هي ٣٠ دقيقة.

Table of Contents

Table of Figurers

CHAPTER ONE

INTRODUCTION

1.1 Background:

Lasers are widely used in medical applications as important parts of different types of equipment. Laser beams of different powers, wavelengths and optical properties are used in surgery, cosmetology, therapy, diagnostics and number of other medical areas. One of the most controversial applications of laser light is its application for the therapy of different disorders, therapy method, commonly known as low level (power) laser therapy (LLLT). Unlike laser surgery, for laser therapy, low power laser light is used, which can not cause significant thermal effects or damage irradiated tissues. Usually red and infrared laser light are used for laser therapy. Recently blue, violet, green and ultraviolet laser-based on therapy system were introduced.

There are various chemical and physical changes caused when materials exposed to electromagnetic radiation. Recent studies demonstrated that not only laser light can have beneficial therapeutic effects. LED light, as well as filtered light of bulbs or other light sources, even natural light of Sun can treat. Therefore, different versions of laser blood irradiation (together with LBI) therapy belong to phototherapy methods. Laser blood irradiation (LBI) therapy employs a modification of blood under the influence of laser light to support faster and better recovery. There are direct and indirect methods of laser irradiation of blood. Direct methods are invasive and require immediate contact of light with blood, while indirect ones are noninvasive. On the scientific side, the processes of light interaction with human skin have a fundamental importance in biomedical sciences.

1.2 Research Problem:

In the past the in-vivo methods were using radiation and chemotherapy for human blood investigation and treatments These methods may result in adverse effects on patients technicians and blood quality itself Therefore studying the effects of laser beams on the different blood components such as red blood cells, white blood cells Might lead to better awareness among physicians and the community. This work to investigate biological effects of diode laser (532 nm) radiation on human blood to figure out the differences between blood parameters such as blood cells and whole blood before and after treatment with the laser radiation and regularly during time of experiment.

1.3 The Objectives of this Dissertation:

1.3.1 General Objective:

- To investigate the biological effects of diode laser (532 nm) radiation on human blood**.**
- To apply one of the medical applications of laser in the laboratory.

1.3.2 Specific Objectives:

- To figure out the differences between blood parameters such as blood cells and whole blood before and after treatment with the laser radiation and regularly during the time of the experiment.
- To compare the effect of different irradiation times on the blood parameters.
- To verify the effect of lasers on complete blood components.
- To investigate the bio summative effect of laser on soft biological tissue.

1.4 Research Methodology:

To achieve the objectives of this dissertation; ten samples of blood will take from one adult healthy person, these samples will be distributed into six EDTA anticoagulant containers by rate of 2.5 ml in every container, five EDTA containers will be irradiated by diode laser beam 532 nm wavelength and output power 1000 mW (Low-level laser therapy) to three different exposure times namely (10, 20 and 30) min. For comparison one in-irradiated blood sample was considered as a control sample. Then the samples will analyze using CBC "Complete Blood Count" machine and UV-Vis spectrometer to study the optical properties.

1.5 Dissertation outline:

This dissertation is consist of four chapters, chapter one introduction and literature review, and chapter tow consist basic concepts of laser and blood, and laser-matter interaction**,** chapter three consist experimental part (The materials and device and method), chapter four consist results and discussion and conclusion, recommendations and finally reference.

CHAPTER TWO

2.1 Previous Studies:

There are numerous research have been studies the interaction of low-level lasers with biological materials such as blood. Haimid , et al. were studied The effect of He-Ne laser (λ= 632 nm, power=2 mW) effect on human whole blood, after irradiated to different times (Haimid, et al., 2019) and investigating the effect of He-Ne laser (λ= 632nm, power=1mW) on human whole blood after irradiated to different times (Haimid, et al., 2019 b)

In 2011 Zahra Al Timimi, et al were studied Green Laser blood Therapy Blood was taken from one hundred adult patients. The sample adding anticoagulant (EDTA), the samples were divided into four groups for irradiating with different laser intensities. Where each sample was subdivided into two groups which one was irradiated and the other considered as a control sample. The samples were made to stand for 30 minutes before determining the change in the rheological properties of blood cells. The study was concluded that low-level laser therapy, affects the rheology of erythrocytes and leucocytes when used on human blood in vitro. It was observed that it changes the erytherocytatory, leucocytatory, BSR, aggregability indices of blood. It was concluded that low-level laser therapy can affect the physical as well as chemical properties of blood cells which is not only helpful in preservation of blood but also in revitalizing the physically and chemically stressed erytherocytatory membranes. It was determined that the laser therapy decreases the viscosity of blood thus increasing the electrophoretic mobility of erythrocytes (Al Timimi, et al, 2011).

In 2014 AL- Timimi were studied the effects of green laser irradiation on red blood cells blood smears from the peripheral blood of two normal individuals (an adult male and female) were prepared and exposed by different laser doses at different intervals. The morphological changes in red blood cells were assessed. The result showed that the green laser at low energy was able to reduce the proportion of the morphologically abnormal red blood cells in the blood smears **(**AL-Timimi, 2014).

The effect of laser irradiation on materials in different environments has been demonstrated by several researchers. Gawbah et al.were used laser to burn agricultural waste in order to obtain and synthesis useful and high-value materials (Gawbah et al., 2017), other many researchers have studied the utilization of laser in irradiation of food like utilization in pasteurize caw's milk (Marouf and Sara, 2018) and the effect of utilization of laser irradiation on physical and chemical properties of bee honey(Al Humira and Marouf, 2017).

2.2 Basic Concepts:

2.2.1 Laser:

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 diode laser in the medical field; for instance, blood cell analysis (cytometry), for the diagnostic and treatment. diode laser is a type of small gas laser with the typical operational wavelength of 532 nm in the red color of the visible spectrum (Mangi *et al*., 2014).

2.2.1.1 Properties of Laser:

i. It Monochromatic: because of only a wave with frequency matching the atomic transition energy and a cavity mode can be amplified.
ii. It Coherent: stimulated emission produces in-phase photons so that there is a set phase relationship between all parts of a laser beam.

iii. It has Good fort you can make it bold: lasers give a beam that propagates with little divergence. This means that the photon energy can be easily collected and focused.

iv. Brightness: brightness is defined as the power emitted per surface area per solid angle, so that, a laser of moderate power is extremely bright because it is directional (John, 2019).

2.2.1.2 Elements of Laser:

The active medium is a collection of atoms or molecules that can be excited to a state of the inverted population; that is, where more atoms or molecules are in an excited state than in some lower energy state.

The excitation mechanism is a source of energy that excites, or "pumps," the atoms in the active medium from a lower to a higher energy state.

The feedback mechanism returns a portion of the coherent light originally produced in the active medium back to the active medium for further amplification by stimulated emission.

The output coupler allows a portion of the laser light contained between the two mirrors to leave the laser in the form of a beam (See Fig.2.1) (Laser Institute of America, 2007).

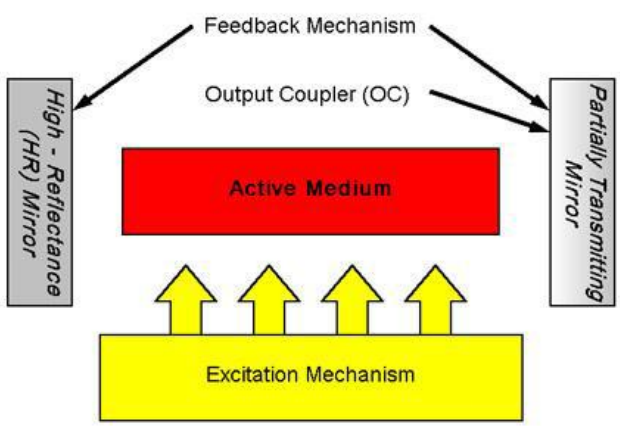


Fig 2.1. Elements of Laser

2.2.1.3 Laser Types:

Lasers are be classified according to the type of active medium, excitation mechanism, and duration of laser output. The following types is depended on the active medium.

2.2.1.3.1 Solid state lasers:

A solid state laser was the first one to come into the laser world. Solid state lasers are a part of the laser system involving solid media

2.2.1.3.2 Semiconductor lasers:

The active medium of a semiconductor laser is the junction between two types of semiconductor material such as Gallium arsenide (Ga - As) which is used in the manufacture of semiconductor laser.

2.2.1.3.3 Gas lasers:

Gas medium are used in this type of laser. Which dived to three types of gas lasers depending up on the active medium? They are atomic, ionic and molecular gas lasers.

2.2.1.3.4 Liquid (dye) laser:

Dye lasers are produced in liquid gain medium. The dyes have very broad emission and spectrum that cover wavelength range from ultraviolet (320nm) to the infrared at about (1500nm) lead to tunable laser output (Nambiar,2004)

4.4.1.1 Laser Applications:

Optical communication -in laser supermarket scanners, a rotating mirror scans a red beam while clerks move packages across the beam.

Laser Machining and cutting-Laser energy can be focused in space and concentrated in time so that it heats, burns away, or vaporizes many materials. (Goswami, 2016).

2.2.1.5 Medical Applications of Lasers:

Surgical removal of tissue with a laser is a physical process similar to industrial laser drilling. Carbon-dioxide lasers operating at 10.6 micrometers can burn away tissue as the infrared beams are strongly absorbed by the water that makes up the bulk of living cells (Goswami, 2016).

2.3 Blood:

Normally 70-80 % of human body weight is from blood. In adults, this amounts to 4.5-6 quarts of blood .Which essential fluid carries out the critical functions of transporting oxygen and nutrients to our cells and getting rid of carbon dioxide, ammonia, and other waste products. Besides, it plays a vital role in our immune system and in maintaining relatively constant body temperature. Blood is a highly specialized tissue composed of more than 4,000 different kinds of components; four of the most important ones are red cells white cells platelets and plasma. All humans produce these blood components there are no population or regional differences as shown in Fig 2.2(**Aljaberi**, 2018).

Fig2.2.The Blood cells

2.3.1 Blood Components:

The human body contains approximately 5liters of blood which is made up of a mixture of red blood cells, white blood cells, and platelets all suspended in a liquid called plasma.

2.3.1.1 Plasma:

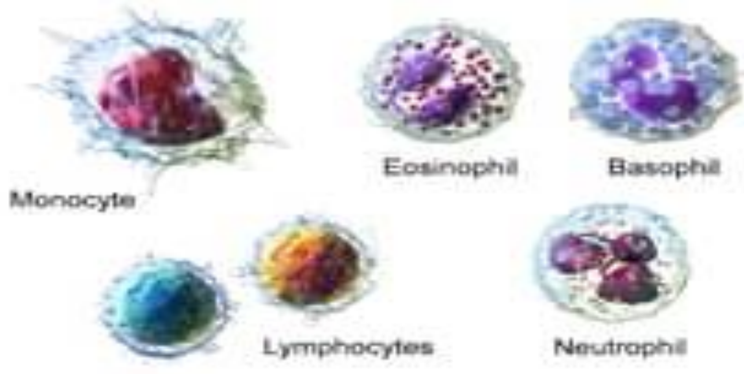
Plasma is the liquid component of Blood, in which the red blood cells, white blood cells, and platelets are suspended. It constitutes of more than half of the bloods volume and consists mostly of water than contains dissolved salts (electrolytes) and proteins .The major protein in plasma is albumin which helps to keep fluid from leaking out of the blood vessels and into tissues .A albumin binds carry substances such as hormones and certain drugs .Other proteins in plasma include antibodies which actively defend the body against viruses, bacteria, fungi, and cancer cells, and clotting factors, which control bleeding (Sarode, 2018).

2.3.1.2 Red Blood Cells (RBCs):

Red blood cells make up about 40% of the blood volume. Red blood cells contain haemoglobin (Sarode, 2018).

2.3.1.3 White Blood Cells (WBCs):

White cells are fewer in number than red blood cells, with a ratio of about 1 white blood cell to every 600 to700 red blood cells in as is shown Fig 2.3 (Sarode, 2018).



White Blood Cells

Fig 2.3 white blood cells.

2.3.1.4 Neutrophil (NEUT):

The neutrophil is usually the most abundant of the WBC in the peripheral blood. It is an amoeba-like phagocyte, loaded with a variety of potent enzymes (Eliot, 2014).

2.3.1.5 Lymphocytes (LYM):

Lymphocytes are typically slightly less numerous than neutrophils in the peripheral blood, except in young children (Eliot, 2014).

S2.3.1.6 Hematocrit (HCT):

It is the volume occupied by packed red cells serial hematocrit is based on the extent hemodilution processes occur following blood loss body hematocrit Hct_0 , signifies the overall average concentration of red cells in blood throughout the body (William , 1966).

2.3.1.7 Hemoglobin (HGB):

Haematologists are not in agreement as to the "normal" amount of hemoglobin in the blood, nor they areagreement as to what amount of haemoglobin can be considered "a haemoglobin value of 100 per cent (John , 1954).

2.3.1.8 Platelets are blood cells (PLT):

That helps with clotting, and it is essential to maintain their levels (Alana Biggers, 2018).

2.3.1.9 Plateletcrit (PCT):

Plateletcrit (PCT) shows a percentage of blood occupied by platelets (medicine, 2019).

2.3.2 Complete blood cell measurements:

CBC for the blood samples of the dilutes are meafured by passeing amount of blood through an aperture delitriccurrent is passing brought a variation in the impedance between the ends. Then lytic reagent for breaking red blood cells was added in the solution. The same aperture did not affect the white blood cells while platelets leaving them intact. The which restring solutions were passed through another detector to for the measurements of red blood cells, white blood cells and platelets. The counter was designed for measuring white blood cells (WBC), red blood cells (RBC), and hemoglobin content (HGB), hematocrit (HCT); mean (red) cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets (PLT), Neutrophil (NEUT) and Lymphocytes (LYMPH).

2.4 Laser-tissue interaction:

The term 'laser light' is a generic, in that one of the defining properties of laser light – that it is monochromatic – requires a qualifying emission wavelength annotation. However, the use of all laser wavelengths in clinical dentistry serves to effect controlled and precise changes in the target tissue, through the transfer of electromagnetic energy. A competent laser dentist will establish predictable laser-tissue interaction and all its definable outcomes, before embarking on that interaction. It is essential that through the correct choice of a given laser wavelength for treating a given target tissue, a minimum level of power is employed both to effect the desired result and to minimise the risk of collateral damage (Parker, 2007).

2.4.1 Basic considerations:

Transmission: in this way, the beam enters the medium, but there is no interaction between the incident beam and the medium. The beam will emerge distally, unchanged or partially refracted.

Scatter: In this phenomenon there is some interaction, but this is insufficient to cause complete attenuation of the beam.

Reflection: Either the density of the medium, or angle of incidence are being less than the refractive angle, results in a total internal reflection of the beam

Absorption: In this case the incident energy of the beam is attenuated by the medium and transferred into another form.

Photopyrolysis: consistent with ascending temperature change from $60^{o}C$ to $90^{o}C$, target tissue proteins undergo morphologic change, which is predominately permanent (Parker, 2007).

CHAPTER THREE

EXPERIMENTAL PART

3.1 Introduction:

To study diode laser (λ= 532 nm, power=1000 mW) effect on human blood, after and before irradiated to different times from 10 min to 30 min.

Study Design: Human blood irradiated by (diode) laser (λ= 532 nm, power=1000mW).

Place and Duration of Study: The irradiation process was done in Institute of Laser, Sudan University of science and technology (SUST), and the samples were taken in the Reyada Hospital, Khartoum- Sudan.

3.2 Materials

Blood samples were taken from one adult healthy volunteer by medical standard laboratory conditions and blood samples were saved in a tube to prevent from coagulation to (EDTA) as is shown in Fig3.1.

Fig3.1 The human blood sample.

One sample used as control and other were exposed to the diode laser with different exposure times as is shown in Fig3.2 .by using stopp watch with alarm as is shown in Fig3.3.

Fig3.2.The Diode Laser

Fig3.3.The Stop Watch

Centrifuges machine to separate the plasma from blood as is shown in Fig3.4.

Fig3.4.The Centrifuges

Complete blood count (CBC) machine to separate the plasma from blood as is shown in Fig3.5.

Fig3.5.The Complete Blood Count (CBC)

UV -Vis spectra. Blood was diluted with normal saline and placed in Kart ell disposable polystyrene cavetto of 10 mm path length. The cavetto is placed in Jasco-670) UV –Vis spectrophotometer for analysis the spectra were scanned in the region between 200 nm to 800 nm using Jasco-670) at Laser institute laboratory, SUST, Khartoum as is shown in Fig3.5.

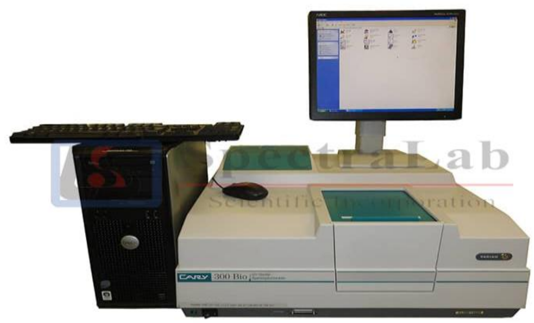


Fig3.5 UV -Vis spectra

3.3 Method:

Apparatus were prepared as shown in the experiment setup, then ten samples of blood were taken from one adult healthy person, these samples were distributed into six EDTA anticoagulant containers by rate of 2.5 ml in every container, five EDTA containers were irradiated by Diode Laser to three different exposure times mainly (10, 20 and 30) min, one in-irradiated blood sample was considered as a control sample.

This treatment was done by a constant power of laser beam “ 532 nm wavelength and output power 1000 mW (Low-level laser therapy), then the samples were analyzed using CBC "Complete Blood Count" machine and UV-Vis spectrometer to study the optical properties, as listed the result in tables and graphs. And as is shown in Fig3.6.

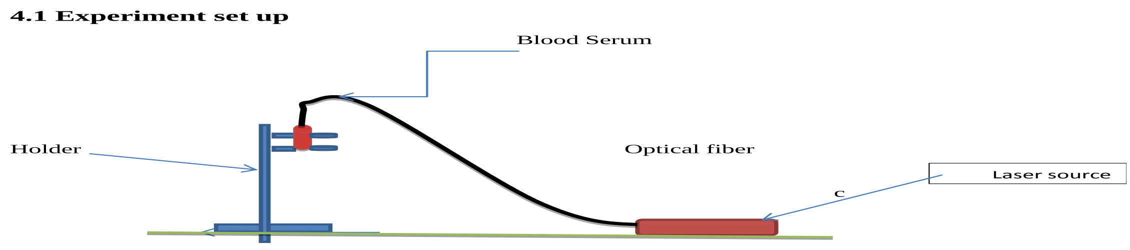


Fig3.6 Experiment set up

A spectrophotometer is a device to measure how much a chemical substance absorbs light by measuring the intensity of light as a beam of light passes through a sample solution. The basic principle is that each compound absorbs or transmits light over a certain range of wavelength.

The optical absorption spectra were taken in the range of (200-800) nm wavelength for blood samples. Irradiation process was done by Diode Laser (532 nm wavelength and 1000 mW power) for exposure time 15 min.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Introduction:

The samples were treated a according to the photochemical interaction. The samples were analyzed by means UV spectrometer to study the optical properties compared to CBC machine listed the result in graphs and tables.

4.2 Results and Discussion:

4.2.1 UV–Vis spectroscopy characterization:

The biological effects of low-level laser light irradiation were first discovered and studied by Mester *et al*. in the 60-s, (Mester, *et al*., 1972).

The first low-level laser therapy systems were based on He-Ne (632.8 nm) lasers. Currently, diode lasers are replacing He-Ne lasers. Due to these lasers are small, simple in maintenance, inexpensive in application and have a long time. Red and infrared lasers are generally used for low-level laser therapy (LLLT). It is reported that clinical trials application laser light in other colors are mainly blue and ultraviolet laser light, but at currently such systems are not widely used. Light therapy with application of LED light instead of laser light (LED therapy) is another novel method of therapy. Sunlight was long known to improve acne, and this was thought to be due to antibacterial and other effects of the ultraviolet spectrum which cannot be used as a long-term treatment due to the likelihood of skin damage (World Health Organization, 2010).

Fig4.1 shown the absorption spectra of the irradiated blood cells with 532 nm for 10 min and non irradiated blood cells counterpart are recorded in the range of 200–800 nm The samples showed absorption bands with λ_{max}= 576.33 and 541.15nm for the both samples. The spectrum of the irradiated sample reveals a degradation appears in the beak of 541.15 nm.

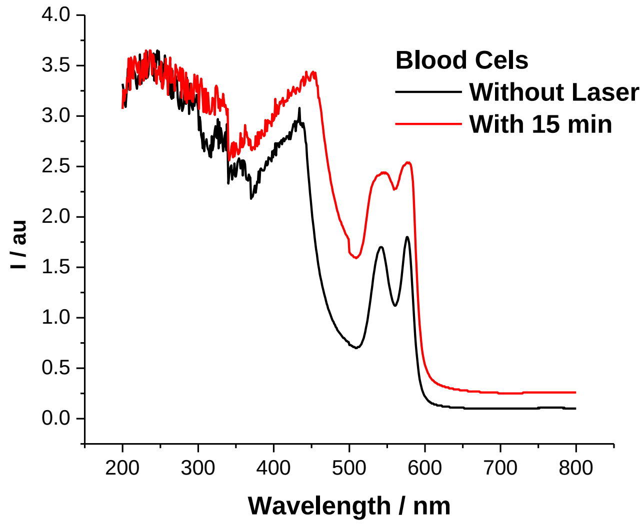


Fig.4.1 Spectra for before and after irradiated blood Cells to Diode laser for 10 min.

In the absorption spectra of the irradiated whole blood with 532 nm for 10 min and non irradiated whole blood are recorded in the range of 200–800 nm Fig. 4.2 shows slight significant changes.

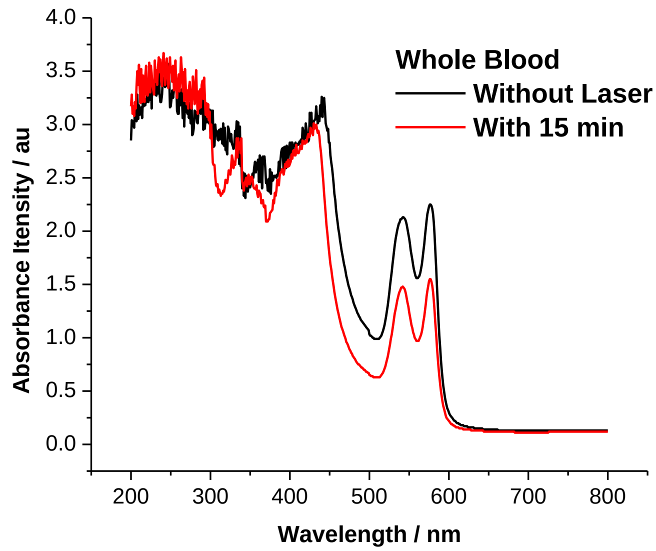


Fig .4.2 Absorbance Spectra for before and after irradiated whole blood to diode laser for 10 min.

The absorption spectra of the irradiated plasma with 532 nm for 15 min and non irradiated plasma are recorded in the range of 200–800 nm as shown in Fig. 4.3 long-term condition cells (LTCs) with λ_{max}= 414.32 nm; the interaction results in degradation of the LTCs.

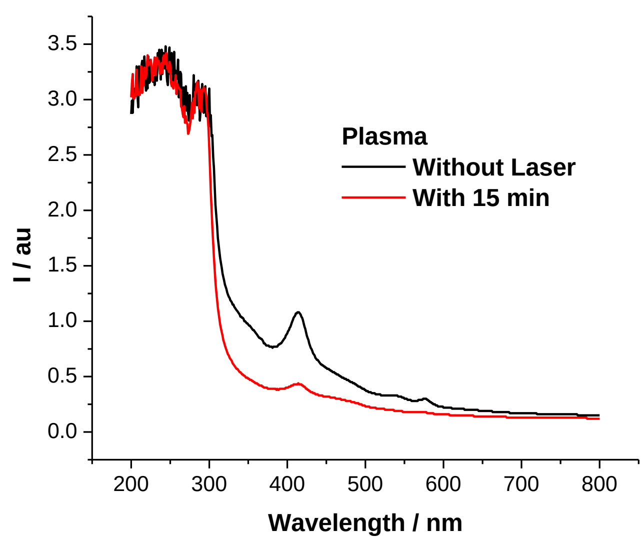


Fig .4.3 Spectrum for before and after irradiated Plasma blood to Diode laser for 10 min

4.2.2 The Effect of Diode Laser Irradiation on CBC

Table 4.1 Complete Blood Count (CBC) for different sample

No	Item	0/min	10/min	20/min	30/min
1	WBC $\times 10^{9}$ /L	2.85	2.64	2.89	3.44
2	RBC$\times 10^{12}$/L	5.73	5.74	5.73	5.67
3	HGB g/Dl	16.3	16.2	16.2	16.1
4	HCT %	47.3	47.2	47.3	46.9
5	MVC fL	82.5	82.2	82.5	82.8
6	MCH pg	28.4	28.2	28.3	28.4
7	MCHCg/Dl	34.5	34.3	34.2	34.3
8	PLT $\times 10^{9/}$/L	156	156	158	168
9	LYM$\times 10^{9}$/L	1.41	1.24	1.39	1.52
10	NEUT$\times 10^{9}$/L	1.09	1.09	1.01	1.42
11	NEUT %	38.6	41.1	35	41.4
12	PCT %	0.164	0.167	0.169	0.18

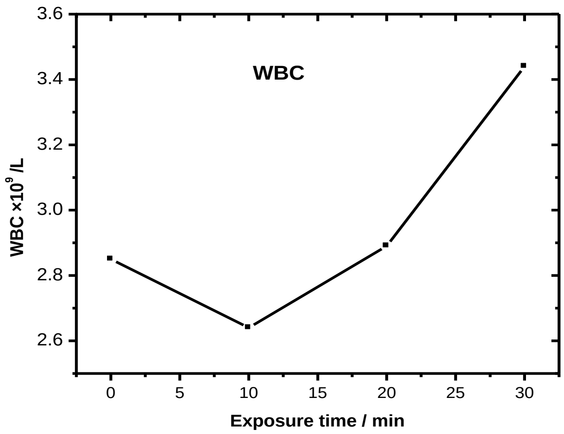


Fig. 4.4 shows the white blood cells (WBC)

The blood were exposed for 10 minutes resulted in decreasing of WBC; however, When espoused for 20 minutes no change was observed of WBC but for 30 minutes were increased the WBC ,values which gave a positive effect on WBC at this laser dose as shown in Fig .4.4.

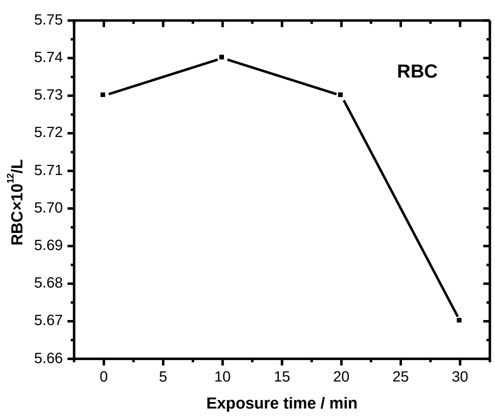


Fig. 4.5 shows the red blood cells (RBC)

Fig .4.5 had shown the blood exposed for10 minutes which results in increasing of RBC which is similar when exposed for 20 minutes. The best laser dose that has a positive effect on RBC is to 30 minutes in comparison to other intervals.

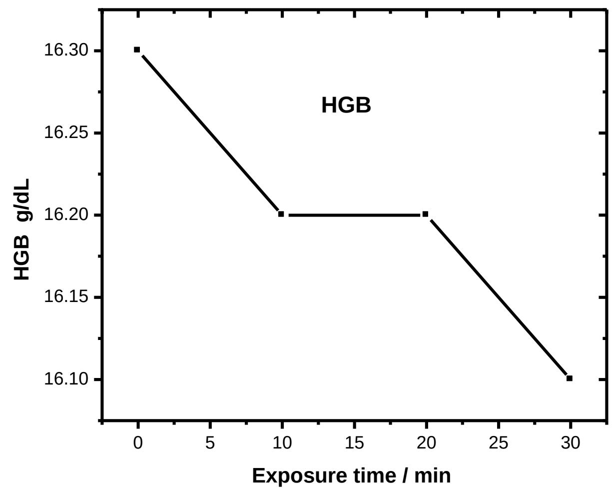


Fig. 4.6 shows the Hemoglobin

As we seen in table 4.1 and **Fig. 4.1 and Fig. 4.6** The decreasing of Hemoglobin (HGB) when irradiated fo10 minutes and 20 minutes without any change .But at the Hemoglobin (HGB) values was decreased again .

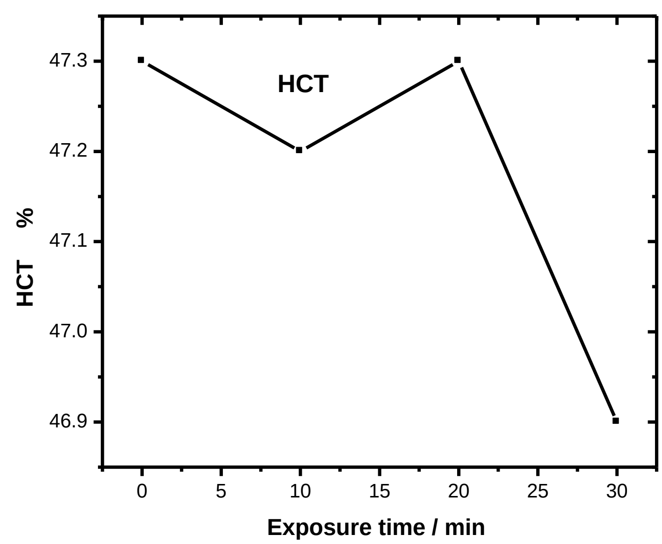


Fig. 4.7 shows the Hematocrit

The blood exposure to 10 minutes results in decreasing of Hematocrit (HCT) however espousing to 20 minutes results no change was observed of HCT but to 30 minutes results in decreasing again of HCT values the best laser dose that has a positive effect on HCT is 30 minutes fig.4.7.

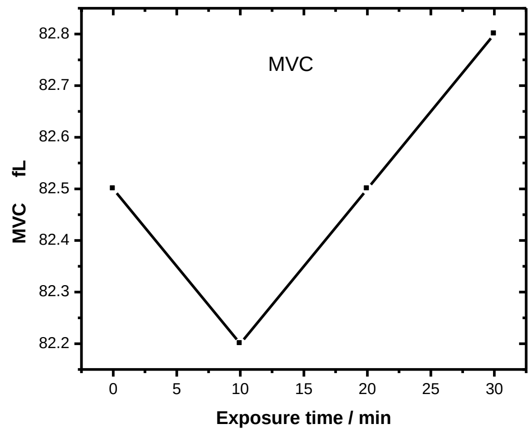


Fig. 4.8 shows the Mean Cell Volume

The blood exposure to 10 minutes results in decreasing of Mean Cell Volume (MVC) however espousing to 20 minutes results no change was observed of MVC but to 30 minutes results in a significant increase of MVC values the best laser dose that has a positive effect on MCV is 30 minutes fig.4.8.

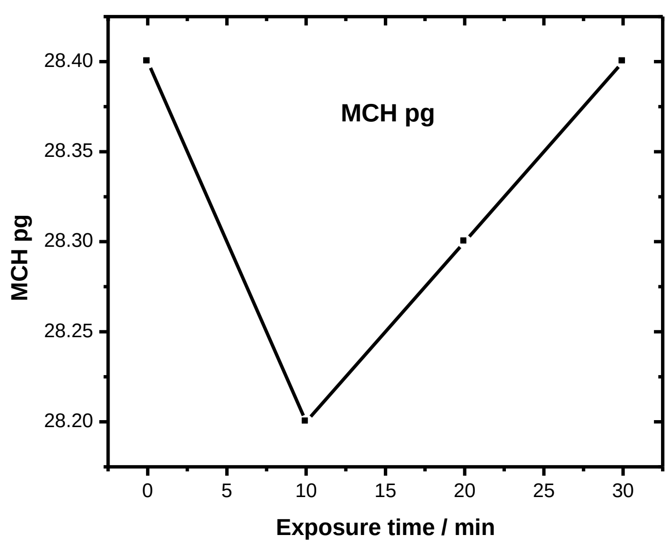


Fig. 4.9 shows The Mean Cell Hemoglobin (MCHpg)

The blood exposure to 10 minutes results in a significant decreasing of Mean Cell Hemoglobin (MCHpg) however to 20 minutes results in increasing of MCHpg but to 30 minutes results no change was observed of MCHpg values the best laser dose that has a positive effect on MCHpg is 20 minutes fig.4.9.

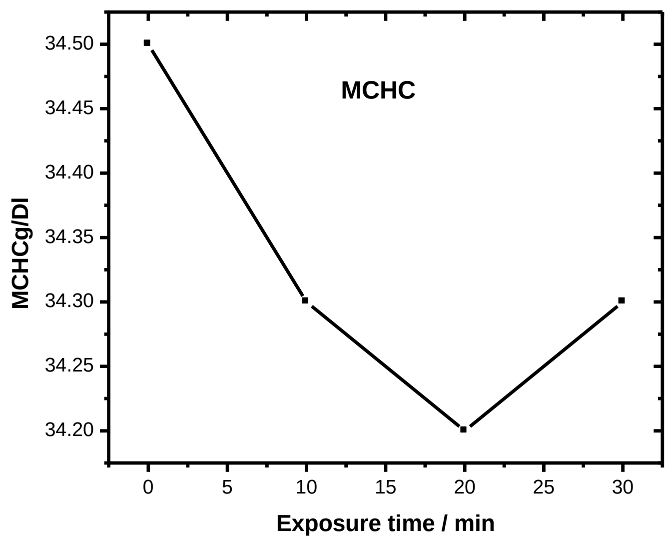


Fig. 4.10 shows The Mean Cell Hemoglobin Concentration (MCHc).

The blood exposure to 10 minutes results in decreasing of Mean Cell Hemoglobin Concentration (MCHc) however to 20 minutes results in decreasing again of MCHc but to 30 minutes results no change was observed of MCHc values the best laser dose that has a positive effect on MCHc is 30 minutes fig.4.10.

The blood exposure to 10 minutes results in stability of the Neutrophil Number (NEUT) but to 20 minutes results no change was observed of NEUT however to 30 minutes results in a significant increase of NEUT values the best laser dose that has a positive effect on NEUT is 10 minutes fig.4.11.

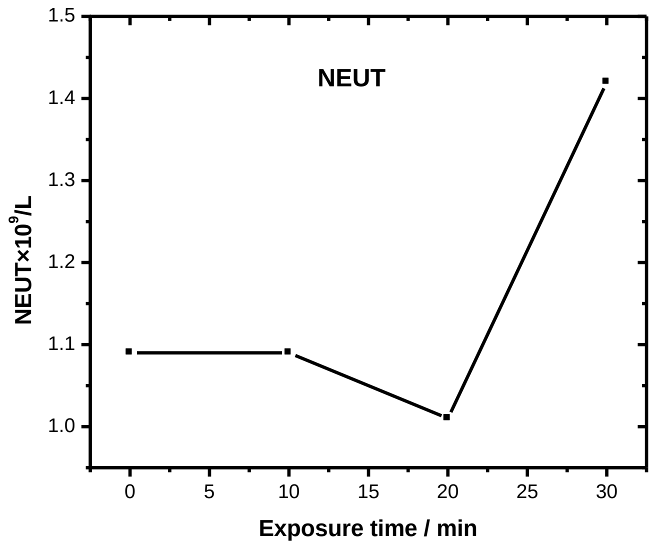


Fig. 4.11 shows the Neutrophil Number

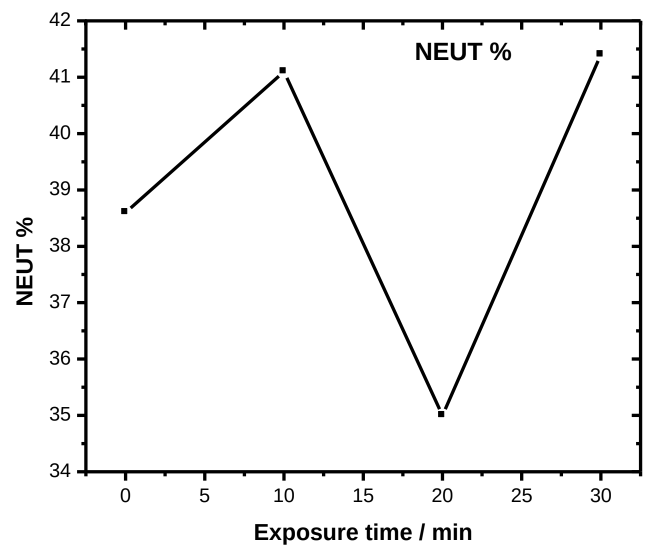


Fig. 4.12 shows the Neutrophil Percent

The blood exposure to 10 minutes results in increasing of Neutrophil Percent (NEUT) but to 20 minutes results in decreasing of NEUT however to 30 minutes results no change was observed of NEUT values the best laser dose that has a positive effect on NEUT is10 minutes fig.4.12.

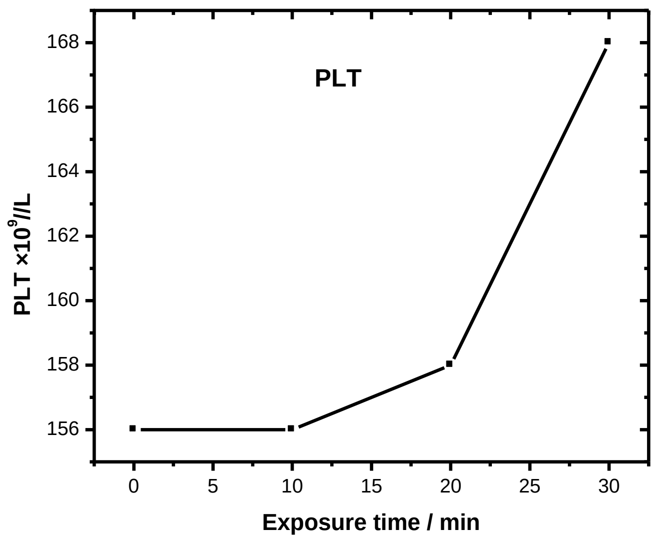


Fig. 4.13 show the Platelet Count

The blood exposure to 10 minutes results in stability of Platelet Count (PLT) however to 20 minutes results in increasing of PLT but to30 minutes results in a significant increase of PLT values the best laser dose that has a positive effect on PLT is 30 minutes fig.4.13.

The blood exposure to 10 minutes results in decreasing of Lymphocyte Number (LYM) however to 20 minutes results no change was observed of LYM but to 30 minutes results in increasing of LYM values the best laser dose that has a positive effect on LYM is 10 minutes fig.4.14.

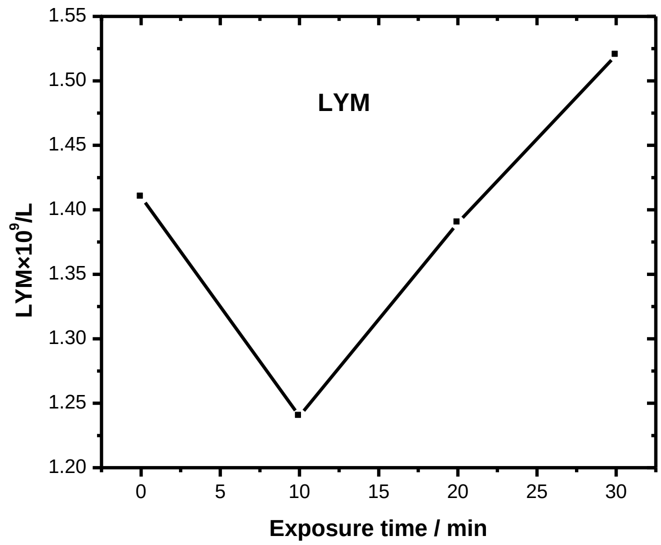


Fig. 4.14 shows the Lymphocyte Number

4.3 Conclusion:

The best laser dose has a positive effect on almost all blood cells and their indicators are approximately 30 minutes as white blood cells, red blood cells ,hemoglobin , mean cell volume , Hematocrit , mean cell hemoglobin concentration , platelets . and the best laser dose has a positive effect on Neutrophil and Lymphocytes are approximately 10 minutes and the best laser dose has a positive effect on mean cell hemoglobin are approximately 20 minutes. Thus our research proved that laser treatment of 532nm diode and high power of 1000 mW is useful for reactivating the functional capacity of preserved blood and also increases the number of blood cells, thus increasing the function of this blood.

4.4 Recommendations:

Recommended for further work; up to involve large number of patient Within 10 minutes, the effect always works but within 30 minutes, it affected all elements except mean cell hemoglobin (MCH) and mean cell hemoglobin concentration

(MCHC). While with 20 minutes there was no effect except for mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) , platelets (PLT), Neutrophil (NEUT) and Lymphocytes (LYMPH).

Those who want to work using a diode laser (532 nm, 1000 mW) in blood with 20 minutes; it does not affect like by 10 or 30 minutes.

Anyone who wants to increase white blood cells (WBC), Lymphocytes (LYMPH), it can be done by exposing to 10 minutes, but if one wants to decrease; it needs 30 minutes.

To increase white blood cells (WBC), mean (red) cell volume (MCV), Neutrophil (NEUT) and mean cell hemoglobin (MCH) it can be done by exposing to 20 or 30 minutes, and when want to decrease; one can expose to 10 minutes.

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