Study the effect of low-power helium-neon laser (632.8nm) on the human red and white blood cells

Ahmed. K.Sameer[1,*], Wisam D.Jalalb[2] and Khalid D. Ahmed[3]

[1]*Department of physics, College of pure Science Education, University of Anbar, Anbar, Iraq*
[2]*Department of physics, College of Science, University of Anbar, Anbar, Iraq*
[3]*Department of General Science, College of Basic Education Haditha, University of Anbar, Anbar, Iraq*
[*]*Corresponding Author:* ahmed.k.s1991@uoanbar.edu.iq

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Abstract: The purpose of this experiment was to investigate the impact of laser radiation on certain blood characteristics of students. Blood samples were collected from healthy individuals who were normal students, under standard laboratory conditions. A total of twenty healthy were obtained. Each sample was divided into three parts, with one part serving as a control (untreated) and the other two parts being exposed to laser radiation with power levels of 0.3mW and 0.9mW. The blood samples were irradiated using a continuous helium-neon laser with a wavelength of 632.8 nm for a duration of 40 minutes. Blood analysis was conducted using a complete blood count (CBC) device, and the results of the exposed samples were compared to the control samples. After 40 minutes, the CBC analysis showed changes in the components and characteristics of the blood. The number of white blood cells significantly increased with higher power levels, and there was also an increase in the number of platelets. Random fluctuations in the sedimentation rate of hemoglobin and red blood cells were observed. However, statistical analysis of the average counts of white blood cells, red blood cells, and platelets for all groups indicated that the observed increases were not statistically significant ($p > 0.05$). This emphasizes the importance of low-power lasers in various biomedical applications for treating different illnesses. The study concluded that laser radiation altered the biological components of the irradiated samples.

***Keywords*:** Laser, Hb, RBC, He-Ne laser, Blood

1. Introduction

Modern medicine uses lasers as essential components of many kinds of apparatus; due to the wavelength's optimization for human tissue response, lasers have become incredibly popular (1-3). Laser technology may be utilized for both surgical and non-surgical(4-6). The biomedical and therapeutic uses of lasers rely on the interactions between light and tissue, which within cells, such as mitochondria, cytochromes, and endogenous chromospheres. This connection leads to many phenomena, including fluorescence, chemical reactions, and thermal impacts (6-8), due of hemoglobin's significant optical absorbance, laser treatments often target red blood cells (9&10). Red blood cells (RBCs) are present in significant quantities within organs. Therefore, the harmful impact of these radiations occurs at the cellular level until hemoglobin is introduced into the cell.

These physiological manifestations trigger many cellular responses, including enzyme deactivation, alteration in metabolic rate, and coagulation, among other structural modifications inside the cell (6,11,12).

Among other therapeutic uses, low-level energy lasers have acquired established therapy in wound healing, nerve restoration, and pain management. They have also been utilised lately in the non-invasive prevention of musculoskeletal and cutaneous issues (13-15). Studies have shown the advantageous effects of low-energy intensity lasers in promoting the healing of damaged wounds in individuals with diabetes (5,16).

Leucocytes are extremely important in disease resistance because they serve as the body's primary immune system protection by utilizing multiple tools such as lysis and phagocytosis (17-19).

Stadler et al., (20) studied in-depth how laser irradiation affects RBCs and peripheral blood lymphocytes in vitro. The research used red light with a wavelength of 660nm and observed a substantial augmentation in the quantities of lymphocytes when exposed to it in whole blood (20). The laser's impact on these immune cells is believed to be mediated via the electron transport chain, leading to an elevation in adenosine triphosphate synthesis and subsequent enhancements in cell metabolism. This, in turn, produces the photobiostimulation effect often associated with laser irradiation (21).

Low level laser therapy is typically performed with a He-Ne laser (wavelength of 632.8 nm).

In this regard, Laser radiation is believed to protect the erythrocyte membranes by stabilizing the cell membranes and thus reducing hypotonic hemolysis. Due to the significant absorption of hemoglobin in this tissue, the activity of the He-Ne laser had a pronounced impact on multiple erythrocytes (RBCs), the amount of hemoglobin (HGB), and the hematocrit (HCT). Lasers that use photochemical influences and do not destroy or fragment tissue, (22). Photochemical -tissue interactions are substantial in therapy medications, alike photodynamic therapy, (20,23). Experiments conducted in a controlled laboratory setting using light wavelengths between 632.8 and 660 nm demonstrated alterations in the behavior of blood cells, including modifications in the clumping together of red blood cells and an enhanced growth of lymphocytes [24-25]. Furthermore, several authors reported biological responses to red lasers, and the observed effects could be linked to changes in blood microcirculation [26-28]. Nonetheless, there has been little understanding of the helpful and harmful threshold values of laser radiation on blood. Furthermore, the permissible limits of radiation and exposure length have not been determined. This study was to investigate whether the low laser energy density (He-Ne Laser) induces the WBCs and RBC number count in the whole human blood (in vitro).

2. Materials and Methods

Whole blood samples (3 ml) were collected from a cohort of 20 , including both males and females , with ages ranging from 18 to 25 years. To prevent clotting, blood aliquots were put in tubes containing an anticoagulant. From the EDTA blood sample, three aliquots were created, One was used as a control (un irradiated) and the other will be irradiated exposed to laser radiation with 0.3mW and 0.9mW. blood samples It was provided by the Biology Laboratory at the College of Basic Education Haditha / Anbar University (Iraq). Blood Irradiation Lasers used in this research are (He-Ne) 632.8 nm red laser gas State with output power, 0.3 and 0.9 mw. Blood samples (~3 ml) were then irradiated with lasers for 40 min exposure time.

During the study the number of white blood cells (WBC), hemoglobin (Hb) were measured**,** means and standard deviations of the different experimental groups were done in the SPSS statistical software. The Significant differences accepted if the P value was less than (0.05) and rejected if the value was exceeded.

3. Results and Discussion

It is clear to us from the below table that there are no significant differences between blood samples (male and female) before and after irradiation, and there is non-significant difference, but there is a very small difference. Table 1 shows us a slight increase in the percentage of white blood cells when exposed to 0.3 mw and 0.9 mw laser radiation, because the increase in radiation power led to the decrease in calcium concentration. Laser light is known to enhance lymphocyte count by increasing cell proliferation and migration and preventing cell death.[29]

Observed during this study that there are no statistically significant differences between blood samples (male and female) before and after irradiation (table 2) , and there is no significant difference, but there is a small numerical difference. Low-power laser beam can induce many biological effects in living cells via biochemical modification rather than thermal effect. Red laser light affects the properties of blood at the molecular level.

Hemoglobin preferentially absorbs laser light at 632.8 nm wavelength, Laser absorption causes partial photo dissociation of hemoglobin under specific circumstances[30]. Blood cell components can be affected by laser light, as the presence of transmembrane proteins can cause protein degradation and cell lysis. The degree of effects is mostly determined by the radiation energy and time. Laser light can cause the following effects when it interacts with blood cells:

1- Increase cell activity.

2- Activate cells by stimulating the redox activity of the cellular respiratory chain

3-Promote cell growth and proliferation

4-Stimulate the degradation of protein RNA and DNA

5- Activation of ATP production.[32,33]

Table 1 : Shows the effect of radiation with a capacity of (0.9mw - 0.3mw) on W.B.C.

Male				
Studied trait	**T_1 (non irradiation)**	**(0.3mw) T_2**	**T_3 (0.9mw)**	**p-Value**
HGB	14.084±1.020	14.23±0.990	14.280±0.962	
Female				
Studied trait	**T_1 (non irradiation)**	**T_2 (0.3mw)**	**T_3 (0.9mw)**	**p-Value**
HGB	12.530±0.377	12.410±0.370	12.470±0.332	

Table 2 : shows the effect of radiation with a capacity of (0.9mw - 0.3mw) on R.B.C.

Male				
Studied trait	**T_1 (non irradiation)**	**T_2 (0.3mw)**	**T_3 (0.9mw)**	**p-Value**
WBC	6.558±0.400	6.655±0.444	6.768±0.384	
LYM	2.216±0.191	2.273±0.216	2.255±0.202	
LYMP	34.380±2.508	34.470±2.654	34.090±6.571	
Female				
Studied trait	**T_1 (non irradiation)**	**T_2 (0.3mw)**	**T_3 (0.9mw)**	**p-Value**
WBC	6.907±0.644	6.927±0.651	7.016±0.635	
LYM	2.096±0.169	2.039±0.164	2.100±0.171	
LYMP	32.040±2.864	30.910±2.762	31.0709±2.912	

4. Conclusion

This study discovered that subjecting blood samples to a 40-minute exposure (in vitro) had a little impact on lymphocytes (white blood cells) and hemoglobin. These effects may become more noticeable as exposure duration increases or storage period, and laser radiation does not have a very significant effect on blood samples. As the statistical analysis of the groups, showed, that the increase was insignificant ($p > 0.05$).

Credit Author Contributions Statement:

Conceptualization: Ahmed. K.Sameer, Wisam D.Jalalb and Khalid D. Ahmed

Data curation: Khalid D. Ahmed

Formal analysis: Ahmed. K.Sameer and Khalid D. Ahmed

Funding acquisition: Ahmed. K.Sameer, Wisam D.Jalalb and Khalid D. Ahmed

Investigation: Wisam D.Jalalb

Methodology: Wisam D.Jalalb and Khalid D. Ahmed

Project administration: Ahmed. K.Sameer, Wisam D.Jalalb

Resources - Ahmed. K.Sameer, Wisam D.Jalalb and Khalid D. Ahmed

Software -Wisam D.Jalalb

Supervision - Khalid D. Ahmed

Validation: Ahmed. K.Sameer, Khalid D. Ahmed

Visualization-Ahmed.K.Sameer, Wisam D.Jalalb and Khalid D. Ahmed: Writing–original draft, Writing–review & editing -

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