Original article

Biostimulation of human blood using He-Ne 632.8 nm and O-Xp 820 nm lasers

Alaa Alabaid, Wafaa Salah, Bashir Algaily, Wasil H.M. Salih*

Al neelain University, Faculty of Science and Technology, Department of Laser and Renewable Energy,

Algamhouria Avenue, Khartoum, the Sudan, 11121

ARTICLE INFO

Article history:
Received 2016 November 17th
Reviewed 2017 March 10th
Accepted 2017 April 10th

Keywords
Biostimulation
low level laser (LLLT)
human blood analysis
He-Ne

Abstract

The human blood biostimulation is of great importance to tackle some blood-related diseases since it showed that it can enhance the count of some blood components. Using two laser sources of different wavelengths and energies, 34 blood samples were irradiated to check for the change in the blood parameters. The results showed that He-Ne laser of 632.8 nm enhances the counts of the human blood components more than using O-Xp of 820 nm. For instance, the white blood counts of the He-Ne irradiated samples increase of about 60% while just 45% is noticed for the O-Xp irradiated samples. However, the O-Xp increases the human blood absorption of light as checked by UV/VIS spectrometer more than for He-Ne irradiated samples. These results agree well with the previous studies, nonetheless the current study makes a comparison between two commonly used laser sources in such field in order to look for the most suitable wavelength to enhance human blood counts.

***Corresponding author**: wasilhashim@yahoo.com

Introduction

The effect of low level laser therapy (LLLT) on blood has attracted the attention of the physicist for the last decades, since they figured out that it changes the physical properties of human blood which may help to tackle human endanger problems. Instead of producing a thermal effect, LLLT may act via no thermal or photochemical reactions in the cells, which is referred to as photobiology or bio-stimulation. Laser radiation and monochromatic light may alter cell and tissue function. Laboratory studies claimed that irradiation stimulates collagen production, alters DNA synthesis, and improves the function of damaged neurological tissue (Csele, 2004 and Dais, 2009.) Accordingly, biostimulation at the tissue level has shown a number of important effects, such as increasing of blood microcirculation during and after the LLLT, formation of new blood and lymphatic vessels, and enhancement of collagen synthesis. Improvement of blood microcirculation facilitates the recovery of oxygen levels in damaged cells, as well as normalization of the delivery of pharmacological preparations employed to treat some diseases (Lim, 2011).

So, microcirculation reduction leads directly to the inflammation of cell tissues, which changes the quantity of blood supply to cells, resulting in ischemic injury. So any influence that can shorten the duration of the ischemic state will have beneficial effects on the course of the disease. Moreover, there are many positive effects at both cellular and tissue level for some diseases treatment such as inhibiting blood supply and distraction of tissue at different levels which decreases regenerative abilities of cells. Additionally, LLLT increases the epithelial activity as well as drainage of interstitial liquids, perception of sensory nerves and the amplitude of action potential of nerves. Nevertheless, LLLT functions to help for the biostimulation of the mitochondrial process with the increase of adenosine triphosphate (ATP) and optimum oxygen consumption, thus stimulating the immune system (Niemz, 2007).

LLLT primarily stimulates the response of the body's cellular damage. In soft tissue injuries, for example, LLLT rapidly increase the proliferation of growth factor cells (i.e. macrophages, fibroblasts and mast cells in particular) and is, therefore, widely used to accelerate tissue repair and mobilize chronic conditions. With appropriate protocols, it can also effectively block pain message signals through nociception suppression, stimulate acupuncture and trigger points and increase endorphin release.

During the first decade after the invention of the laser by Mailman (1960), many studies were conducted investigating potential interaction effects using all types of laser systems and tissue targets. Although the number of possible combinations for the experimental parameter is unlimited, mainly five categories of interaction type are classified today; these are photochemical interaction, thermal interaction, photo ablation, plasma-induced ablation, and photo disruption. In particular, the physical principles governing these interactions are reviewed. Emphasis is placed on microscopic mechanisms controlling various processes of laser energy conversion (Niemz, 2007).

Numerous studies have been conducted during the last two decades to investigate the usefulness of laser blood biostimulation. Recently, Rathore and Ali, 2013, have investigated the effect of LLLT on rheological parameters of human blood, they noticed a change in both viscosity and size of erythrocytes (Rathore and Ali, 2013). Moreover, Al Timimi et al. 2011, have studied the photodynamic therapy and green laser therapy on different blood cells *(Al Timimi et al*., 2011), they exposed human blood to a laser source with a wavelength of 532 nm using various energy fluencies to see the effects on blood samples. Their results showed that exposing blood to certain kinds of laser can help to improve some components of blood, in retain it helps to tackle some diseases. In addition, they showed that laser therapy decreases the viscosity of blood, thus increasing the electrophoretic mobility of erythrocytes (Al Timimi et al., 2011). Mi *et al.* 2004, have conducted a study to point out the effects of laser irradiation on rheological properties of blood in vitro. They showed promising results in the modulation of hemorheological properties; when blood samples from patients with abnormally high values of ESR were irradiated, the values of ESR were lowered significantly by either of the two laser wavelengths that they used (632.8 nm and 532 nm). The laser irradiation reduced blood viscosities at different shear rates for the hyper-viscosity blood samples. However, their results showed that the laser irradiation increased the electrophoresis mobility of erythrocytes (Mi *et al*., 2004). On the other hand, Golub et al. 2003, have studied the influence of laser blood photo-modification on dynamic characteristics of surgical stress. They investigated the laser photo-medication of blood which has been reported to reduce the number of medicaments required for a certain level of patient's anesthesia (Golub *et al.,* 2003).

One sees that the LLLT devices normally are occupied with gallium arsenide (GaAs), gallium aluminum arsenide infrared semiconductor (GaAlAs), and helium neon (He-Ne) lasers. The 632.8 nm wavelength He-Ne laser emits visible red light and may have a shallow penetration into skin. The GaAlAs, infrared laser has a longer wavelength than red beam laser and may have deeper tissue penetration. The 904 nm wavelength GaAs laser is most commonly used for pain and inflammation because it has the deepest tissue penetration. As a result, it may be less suited for wound healing. Varying treatment parameters may involve altering pulse rate, applicator placement, wavelength, irradiance (power/unit area), beam divergence, spot size, delivery (fiber optic, direct), polarity, pulse duration, and duty cycle (Csele, 2004, Dais, 2009 and Rathore and Ali, 2013). Consistently, the current study comes as continuation of the previous efforts to study the effects of LLLT on human blood using two sorts of lasers; He-Ne laser with a wavelength of 632.8 nm and Omega XP laser with a wavelength of 820 nm. In this study 3B Lasers were used to tackle some technical problems. The infrared light at 820 nm provides a penetrative, highly effective wavelength for soft tissue and sports injuries, pain relief, acupuncture point stimulation and smoking cessation (Dais, 2009 and Al Timimi, *et al.,* 2011).

Materials and Methods

Samples Collection

34 blood samples (5 ml for each) were taken from 34 healthy volunteer adults. Next, blood samples were saved in an anti-coagulant tube to prevent coagulation. Each blood sample was further divided into three sub-samples (two of 2 ml each and one of 1 ml). Both 2 ml samples were irradiated to two different sorts of laser whereas the other one was kept as control, cf. section 3.2. Blood components for the irradiated samples were compared to the control sample.

The blood samples were investigated using two approaches; automate haematology analyzer to count the complete blood cells, and UV/VIS spectroscopy to check the absorption. All measurements were conducted within 3 hours after collecting the blood samples to avoid artefacts causes.

The entire manipulations of this study, including sample collection and preparation, were carried out according to local; at Al Neelain University, the Sudan, and international code of conducts and Guiding Principles for Research Involving Animals and Human Beings as adopted by The American Physiological Society.

laser irradiation

The two major groups of the blood samples were exposed to two types of laser; medical He-Ne laser and Omega-XP laser. He-Ne Intra-vascular low power laser irradiation therapy was used to irradiate the blood samples, since it has been used widely in the biostimulation of humans blood (Csele, 2004). KZ-350-LB setup was used. He-Ne laser with a wavelength of 632.8 nm, in a continuous wave mode was applied to the samples. The parameters of the laser device were set to the following; beam diameter at aperture ~2.3×10^{-6} mm, power density equals 3.3 mW/cm^2. 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.

The second type of laser was Omega XP laser. It was used to irradiate the second group of the blood samples, because Omega Laser Systems is one of the well-known manufacturers

in the field of LLLT. The parameters of the laser device were set to the following; wavelength of 820 nm at 6 mW and a power density of 3.3 mW/cm^2 in a continuous wave mode. The beam mode was TEM00. The distance between the laser source and the samples was kept to 10 cm.

Measurement tools

After irradiating samples to both laser sources, each sample was divided again into two sub-samples for further investigations using both automate hematology and UV/VIS Spectrophotometer. Some of the human blood components such as the: Red Blood Cells (RBCs), White Blood Cells (WBCs), Platelet (PLT),

Hematocrit (HCT) and Hemoglobin (HGB) were examined to see

Whether it has been changed after irradiation with lasers or not. To do so, automate haematology analyzer machine (Mindary, 2800) was used. For the analysis, dilute samples of blood were passed through an electric current aperture, to make an impedance variation between the ends. Then a lytic reagent was added in the solution to break the red blood cells. Whereas it did not affect the white blood cell and platelets. Further, these solutions were passed through another detector, to get the counts for the red blood cells, white blood cell and platelets. Blood samples were kept in a shaking device at room temperature 25 °C during the experimental setup. Next, UV/VIS Spectrophotometer (Shimadzu ™ min 1240) was used to analyze the second part of both irradiated and non-irradiated blood samples. The UV/VIS Spectrophotometer checks the change in the optical properties relative to normal blood optical properties. To analyze samples using the UV/VIS spectroscopy, 5 µl of the blood was added to 10 ml of distal water in plane container.

Results

The results of the entire measurements of both control and irradiated blood samples are presented in figures 3.1 – 3.5. Figure 3.1(a) presents the RBCs, figure 3.2(a) presents WBCs. The PLT, HCT and HGB are presented in figures 3.3(a), 3.4(a) and 3.5(a) respectively. Moreover, the percentages of the increase in each tested blood parameter relative to their controls are presented as well in figures 3.1(b), 3.2(b), 3.3(b), 3.4(b) and 3.5(b) for the RBCs, WBCs, PLT, HCT and HGB respectively.

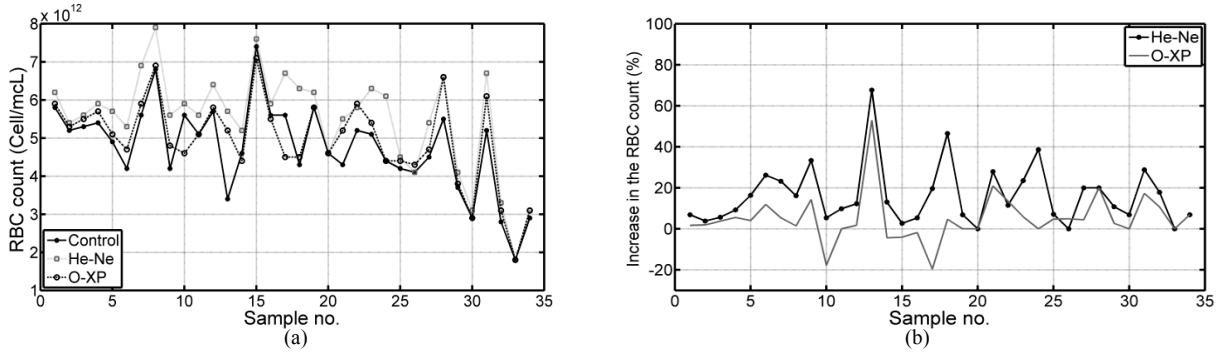


Figure 3.1: (a) The RBCs count for the control samples and the irradiated samples to both He-Ne and O-Xp laser sources. (b) The RBCs increment percentage for the irradiated samples to both He-Ne and O-Xp laser sources respectively to their controls.

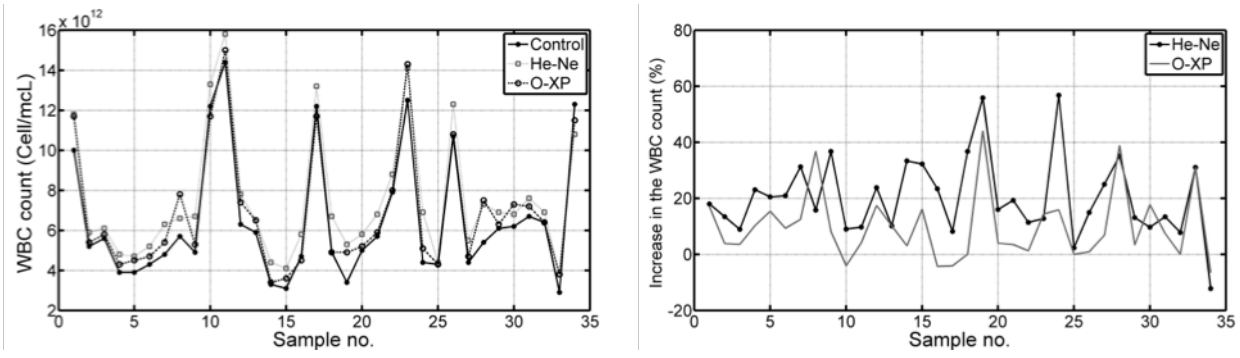
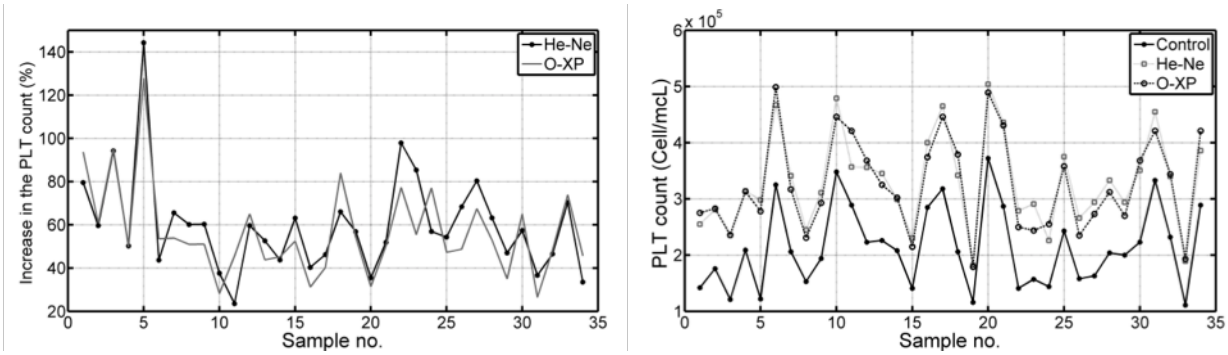
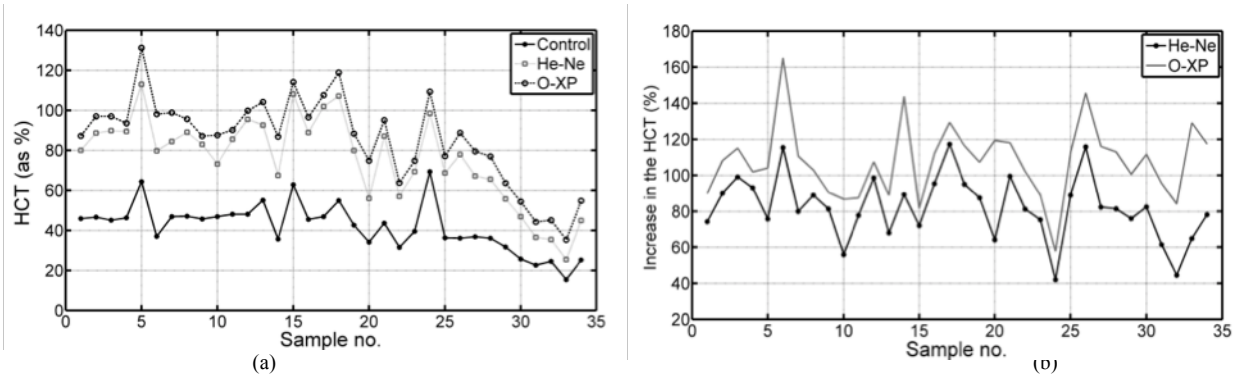


Figure 3.2: (a) The WBCs count for the control samples and the irradiated samples to both He-Ne and O-Xp laser sources. (b) The WBCs increment percentage for the irradiated samples to both He-Ne and O-Xp laser sources respectively to their controls.



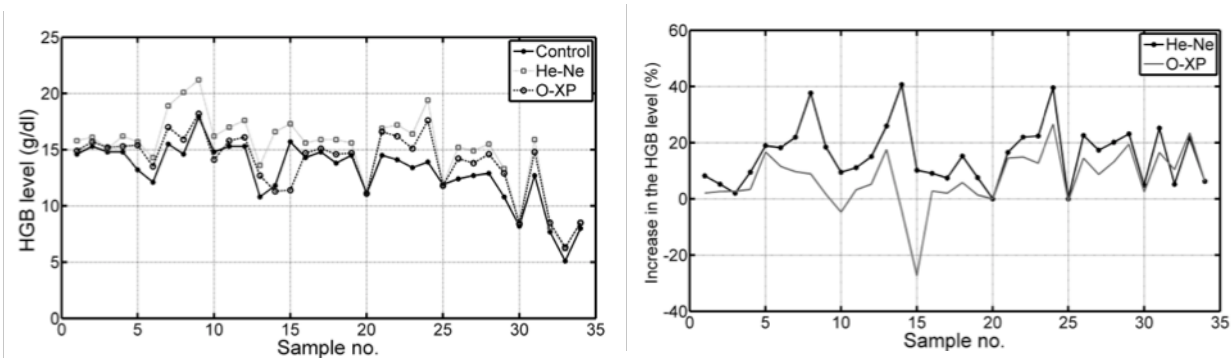
(a) (b)

Figure 3.3: (a) The PLT count for the control samples and the irradiated samples to both He-Ne and O-Xp laser sources. (b) The PLT increment percentage for the He-Ne and O-Xp irradiated samples relative to their controls.



(a) (b)

Figure 3.4: (a) The HCT percentage for the control samples and the irradiated samples to both He-Ne and O-Xp laser sources. (b) The HCT increment percentage for the irradiated samples to both He-Ne and O-Xp laser sources respectively to their controls.



(a) (b)

Figure 3.5: (a) The HGB level for the control samples and the irradiated samples to both He-Ne and O-Xp laser sources. (b) The HGB increment percentage for the irradiated samples to both He-Ne and O-Xp laser sources respectively to their controls.

As mentioned earlier, the UV/VIS analyses were conducted to examine the absorption of blood samples understudy. It is well known that normal blood has a common absorption profile according to the wavelengths, so any change in such phenomena indicates change in the internal components of the sample under investigations. After doing so, some results of these investigations are shown in figure 3.6(a-f)

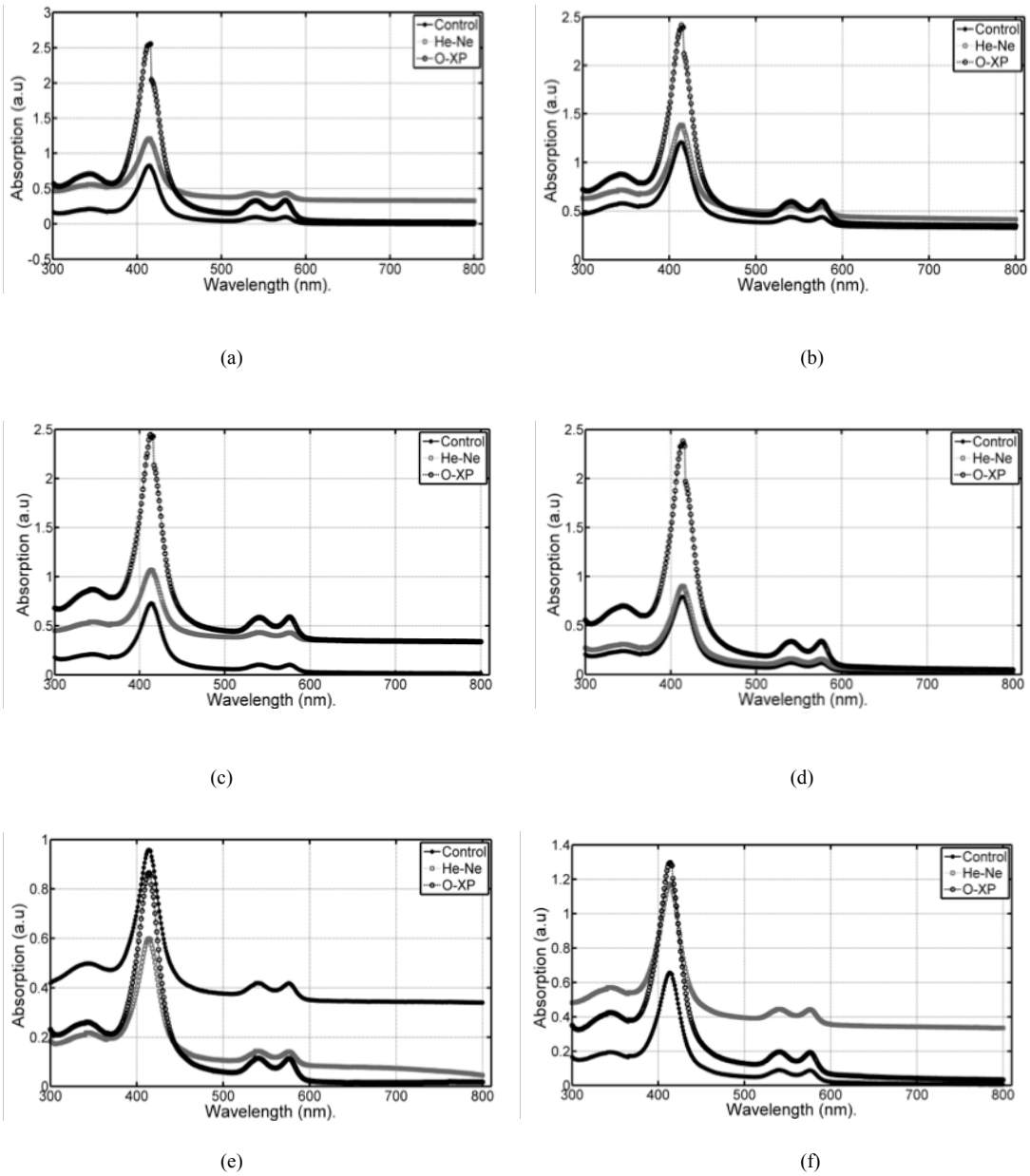


Figure 3.6: The absorption of human blood as a function of wavelength using UV/VIS spectrometer for control and irradiated samples to both He-Ne and O-Xp laser sources for the samples: (a) S#7, (b) S#13, (c) S#18, (d) S#19, (e) S#20 and (f) S#31.

Discussion

Figures 3.1 – 3.5 showed the increment in the entire blood components understudy. The RBCs counts show fluctuations between 2 X 1012 Cell/mcl to 7 X 1012 Cell/mcl for the control blood samples. After irradiating samples to He-Ne laser source, these values rise to vary from 2X1012 Cell/mcl to 8x1012 Cell/mcl, cf. Figure 3.1(a). However, less increment in the RBCs counts are noticed when O-Xp laser source was used. In addition, the RBCs increment shows percentages up to $\approx$ 70% relative to the control blood samples when He-Ne was used. Nonetheless, most of the samples are fluctuating between 10%and 40. Sample with percentage of 70% and the ones with less than 10% can be considered as outlier, cf. Figure 3.1(b). On the other hand, O-Xp irradiated blood samples show an increment percentage ranging from 0% to 20%. Nonetheless, two samples show a decrement of about -20% and one sample shows an increment for more than 60% for unknown reasons.

Concerning WBCs, somehow, similar phenomena were observed. The WBCs count for the control samples show values fluctuating between 3 X 10^{12} Cell/mcl and 14 X 10^{12} Cell/mcl. These values increase to 16 X 10^{12} Cell/mcl when exposed to He-Ne laser source. However, the maximum value for the WBC when O-Xp was used is limited to 14 X 1012 Cell/mcl, which far less than the value of He-Ne laser source, cf. figure 3.1(a). Additionally, figure 3.2(b) shows increment percentages of 4% to 60% when blood exposed to Ne-He compared to the WBC count of the control blood samples. These percentages show values of 3% to about 45% for O-Xp laser source. Nevertheless, for unknown reasons one He-Ne irradiated sample shows a drop of about 10% and 4O-Xp irradiated samples are also decreased for not more than 10%. The analysis for the PLT values indicate an increment of about 25% up to 100% when human blood samples were irradiated to He-Ne laser source compared to the control samples. Less increment is seen for O-Xp irradiated blood samples ($\cong$25% to $\cong$85%). Nevertheless, one sample increases for more than 140%when exposed to both He-Ne and O-Xp sources. This can be considered as artifact. Consistently, HGB level increases a bit when blood samples were exposed to He-Ne laser sources. The samples increase up to 40%. Less value, not more than $\cong$25%is observed for the O-Xp irradiated samples, cf. figure 3.5(b). However, unlike the previous blood parameters, the HCT increases more when O-Xp is used; 60% up to 160%, rather than He-Ne; 40% to $\cong$120% as seen in figure 3.4(b).

The UV/VIS spectrophotometer analysis showed an increment in the absorption of the laser-exposed samples compared to their controls; this confirms the results obtained by the blood flow-cytometer. The blood absorption is reported to have a common profile as a function of wavelength. In the biomedical optics, this phenomenon is so important to study the composition of human blood (Niemz, 2007). So as seen in figure 3.6, the samples are consistent in their peaks of absorption around 400 nm for the control samples and the laser exposed samples as well. Nevertheless they vary in the absorption level. Unlike flow-cytometer results, figures 3.6(a) – 3.6(e) show that the O-Xp exposed samples have more absorption level than the He-Ne exposed samples. Simply, this can be attributed to what is known as “Near-infrared window in biological tissue” (Wang and Wu 2007) since O-Xp has a near-infrared (NIR) wavelength. This phenomenon shows how the NIR wavelengths have more depth of penetration in tissue, therefore, one sees more absorption level compared to He-Ne exposed samples, cf. figures 3.6(a) – 3.6(e). However, figure 3.6(f) shows somewhat different behavior for the absorption. Here, the control has more absorption level than both laser exposed samples. Again this occurs due to unknown artifact. Nevertheless, the results obtained from the UV/VIS analysis agrees well with the literature (Wang and Wu 2007).

From the previous paragraphs one sees that the blood parameters understudy are increased after irradiating to laser. So, it is noticeable that, except for the HCT, their values increase more when He-Ne is applied than using O-Xp. This agrees well with the literature e.g. Al Timimi *et al.* (2011). The samples that didn’t increase are limited and could be attributed to the interspecies variation as well as could be caused by unknown reason artifacts. Concerning WBCs, somehow, similar phenomena were observed. The WBCs count for the control samples show values fluctuating between 3 X 10^{12} Cell/mcl and 14 X 10^{12}Cell/mcl. These values increase

Acknowledgement

The authors would like to thank the volunteers who agreed to give blood samples, Dr Abdelfatah Ahmed for his help and putting the UV/VIS equipment at our disposal and the the Department of Hematology, Faculty of Medical Laboratories for the blood cytometry analysis.

References

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

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

Golub, I.E. A. N. Malov, A. V. Neupokoeva, L. V. Sorokina, and Yu., (2003). The Influence of Laser Blood Photomodificationon Dynamic Characteristics of Surgical Stress. *Laser Physics,* 13(1), 106–111.

Judy, M.M., (2000). Biomedical Lasers. In J. D. Bronzino, ed. The Biomedical Engineering Handbook.

Lim, L., (2011). Biostimulation Mechanism with Intranasal Light Therapy – What really happens under the surface, Toronto, Ontario, Canada.

Mi, X.Q., Chen, J.Y. & Cen, Y., (2004). A comparative study of 632 . 8 and 532 nm laser irradiation on some rheological factors in human blood in vitro. Journal of Photochemistry and Photobiology B: *Biology*, 74, pp.7–12.

Niemz, M.H., (2007). Laser-Tissue Interactions: Fundamentals and Applications Third, Enl., Springer Berlin / Heidelberg.

Rathore, S. & Ali, B., (2013). Effect of He-Ne Laser Radiation on Viscometric Behavior of Human Blood. *Journal of Chemical, Biological and Physical Sciences*, 3(3), 2124–2129.

Al Timimi, Z. Z, M.S. Jaafar, Mohd Zubir Mat Jafri.., (2011). Photodynamic therapy and Green Laser blood Therapy. *Global Journal of Medical Research*, 11(5), 22–28.

Walker, M., 2002. The Beneficial Applications of Low Level Laser Therapy, USA.
Wang, L.V. & Wu, H., 2007. Biomedical Optics: Principles and Imaging, Wiley-VCH.