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Effect of Low-Power DPSS Laser Irradiation upon the Stability of Packed RBCs after Different Storage Periods

Safa H. Mohammed
Department of Health Physics and Radiation Therapy, College of Technical Engineering, Sawa University, Almuthana, Iraq

Ahmed U. Kareem
Department of Air Conditioning and Refrigeration, College of Technical Engineering, Sawa University, Almuthana, Iraq

Abstract: Numerous studies have demonstrated that laser therapy significantly enhances biological systems; low- power lasers have been frequently used in medicinal applications. The aim of the research is to determine whether the stability of stored RBCs is impacted by low-power DPSS laser irradiation. Venipuncture was used to collect human blood samples, which were then put in tubes with the anticoagulant citrate-phosphate dextrose-adenine (CPDA-1). After being separated into eight equal aliquots, the blood sample was kept at 4ºC for 21 days. The stability test was conducted on days 0, 7, 14, and 21 of storage for both radiated and non-irradiated aliquots. The test measures the percentage of hemolysis of an overnight sored RBC in saline solution. The irradiated aliquots were exposed to a DPSS laser at a wavelength of 650 nm with dosages of 30, 50, and 70 J/cm² for 15 minutes. Exposure of RBC suspension to a 650 nm wavelength low-power laser and a radiation doses of 70 J/ cm², 50 J/ cm², and 30 J/ cm² was associated with a significant reduction in the percentage of hemolysis that ranged from 3% to 11% throughout the whole storage time. In conclusion, it is observed that throughout storage periods, the 70 J/ cm² has a greater impact on the stability of the RBC suspensions.

Keywords: Stored RBCS. Low-power DPSS laser. Blood stability.

1. INTRODUCTION

In recent years, a wide range of medical applications employ low-power laser light [1]. The influence of low-energy laser light upon RBCs has been the subject of numerous prior investigations [2]. Due to their capacity to modify blood theology and enhance microcirculation, several low level lasers have been utilized for blood therapy for various clinical uses. These wavelengths also vary in power and exposure period [3][4]. Hydrogen bonds in tissues do not break down at low power, with the exception of the photochemical effects brought on by cell agitation through an increase in cell metabolism [5]. LLL treatment does not cause heat damage to living cells [6]. Photons must be absorbed by electrical absorption bands linked to a molecular chromophore or a photoacceptor in order to assess the impact of LLL irradiation upon living biological systems [7]. The specific mechanism underlying the blood-LLL radiation interaction remains unclear, according to Mi et al. (2004). Thus, a thorough knowledge of the effects of laser radiation on biological tissues requires an investigation into the effects of LLL radiation on blood. Further research will be required to understand how the human blood cells respond to LLL irradiation [8]. Although the blood's reaction to low-level laser light (LLL) offers crucial insights into the process of cell-cell contact, relatively little work has been done on this topic in living tissues. Furthermore, limited data currently exists regarding the nature of the reaction, especially how RBCs react to light from LLL lasers. [9]. Because red blood cells become more sensitive when they absorb more photons, hemoglobin (Hb) may be the target of laser irradiation [10][11]. The application of laser irradiation to revitalize preserved blood has been proposed in the field of hematology. This is so that blood contamination can be ensured as laser blood irradiation is a cost-effective, non-invasive, practical, and efficient method [12]. The quality of the blood may be impacted by distinct alterations

known as storage lesions that take place during storage [1]. RBC membranes undergo morphological and physiological alterations as a result of storage circumstances such low ATP concentration. RBCs that have been preserved generally exhibit decreased deformability and increased osmotic fragility [13]. This research examines the impact of varying low-power diode-laser radiation doses (650 nm wave length) upon the stability of packed RBCs after different storage periods.

2. MATERIALS AND METHODS

2.1. Blood sample

The samples have been collected from 12 volunteers of healthy adults through vein puncture into citrate-phosphate dextrose-adenine (CPDA-1) tubes containing an anticoagulant. This study's volunteers were all given informed consent. Following blood collection, the samples were promptly processed.

2.2. Preparing blood samples

After collecting (10 ml) Fresh blood samples through venipuncture and combined them with CPDA -1 (1.4 ml of CPDA-1/ 10 ml of blood) as an anticoagulant. Under our experimental conditions, this CPDA-1 (MacoPharma) was used to store human blood for a period of 21 days at 4ºC. Each sample of blood was divided into eight equal aliquots of 1 ml each, four of which will serve as controls, while the remaining four aliquots will be irradiated by laser beam for 15 min. Components of blood are separated by centrifuging the blood for ten minutes at 3000 x g. The top sections of packed RBCs, buffy coat, and plasma were thrown away. The RBCs were washed once again with a 0.9% NaCl solution after being re-suspended and centrifuged.

2.3. Laser irradiation

We utilized a low-power, 650 nm diode pump solid state (DPSS) laser (Model HLPS-831 china) as the primary source of radiation. The laser was operated at 50 mW for 15 minutes with radiation dosages of 30, 50, and 70 J/cm². The middle of the test tube holding a blood sample was where the beam of light was aimed. A power meter (Gentec-E, Maestro) was used to measure a laser's output power. The irradiation was performed at room temperature (23 + 2°C).

2.4. MEASUREMENT OF BLOOD STABILITY

The stability of RBC suspension was indirectly measured by measuring the hemoglobin (Hb) released after 24 hours of storage at 4ºC with or without irradiation with a laser wavelength of 650 nm. Two blood samples were assigned to each of the storage periods of 0, 7, 14, and 21 days. Each storage day, a non-irradiated sample served as a control. The aliquots received radiation dosages of 30, 50, and 70 J/cm² from a low-level DPSS laser with a continuous wave wavelength of 650 nm. After the wash process, add 1ml of normal saline to the packed RBC at the control sample and irradiate it. On day 0, 50 ul of blood from the control sample was mixed with 5 ml of distilled water to achieve complete hemolysis. Another 50 ul of blood from the control sample was drawn and mixed with 5 mL of normal saline. The stability test, described by Clznar and Shands (1971), was performed on both RBC suspensions at 4ºC for 24 hours. Following this period, a bench centrifuge was used to centrifuge both blood suspensions for ten minutes at 3000 g. A spectrophotometer (OPTIMA SP-300 OPTIMA, JAPAN) operating at 540 nm was used to measure the O.D. of the supernatants. By comparing the optical densities of the blood suspension in normal saline and distilled water (i.e., 100% hemolysis), the percent hemolysis in the sample was determined. The irradiated sample underwent this procedure once again. Every O.D. measurement was performed twice, and the average was calculated for comparison. After being kept, every blood sample went through the identical procedure once more. Throughout the course of storage, all blood samples that were designated as irradiated, along with the sample from the same testing day, were exposed to a laser light at a particular wavelength and dosage.

STATISTICAL EVALUATION

The mean ± SD is used to represent all data. To display differences between variables, data analysis was done using a pured Student's t test. Less than 0.05 indicated a significant P value.

RESULTS

The goal of the current study was to determine how a low-power diode-laser, with an output power of 50 mW, affects the stability of packed RBCs during different storage periods. In comparison to their non-irradiated counterparts at 0, 7, 14, and 21 days of storage time, respectively, exposure of RBC suspension to 650 nm wavelength laser beams at a radiation dose of 70 J/cm² was associated with a significant reduction in the percentage of hemolysis by 11%, 8%, 7%, and 10% (figure 1). In contrast, when the RBC suspension was exposed to a radiation dose of 50 J/ cm² was associated with a significant reduction in the percentage of hemolysis by 8%, 5%, 5%, and 3% relative to their non-irradiated sample counterparts at 0, 7, 14, and 21 days of storage, respectively (figure 2). Furthermore the same observations were noted when RBC suspension was exposed to a radiation dose of 30 J/ cm² in which there were significant reductions in the percentage of hemolysis by 2%, 2%, 3%, and 2% relative to non-irradiated sample counterparts at 0, 7, 14, and 21 days of storage time, respectively (figure 3). In conclusion, it was found that 70 J/ cm² has a greater impact on the stability of stored RBC suspensions during storage periods.

DISCUSSION

Several investigations and evaluations have demonstrated that utilization of LLL treatment can change the theological characteristics of blood according to low-energy radiation parameters [5]. Despite its contentious use in radiation therapy, low intensity lasers have become more common in therapeutic applications recently [6]. The interaction of low-energy laser light with biological tissues is still mostly unknown and lacking [14] Red blood cell irradiation with low-energy lasers has been the subject of numerous experiments that have shown excellent results [10]. Our research supports previous investigations on low-power DPSS laser irradiation of RBCs, showing a decrease in hemolysis percentage at different doses 30, 50, and 70 J/cm², demonstrating sufficient laser fluency for human blood stability [15]. The maximum decrease in the percentage of hemolysis is observed with a dose of 70 J/cm². The obtained results are consistent with the biphasic dose-response concept. The biphasic curve can help you determine the threshold dose, or the amount of energy required to produce optimal bio-stimulation. Bio-inhibition takes the place of bio-stimulation when the dosage level is significantly greater than the threshold dose [16][17]. In this investigation, the association between blood stability and wavelength was proven, with a significant decrease in hemolysis at different doses [18]. Laser light has the potential to modify the structure of RBC membrane proteins, resulting in protein denaturation and cell lysis. The time of irradiation and the energy doses absorbed have the greatest influence on the severity of these effects [2]. Nonetheless, the underlying pathways that control LLLI activity in tissues are unknown. The laser light is absorbed by hemoglobin (Hb), the major target of red blood cells. As a result, as more photons are absorbed, the response grows stronger [5]. Photons are absorbed by hemoglobin and the RBCs membrane. The most important indicator of RBC deformability is Hbm membrane-bound hemoglobin [10]. Due to weak connection between Hb and RBC membrane, low-level laser photons absorb by Hbm, vibrating the Hbm link and transforming Hbm into Hb [19]. Laser action significantly impacts red blood cells (RBCs) due to high absorption, but does not destroy them. Low-power laser irradiation protects RBCs membranes, reduces hypotonic hemolysis, and stabilizes cell membranes [20] As a result, utilizing a low-level laser beam to increase the length of blood preservation without haemolysis of blood cells is a major concern. DPSS lasers are being utilized more frequently in medical applications, especially blood irradiation, because of the long-term stability of the absorbed radiation light source [21]. In conclusion, we think that if time is included in our investigation, future research may maximize the use of a low-power diode laser for long-term storage and protection of RBCs.

CONCLUSION

The current investigation reveals that the percentage of hemolysis in RBC suspension significantly decreases when it is exposed to low-power lasers at 650 nm, as opposed to its non-irradiated counterparts during various periods of storage. The outcome demonstrates that irradiating RBC

suspensions at a dose of 70 J/cm² has a higher effect on the stability of the suspensions during storage times.

Figures

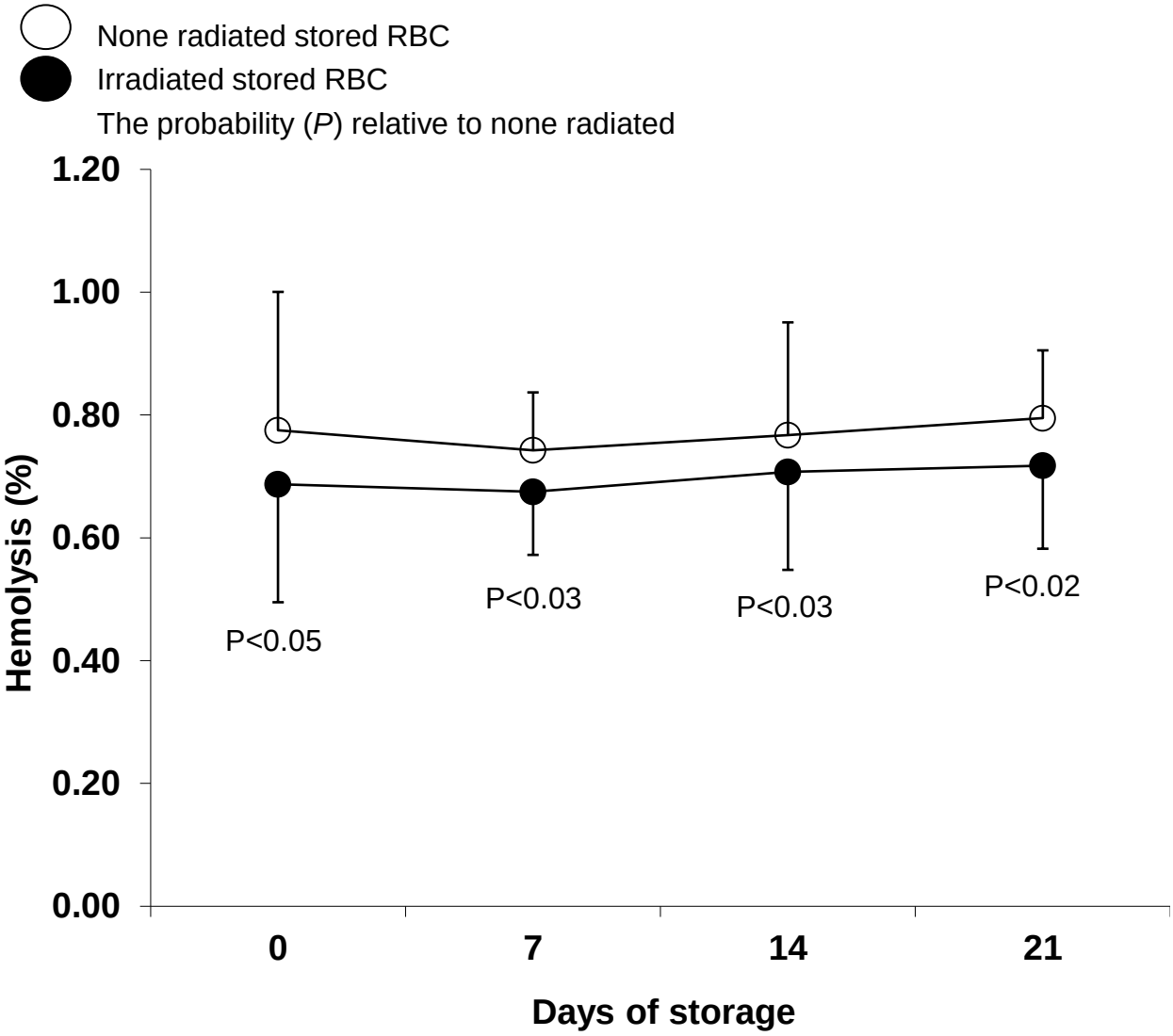

Figure 1: The percentage of hemolysis of stored RBC suspension irradiated with 650 nm wavelength laser light at a dose of 70 J/cm². N = 4.

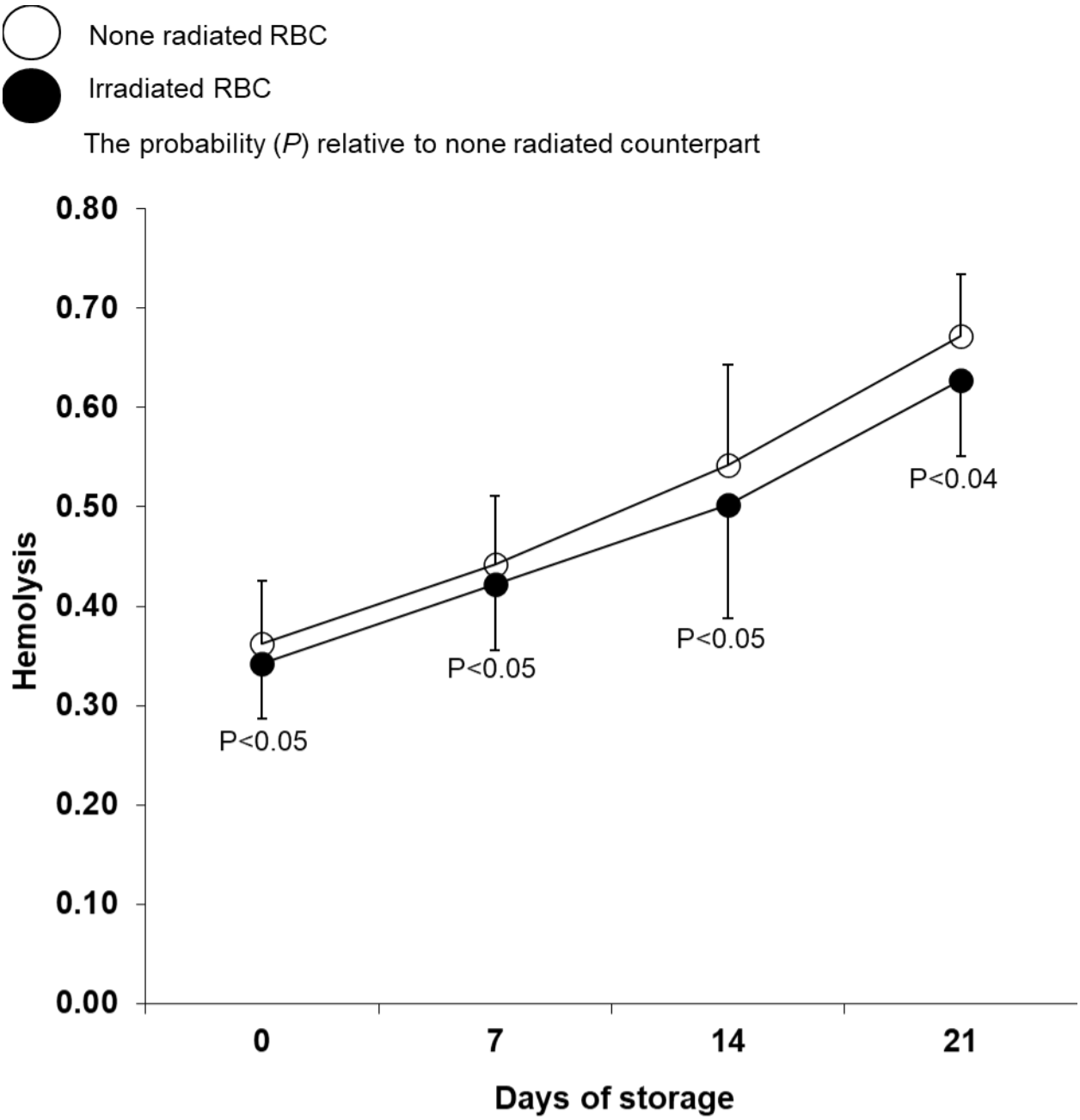

Figure 2: The percentage of hemolysis of stored RBC suspension irradiated with 650 nm wavelength laser light at a dose of 50 J/cm². N = 4.

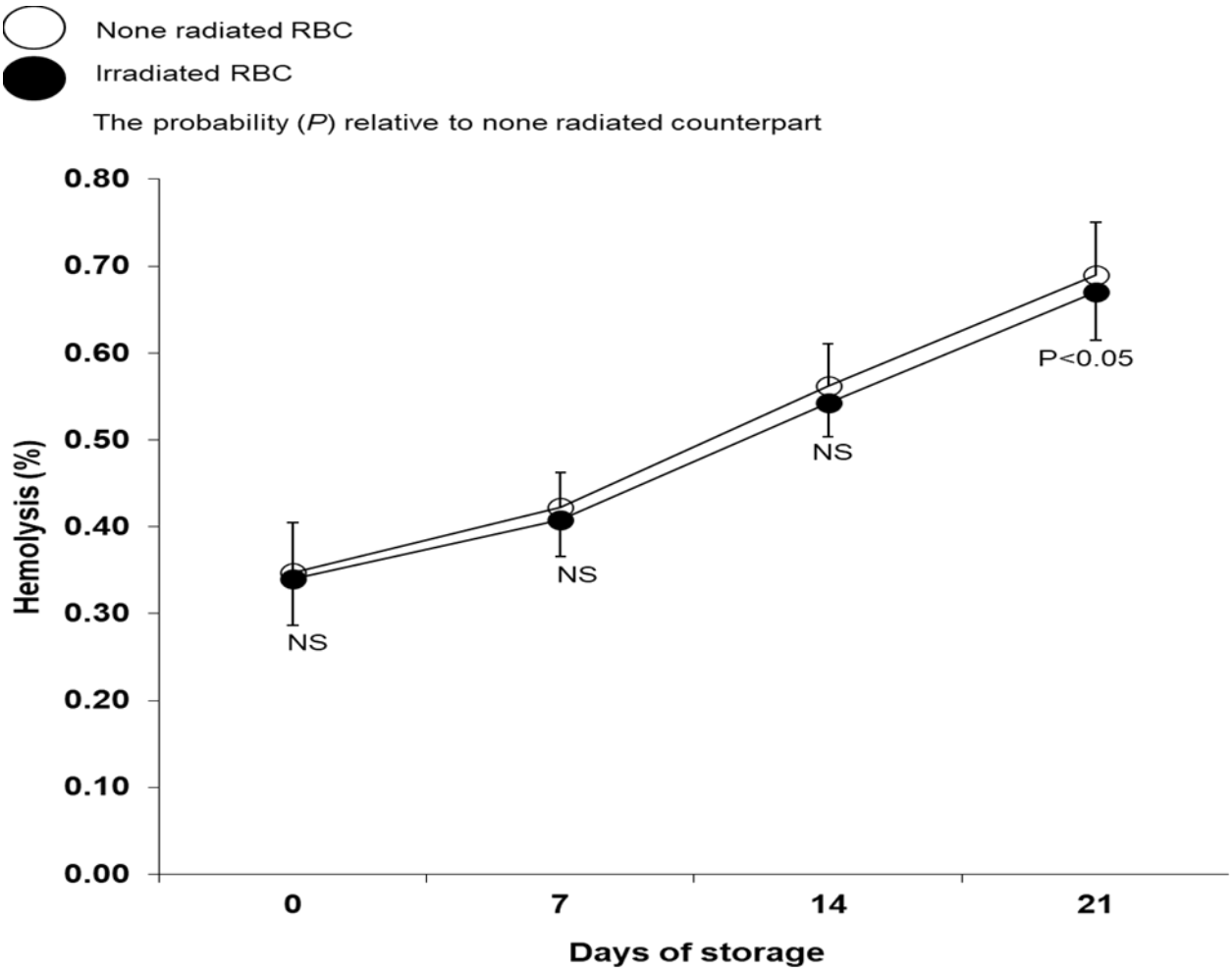

Figure 3: The percentage of hemolysis of stored RBC suspension irradiated with 650 nm wavelength laser light at a dose of 30 J/cm². N = 4.

References

1. H. K. Mohseen, K. N. Madlum, and H. A. Jabbar, "Effect of low level laser irradiation on white and red blood cells after different storage periods," *Int. J. Drug Deliv. Technol.*, vol. 10, no. 4, pp. 617–619, 2020, doi: 10.25258/ijddt.10.4.19.
2. A. Y. Al-Yasiri, "In Vitro Influence of Low-Power Diode Laser Irradiation Time on Human Red Blood Cells," *Photomed. Laser Surg.*, vol. 36, no. 5, pp. 253–257, 2018, doi: 10.1089/pho.2017.4395.
3. T. I. Karu, N. I. Afanasyeva, S. F. Kolyakov, L. V. Pyatibrat, and L. Welser, "Changes in absorbance of monolayer of living cells induced by laser radiation at 633, 670, and 820 nm," *IEEE J. Sel. Top. Quantum Electron.*, vol. 7, no. 6, pp. 982–988, 2001, doi: 10.1109/2944.983303.
4. A. N. Korolevich, N. S. Dubina, and S. I. Vecherinski, "<title>Influence of low-intensity laser radiation on human blood microcirculation</title>," *Opt. Diagnostics Biol. Fluids V*, vol. 3923, no. May 2000, pp. 175–179, 2000, doi: 10.1117/12.387140.
5. M. S. Al Musawi, M. S. Jaafar, B. Al-Gailani, N. M. Ahmed, F. M. Suhaimi, and M. Bakhsh, "Erythrocyte sedimentation rate of human blood exposed to low-level laser," *Lasers Med. Sci.*, vol. 31, no. 6, pp. 1195–1201, 2016, doi: 10.1007/s10103-016-1972-1.
6. M. L. Esmaiel, M. S. Al Musawi, and B. T. Al-Gailani, "Influence of low power diode pumping solid state laser radiation on the red blood suspension viscosity and deformability," *Mater. Today Proc.*, 2021, doi: 10.1016/j.matpr.2021.02.770.

7. J. C. Sutherland, “Biological Effects of Polychromatic Light¶,” *Photochem. Photobiol.*, vol. 76, no. 2, pp. 164–170, 2007, doi: 10.1562/0031-8655(2002)0760164beopl2.0.co2.
8. G. A. Zalesskaya and E. G. Sambor, “Interaction of low-intensity laser radiation with blood and its components,” *J. Appl. Spectrosc.*, vol. 72, no. 2, pp. 242–248, 2005, doi: 10.1007/s10812-005-0062-0.
9. J. Kujawa, L. E. U. Zavodnik, and I. Zavodnik, “(810 nm) Laser Radiation on Red Blood Cell ATPase,” *J. Clin. LaserMedicine Surg.*, vol. 22, no. 2, pp. 111–117, 2004.
10. X. Q. Mi, J. Y. Chen, Y. Cen, Z. J. Liang, and L. W. Zhou, “A comparative study of 632.8 and 532 nm laser irradiation on some rheological factors in human blood in vitro,” *J. Photochem. Photobiol. B Biol.*, vol. 74, no. 1, pp. 7–12, 2004, doi: 10.1016/j.jphotobiol.2004.01.003.
11. M. S. A. Al Musawi, M. S. Jafar, B. T. Al-Gailani, N. M. Ahmed, F. M. Suhaimi, and N. Suardi, “In Vitro Mean Red Blood Cell Volume Change Induced by Diode Pump Solid State Low-Level Laser of 405 nm,” *Photomed. Laser Surg.*, vol. 34, no. 5, pp. 211–214, 2016, doi: 10.1089/pho.2015.4043.
12. S. M. Sallam, A. M. Sallam, E.-S. M. El-Sayed, L. I. A. Salem, and M. M. Rizk, “Enhancement of Human Blood Storage Period by Irradiation of Low Level He-Ne Laser,” *J. Biophys. Chem.*, vol. 06, no. 03, pp. 77–86, 2015, doi: 10.4236/jbpc.2015.63008.
13. A. C. Zubair, “Clinical impact of blood storage lesions,” *Am. J. Hematol.*, vol. 85, no. 2, pp. 117–122, 2010, doi: 10.1002/ajh.21599.
14. X. Q. Mi, J. Y. Chen, Z. J. Liang, and L. W. Zhou, “In vitro effects of helium-neon laser irradiation on human blood: Blood viscosity and deformability of erythrocytes,” *Photomed. Laser Surg.*, vol. 22, no. 6, pp. 477–482, 2004, doi: 10.1089/pho.2004.22.477.
15. A. Jain *et al.*, “Storage stability of commonly used haematological parameters at 33 °C: Electronic supplementary material available online for this article.,” *Biochem. medica*, vol. 28, no. 2, p. 020901, 2018, [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/29666560%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5898956.
16. M. R. Hamblin, Y. Y. Huang, S. K. Sharma, and J. Carroll, “Biphasic dose response in low level light therapy - an update,” *Dose-Response*, vol. 9, no. 4, pp. 602–618, 2011, doi: 10.2203/dose-response.11-009.Hamblin.
17. A. P. Sommer, A. L. B. Pinheiro, A. R. Mester, R. P. Franke, and H. T. Whelan, “Biostimulatory windows in low-intensity laser activation: Lasers, scanners, and NASA’s light-emitting diode array system,” *J. Clin. Laser Med. Surg.*, vol. 19, no. 1, pp. 29–33, 2001, doi: 10.1089/104454701750066910.
18. A. Orbach, O. Zelig, S. Yedgar, and G. Barshtein, “Biophysical and Biochemical Markers of Red Blood Cell Fragility,” *Transfus. Med. Hemotherapy*, vol. 44, no. 3, pp. 183–187, 2017, doi: 10.1159/000452106.
19. X. Q. Mi, J. Y. Chen, and L. W. Zhou, “Effect of low power laser irradiation on disconnecting the membrane-attached hemoglobin from erythrocyte membrane,” *J. Photochem. Photobiol. B Biol.*, vol. 83, no. 2, pp. 146–150, 2006, doi: 10.1016/j.jphotobiol.2005.12.018.
20. Y. A. Vladimirov, A. N. Osipov, and G. I. Klebanov, “Bcm_0081,” vol. 69, no. 1, 2004.
21. M. S. Al Musawi, M. S. Jaafar, B. Al-Gailani, N. M. Ahmed, and F. M. Suhaimi, “Laser-induced changes of in vitro erythrocyte sedimentation rate,” *Lasers Med. Sci.*, vol. 32, no. 9, pp. 2089–2095, 2017, doi: 10.1007/s10103-017-2340-5.