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Influence of The He-Ne (632.8 nm) laser Radiation on a Human Whole Blood Stored

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THE aim of the present work is to study the effect of He-Ne laser (632.8 nm, 2 mm spot diameter, 30 mW, and continues wave) on the properties of human blood during different storage periods and seeking for the optimum laser dose (0.198 J/cm^3) to enhance blood shelf life. Human blood from five healthy donors was collected in human blood bags under the supervisionof thecentral blood bank. The control and treated samples (by three doses of He-Ne laser) were stored incentral blood bank refrigerator at 2-6^{0}C. The collected blood was divided into 4 groups, one for the control sample and the three other groups for the irradiationwith different doses of He-Ne laser (0.0287, 0.0563 and 0.198 J/cm^3. Each group was subdivided into 6 samples to perform during storage days (9, 24, 30, 35, 42, & 50). The measurements of the fragility (F) and viscosity (η) values of all samples during the storage days suggested that the He-Ne laser at a dose 0.198 J/cm^{3}is working to increase the resistance of the membrane of the red blood cells (RBC) and consequently the storage period increases form 35 day to 42 days. I n addition to viscosity for human blood before and after exposure to He-Ne laser dose of 0.198 J/cm^3 reflects the validity of the blood during the storage period. The obtained data proved that the ability to use the recommended He-Ne laser dose of 0.198 J/cm^3 the rheological properties and prolong the storage period of human blood from 35 days to 42 days.

Keywords: He-Ne laser, human blood, fragility, viscosity, storage period.

Introduction

Low-intensity He-Ne laser radiation has been found to have a lot of applications in the medical field, such as wound healing, tissue repair ,vascular restenosis, musculo-skeletal complications, and pain control[1-5]. The experimental medicine requires detailed information on the mechanisms of their biological effects [6]. A commonly used source of light is a He-Ne laser with radiation at 632.8 nm (red light). The main reason for using the lasers radiating in the red and near-infraredspectral regions is the fact that hemoglobin does not absorb light in this region and thus light can penetrate deeply into living tissue[7] . Low-energy laser is capable of producing so low energy density that all biologic effects are the result of direct irradiation and not of thermal events [7,8].

The exposure of the blood to He-Ne laser improves its rheological properties and decreases the osmotic fragility of erythrocytes (increases the resistance of erythrocytes to hypotonic)[9] .Low powered lasers stabilized stored erythrocytes in hypotonic solution and reduced the drop in deformability for stored erythrocytes [10]. Low powered He-Ne laser irradiation produced a protective effect on RBC membranes, reducing hypotonic hemolysis and stabilizing the cell membrane [11]. El Batanouny and coworkers [12] have reported that low dose of He-Ne laser caused a decrease in cells damage percentage and promote the cell cycle of lymphocyte cells. Gulosoy et al. [13] reported that He-Ne laser increasing proliferation of blood mononuclear cells after 7 days of laser irradiation and suggested the optimum He-Ne dose of 2.5 J/cm^2. Our obtained data proved that the ability to usethe recommended dose 0.198J/cm^{3}of He-Ne laser to improve the rheological properties and prolong the storage period of human blood from 35 to 42 days.

Material and Methods

A) Preparation of the samples

Blood samples were prepared from five

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healthy donors, both male and female, preserved in macro pharms bags, and treated withCitrate phosphate dextrose adenine (CPDA-1. CPDA-1 is an anticoagulant solution used to store human blood for periods of about 35 days. To have more samples per volunteer, the original bags were separated into smaller bags prior to getting the blood each sample being able to collect about 10 ml of blood. Twenty four small bag samples were papered to determine viscosity and fragility of blood before and after irradiation. They are divided into four groups stored, one control and three groups irradiated with He-Ne laser of different doses (0.0287, 0.0563 and 0.198 J/cm^3). The blood samples were storage before and after irradiation in transfused bags at 2-6^{0}C for (9, 24, 30, 35, 42 & 50) days.

B) Irradiation procedure

Irradiation was carried out using a system designed in science faculty ,Benha university .A source of He–Ne laser (U.S.PAT.311,969) had a beam of (632.8 nm, 2mm spot diameter, 30mW, and continues wave) is used in contact with the rubber narrow tube of blood bag during the blood flow, as in Fig. (1).

In this method of irradiation dose is depend on the blood flow rate which is controlled by theapparatus's valve

1-Flow rate can be calculated by the relation :

Flow rate (cm^3/sec) = v* A

where v = sample velocity, A=cross section area of the tube

2-Dose value can be calculated by the relation [9]:

Dose = power/(flow rate)=(Power(Watt) * time((second))/Volume

C) The osmotic fragility test

In this test, blood (0.1ml) was added to 9. 9 ml of varying concentrations from sodium chloride solution (0, 0.1, 0.2 up to 1%) and allowed to incubate at room temperature for half an hour. The tubesarecentrifuged, and the amount of hemoglobin was estimated based on absorbance at 540 nm for the supernatant of saline. The level of hemolysis of erythrocytes was determined by measuring hemoglobin released from the cells, relative to the total cellular hemoglobin content.

The percentage of hemolysis is calculated according to the following equation [14]:

$$\text{Hemolysis \%} = [(A_{x\%} - A_{0.9\%}) / (A_{0.0\%} - A_{0.9\%})] \times 100$$

Where, $A_{x\%}$ is the absorbance of the tube to be assessed, $A_{0.9\%}$ is the absorbance of the isotonic tube (0% hemolysis), $A_{0.0\%}$ is the absorbance of the pure water tube (100% hemolysis).

D) Viscosity measurements

The viscosity of whole blood samples was measured at room temperature(22±1)^{0}C by Brookfield Model LVDV-I Prim TCP cone and plate viscometer with digital screen monitor display. The applied shear rate was (2.25 to 225) sec^{-1}, this measure is repeated during blood storage periods (9,24,30,35 ,42 and 50 days) for control and irradiated samples with different doses (0.0287, 0.0563 and 0.198 J/cm^3) of He-Ne laser.

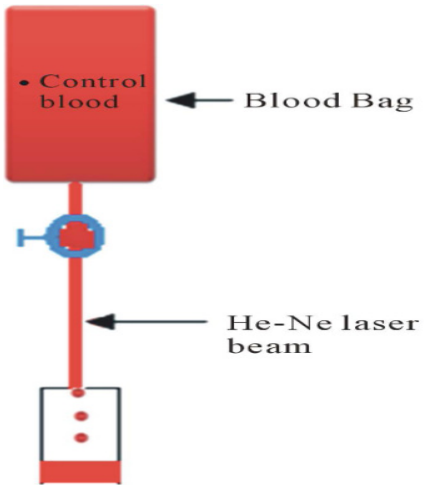

Fig. 1. Irradiation method.

Results and Discussion

In this study, different measurement such as fragility test and viscosity were used to show the effect of different doses of He-Ne laser (0.0287, 0.0563 and 0.198 J/cm^3) on human blood during storage days (9, 24, 30, 35 ,42and 50 days).

1)-Fragility: Figure [2–(a), (b), (c) and (d)] represent the variation of the hemolysis percentage (%) of control and treated human blood samples with He –Ne laser with different doses (0.0287, 0.0563 and 0.198 J/cm^3) with different concentration of NaCl(%) during storage period (9, 24, 30, 35, 42 and 50 days).

Figure [3] represents the relation between hemolysis (H_{50}) and storage period (9,24,30,35,42 and 50 days) for control and irradiation He-Ne laser samples with different doses (0.0287,0.0563 and 0.198 J/cm^3). It is noted that the average hemolysis percentage (H_{50}) increases by increasing the storage period.

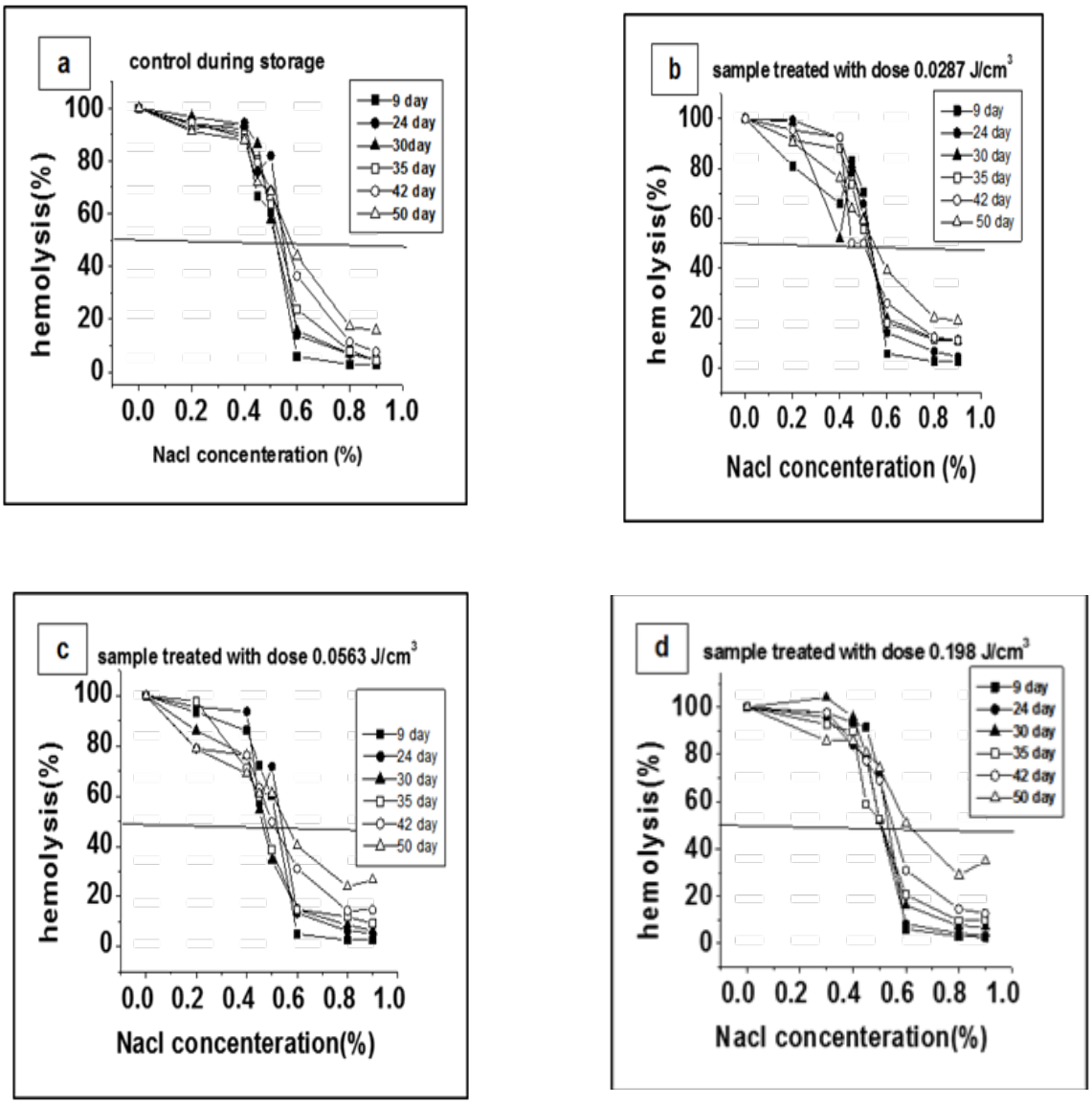

Fig. 2. The variation of the hemolysis percentage of control sample (a) sample treated with dose 0.0287J/cm^3(b) sample treated with dose 0.0563 J/cm^3(c) sample treated with dose 0.198 J/cm^3(d) with the concentration of Nacl solution during storage periods (9, 24, 30, 35, 42 & 50 day).

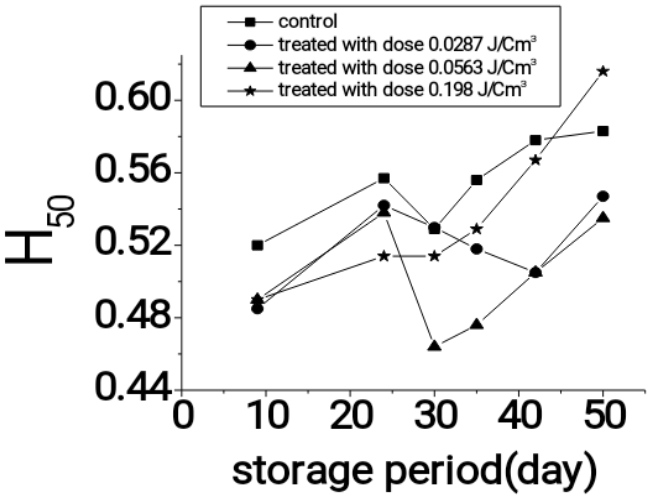

Fig. 3. The variation of NaCl concentration (%) causing 50% Hemolysis (H_{50}) with storage period (9, 24, 30, 35, 42 and 50 day) for control and treated human blood samples with He –Ne laser of different doses (0.0287, 0.0563 and 0.198 J/Cm^3).

There is a variation in (H_{50}) values of control and treated samples during storage periods in agreement with I. Z. Al Khalid et el. [15] who investigated that fragility changes by time and RBCs hemolysis increases the timeof storage. The same figure shows that there is an effect on the osmotic fragility of the blood due to exposure with He-Ne laser of different doses because the erythrocytes demonstrate rapid, dose-dependent lysis, as determined by release of free hemoglobin in agreement with Glassberg et al. [16]. These results were also in agreement with Yousry M et al. [9] who investigated that the exposure with He-Ne laser decreases the osmotic fragility of erythrocytes (increases the resistance of erythrocytes to the hypotonic solution.

The normal red blood cell isa relatively impermeable biconcave disc which maintains osmotic equilibrium with the surrounding medium. As the surrounding medium becomes hypotonic, fluid will be taken into the cell to maintain stability [9].

He-Ne laser (632.8 nm wavelength) has low photon energy and power output, which may produce minimum biomoleculardamage. Because it elevates the temperature of the irradiated cellsto less than 0.5ºC [17], therefore the irradiation of He-Ne laser cause photochemical interaction with the cells rather than thermal effect. Laser irradiation in this red spectral area increases the proliferative activity of cells and promotes tissue repair [18,19].

Figure [3] shows that H_{50} at dose 0.198 J/cm^3 was near control values referring to that blood sample exposed to that dose were improved than other two doses.

2)-Viscosity measurements

Blood is a non-Newtonian fluid as it is a multiphase system such that viscosity depends on the viscosity of each of the individual phases. Figure [4] show that viscosity decreases with increasing the shear rate value. From a physical point of view, blood can be defined as a "non-Newtonianshear-thinning fluid," reflecting its composition (i.e., a suspension of blood cells in plasma), and the special behaviour of red blood cells (RBC) that constitute 99.9% of the cellular elements [20]. These figures show that viscosity values decreaseatalow shear rate and it takes exponential values, it is nearly constant with higher shear rates.

Figure [5] shows that the viscosity depends on the storage days andlaserdoses. It is shown that the value of viscosity for the control and treated samples with different doses behaves the same behaviour that it increases with increasing the storage period.

These results were in agreement with Li, Guolin et al., who reported that there were

significant increases in blood viscosity during storage [21]. RBC suspensions from both genders demonstrated progressive increases in viscosity, elasticity and relaxation time at equivalent shear rates over seven weeks of storage indicating a decrease in RBC deformability [22].

From our obtained data and figures, it is noted that there is a variation effects of different He-Ne laser doses on the viscosity of whole human blood. It has improved values at dose 0.198 J/cm^3 because it recorded lower values than control value during storage period as shown in Fig [5] while the viscosity values of the samples treated with doses 0.0563 J/cm^3 and 0.0287 J/cm^3 were higher than control values.

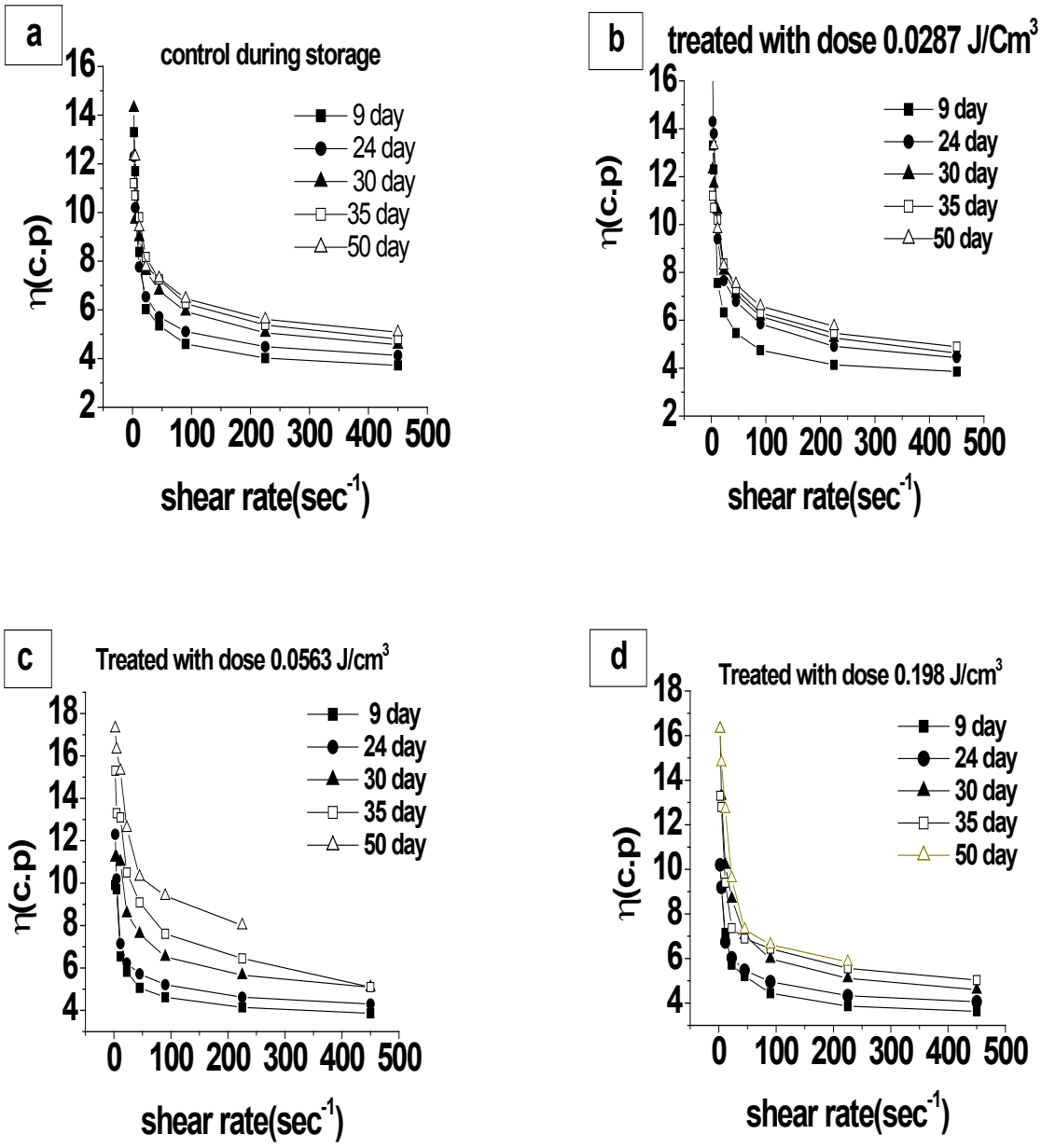

Fig. 4 . The relation between viscosity (η) and shear rate from 2.25 to 225 sec^{-1} for control sample (a) treated samples with He-Ne laser of different doses 0.0287 J/cm^3 (b), 0.0563 J/cm^3 (c) and 0.0198 J/cm^3(d) for storage periods (9, 24, 30, 35, 42 and 50 days).

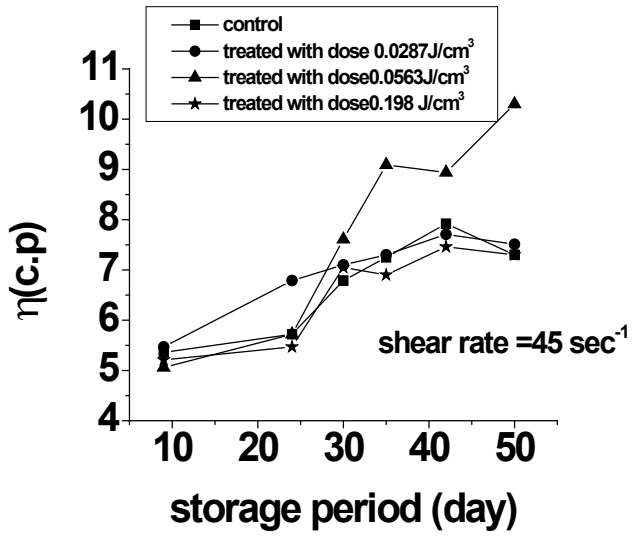

Fig. 5. The relation between viscosity (at shear rate = 45 sec^{-1}) and storage period (9, 24, 30, 35, 42 and 50 days) before and after irradiationof blood by He-Ne laser with different doses (0.0287, 0.0563 and 0.198 J/cm^3).

Conclusion

The obtained results showed that :

- Fragility study explained that the He-Ne laser at a dose 0.198J/cm^3 is working to increase the resistance of the membrane of the red blood cells (RBC) and consequently the storage period increases form 35 day to 42 days.
- Viscosity for human blood before and after exposure to this selected dose reflects the validity of the blood during the storage period.
- The obtained data proved that the ability to usethe recommended dose of 0.198 J/cm^3 to improve the rheological properties and prolong the storage period of human blood from 35 days to 42 days.

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تأثير شعاع الليزر He-Ne (نانومتر632.8)

على تخزين الدم البشري

سميرة سلام, لطفي أبو سالم, عبدالفتاح بكر, منى رزق, مصطفى إبراهيم

قسم الفيزياء - كلية العلوم - جامعة بنها - بنها - مصر

أن استمرارية الدراسة والبحث علي دم الانسان تكتسب أهميتها من أن الدم هو المائع داخل الجسم المنوط به امداد الخلايا بالمواد الغذائية والاكسجين وكذا نقل مخلفات الايض من السائل الخلوي الخارجي. ان عملية حفظ الدم لها أهميه كبري ليس فقط من ناحية الاستخدامات الطبية ولكن ايضا علي المستوي الاستراتيجي القومي.

تهدف هذا البحث الي دراسة تأثير ليزر الهليوم – نيون (٣٠ مللي وات, ٦٣٢,٨ نانومتر) بجرعات مختلفة علي خواص دم الانسان أثناء فترات حفظ حتي ٥٠ يوما والبحث عن الجرعه المناسبة التي تعمل علي زيادة فترة حفظ الدم.

تم تجميع دم من خمسة اشخاص اصحاء في اكياس الدم الصحية المناسبة لذلك وتحت اشراف طبي في مركز بنك الدم بالمستشفي الجامعي لجامعه بنها، كما أن العينات الحاكمة والمعالجة بثلاث جرعات من ليزر الهليوم نيون (٠,١٩٨,٠,٠٥٦٣,٠,٠٢٨٧ جول/سم٣) تم تخزنها في ثلاجات مركز بنك الدم عند درجة حرارة ٢-٦°م.

تم تقسيم كمية الدم الي اربع مجموعات ، واحدة منها كمجموعه حاكمة والثلاث مجموعات الاخري تم تعريضهم لجرعات الليزرالثلاث ٠,١٩٨,٠,٠٥٦٣,٠,٠٢٨٧ جول/سم٣) ، كما ان كل مجموعه تم تقسيمها الي ست عينات.

كل العينات تم استخدامها أثناء ايام الحفظ ٥٠,٤٢,٣٥,٣٠,٢٤,٩ يوما. تم اخذ القياسات العملية قبل وبعد التعريض لاشعه الليزر باستخدام تقنيات (اللزوجة ، الهشاشة).

بينت النتائج التي تم الحصول عليها:

- الجرعه المناسبة التي عملت علي زيادة فترة حفظ الدم كانت٠,١٩٨جول/ سم٣وهذه الجرعة المثالية عملت عى زيادة فترة التخزين الى ٤٢ يوم ، حيث المعروف عالميا ان فترة تخزين الدم ٣٥ يوم باستخدام مادة .(١-CPDA)
- حدوث زيادة في لزوجة عينات الدم الحاكمة بنسبة ٣٦,٠١٪ عند معدل قص ٤٥ث-١ خلال فترة حفظ ٥٠ يوما بينما حدث نقص في اللزوجة بعد التعريض باشعه الليزر بحوالي ٣,٦٪ في المتوسط
- قياسات الهشاشة اثبتت أن الجرعه المناسبة ٠,١٩٨ جول/سم٣ لها تاثير في زيادة مقاومة خلايا الدم الحمراء ضد التحلل.