Effects of low- and high-level pulsed Nd:YAG laser irradiation on red blood cells and platelets indices of albino rats *in vitro*

Amer Khazal Jaber Al-Hasan

Department of Pharmacology and Toxicology, College of Pharmacy, Basrah University, Basrah, Iraq.
Correspondence to Amer Khazal Jaber Al-Hasan (email: amer.khazal@gmail.com).

(*Submitted: 22 December 2016 – Revised version received: 17 January 2017 – Accepted: 22 February 2017 – Published online: 26 March 2017*)

Objectives Erythrocyte cell membrane and enclosed haemoglobin content being a target with a consistent wavelength in the laser absorption spectrum. In this study, intension was made to clarify whether irradiation with high energy densities of pulsed laser; Neodymium-doped yttrium aluminium garnet (Nd:YAG) with different pulse rates can improve the haemorheological parameters of red blood cells (RBCs) and platelets.

Methods Pulsed Nd: YAG laser ($\lambda = 532$ nm) performance was measured and calibrated to either low (LLLR) or high (HLLR) level laser radiation schemes. Albino rat blood samples were collected and irradiated with low- and high-level energy laser (Fluence = 2.38–47.77 J/cm^2). Complete blood picture was done for all samples and blood parameters were assessed and compared for both control and irradiated groups using paired *t*-test.

Results Blood parameters like haemoglobin (HGB) and haematocrit (HCT) increased significantly ($P \leq 0.05$) by low laser fluence 2.38–4.77 J/cm^2. High-level laser irradiation significantly decreased ($P \leq 0.05$) RBCs distribution width (RDW) and platelet distribution width (PDW) for fluencies from 11.94/cm^2 to 47.77 J/cm^2. Stained blood films showed improved rheological properties of both RBCs and platelets irradiated with laser energy as low as 7.16 J/cm^2. Both LLLR and HLLR fluencies significantly decreased ($P \leq 0.05$) and normalized platelets aggregatory properties.

Conclusion High-level laser irradiation reduced RBC deformability, aggregability and platelet aggregation resulting in improvements of cell membrane revitalizing, decreased viscosity and stress adaptation. Laser also enhanced both RBC and platelet life span with high-energy fluencies. HLLR using pulsed Nd:YAG is potentially a useful technique for the clinical intervention of many blood disorders.

Keywords Nd:YAG laser, RBCs indices, platelets aggregation

Introduction

Utilizations of low-intensity laser light had spread generally amid the most recent decades in different molecular and medicinal research fields, contemplates have been accounted for on the impact of low power laser illumination on mammalian red blood cells (RBCs) and platelets biological parameters, a few scientists inform for illumination concerning blood cell parts during blood storage for donating,[1] others showed on green laser Neodymium :Yttrium Aluminum Garnet (Nd:YAG) high-power dosage impact that can trigger stimulus transduction, prompting both biostimulation and initiation reactions.[2] RBCs and platelets *in vitro* challenge many hard conditions and biomodulation effects that impact their trademark components and conveyance and with the guide of an aligned energy of photons (e.g. Laser beam), such changes could be guided towards valuable scope of bio-incitement components.[3] Vast scope of treatment parameters influence laser treatment, i.e., wavelength, transient method of operation (beat or persistent), exposure irradiance, timing, recurrence, length of treatment and optical properties of tissues and cells.[4] The wavelength could be a vital parameter, revealing that constant wave laser (He-Ne, 632.8 nm) has been the most prevalent laser utilized with many reviews and the relative absence of transdermal infiltration of this wavelength have assumed a critical part in the disability of laser treatment to create blood parameter changes in a many cases.[5] The *in vitro* impact with pulsed laser of wavelengths in the vicinity of 632.8 and 660 nm indicated consequences for RBCs viscosity changes, and expanded multiplication of lymphocytes.[6,7]

The *in vitro* impacts of pulsed Nd:YAG laser illumination of wavelength 532 nm. on many hemorheological factors, like blood consistency, erythrocyte sedimentation rate, RBCs deformability and their electrophoretic versatility were explored similarly with continuous wave (CW) lasers of 632.8 and 532 nm. It was found that other than the 632.8 nm laser (which is often utilized as a part of scientists line of study), the pulsed 532 nm laser is additionally encouraging and proved experimentally.[8]

Materials and Methods

All experiments were carried out on blood samples drawn from five adult healthy male Albino rats of the Wistar strain. Initial body weight was approximately 250 g. The animals were kept in separate cages in the animal house at the Biology Department, College of Science, Basrah University prior to study with free access to food pellets and water bottles in room temperature with a 12-h light/dark cycle. The animals were then maintained in a controlled environment room, 25°C, (Biophysics Lab, Physics Department, College of Science, Basrah University) for 24 h to allow sufficient adaptation time prior to sacrifice. Experimental rats were then anaesthetized successively by 30-s inhalation in a closed chloroform jar, then mounted supine on a retractor holding their extremities and sacrificed with opening the chest and direct heart puncture with a sterile syringe to withdraw blood (approx. 2.5 ml). Blood samples were then transferred to E.D.T.A. tubes (Merck Chemicals Ltd.), and put immediately for tube shaking (Krackeler Scientific, USA) at room temperature, then test samples were handled sequentially for laser irradiation. Then all samples were transported in closed container (8–10°C) to the hematology analyzer lab within the university campus.

Instrumentation

1. Laser radiation source used: Q-switched Nd:YAG laser unit (DIAMOND-288 pattern ELPS, Huafei Tongda Technology), wavelength (1064/532 nm) with pulse energy (≤800 ≤400 mJ), pulse width (10 nano.sec.), Frequency = 6 Hz, beam spot diameter (4 mm) present in Biophysics Lab, Physics Department, College of Science, Basrah University.
2. Horiba hematology analyzer (ABX SAS-Micros ES-60-HORIBA Medical Distributors Network, France), present in Main Research Laboratory, Pharmacy College, Basrah University.
3. Calibration Joule Meter (Lab-Max TOP, COHERENT high performance laser power and energy meter, U.S.A), present in Biophysics Lab, Physics Department, College of Science, Basrah University.

Preparation of Samples

Anticoagulated blood from each EDTA tube was separated into equal volumes (200 µl) and inserted into 12 (300 µl) Eppendorf tubes two non-irradiated controls and ten laser-irradiated (five tubes for low-level, and five tubes for high-level laser irradiation). Leishman's stained blood films were done for separate control and laser-treated blood samples for further evaluation of RBC morphology and aggregatory changes.

Laser Dose Calibration

Measurement of the spot size and assessment of the laser beam profile from the hand piece output crystal of the unit was done with the COHERENT-joule meter with different energy settings and exposure distances prior to irradiation of samples to calibrate and calculate the absorbed dose, using the information below:

Laser wavelength (λ) = 532 nm
Irradiation distance from aperture = 4 cm
Beam width = 4 mm (observed)
Beam radius = 2 mm = 0.2 cm
Irradiation area = πr^2 = 0.1256 cm^2 (calculated)
Pulse rate frequency (PRF) = 5, 10, 15, 20, 25 (For low-level irradiation)
= 10, 20, 30, 40, 50 (For high-level irradiation)

Using the laser unit control panel:

Laser output (Low-energy setting) = (100 mJ), Frequency = 1 Hz, Pulses = 5

Laser output (High-energy setting) = (200 mJ), Frequency = 1 Hz, Pulses = 10

Using COHERENT Joule Meter:

Laser-calibrated energy density (mJ/cm^2) = Laser-calibrated energy/Area irradiated

Laser-calibrated power (mW) = Calibrated energy density (mJ/cm^2) × PRF

Calibrated laser fluence (mJ/cm^2) = Calibrated laser power (mW)

Area (cm^2)

N.B. : For CW lasers

Fluence = Irradiance (Watt/cm^2)

Laser radiation dose (J/cm^2) = Laser irradiance × Exposure time (sec.)

Since we are using volumes of blood (200 µl), one can divide laser fluence over volume of blood sample irradiated to produce = Laser fluence per volume blood (Joule / ml).

Laser Irradiation of Samples

Irradiation was carried immediately after blood drainage to the Eppendorf tubes sequentially, with the laser hand piece vertically oriented over the opened and isolated tube. With the aid of an attached aiming device the distance was kept constant (4 cm) along with wearing special laser safety eye glasses (goggles), laser pulses were delivered while blood was stirred gently by agitating Eppendorf tubes in a circular horizontal motion to ensure homogeneity distribution of laser dose to whole blood sample. Radiation scheme was carried according to Table 1.

Blood Picture Measurement

Laboratory measurements included 11 parameters performed using Horiba Hematology analyzer providing the following data : Total RBC count in (10^6/mm^3), haemoglobin quantity (HGB) in g/dl, haematocrit concentration %(HCT), mean corpuscular volume MCV in µm^3, mean corpuscular haemoglobin (MCH) in picograms (p.g), mean corpuscular haemoglobin concentration %(MCHC), RBC distribution width %(RDW), Total platelet count (PLT) in (10^3/mm^3), Mean platelet volume (MPV) in µm^3, Platelet crit %(PCT), Platelet distribution width %(PDW).

Statistical Analysis

In this work, our concern is mainly centred on finding a significant difference between non-irradiated controls and laser-irradiated samples thus paired *t*-test was used for all low and high levels of laser treatments along with calculating the relative variation (RV) percentage. (RV of a given blood parameter (X) is the percentage difference between its value after irradiation and before irradiation, divided by the value before irradiation (i.e. RV = ΔX/X%) a negative (−) sign RV means a relative decrease and vice versa. Taking into account all the range of normal values will be a laborious work since many rat studies revealed a big difference in blood parameters between peripheral (tail) and systemic (cardiac) blood along with diurnal changes (parameters fluctuate; these are active during the day rather than at night). The relevant interval (RI %) is the ratio between the difference of parameter maximal and minimal normal value (divided by 2) and the mean value of the same maximal and minimal normal value RI = (max − min normal value/max + min normal value)/2. A positive result appears in which the RV exceeded its RI.

Results

The *in vitro* effects of LLLR and HLLR on the rat RBCs and platelets parameters will be presented in separate tables according to the calculated laser fluence (J/cm^2).

Table 1. **Laser radiation scheme**

Abbreviation	Energy level	Laser unit energy set	Number of laser pulses
Cont.	Control	0	0
LLLR	Low-level laser	100 mJ	5, 10, 15, 20, 25
HLLR	High-level laser	200 mJ	10, 20, 30, 40, 50

Laser Energy Calibration

From experiments done using the COHERENT joule meter, reading averages were calculated as LLLR = 100mJ Unit set (calibrated = 60 mJ), and as HLLR = 200 mJ Unit set (calibrated = 120 mJ) as in Table 2.

Effects of Low Level Laser Radiation on RBC and Platelets

The experimental data at the beginning of laser irradiation (Table 3) with low fluence (2.38 J/cm^2) showed a mean RV of 10.4%, 9.2%, 10.2% observed in (RBC), (HGB), (HCT) indices, respectively, and non-significant decrease ($P > 0.05$) in (MCV), (MCH), (MCHC) ranges. The RV of erythrocytes distribution width (RDW) ranged between −3.3% and +1.2% with no relevant effect. Platelet or thrombocytes count (PLT) showed non-significant decreased RV ranged between 20% and 80% ($P > 0.05$), only one rat showed increased RV = 9.9%. Plateletcrit (PCT) is a measure of total platelet mass whose values may vary depending on mean platelet volume (MPV). Normal platelet count has a PCT within the range of 0.20–0.36%. PCT is an effective screening tool for detecting platelet quantitative abnormalities. MPV showed RV ranged between −1% and 27.9%, and according to relative interval (RI = 0.285), laser effect was relevant in two rats. PDW showed significant decrease ($P \leq 0.05$) with RV ranged from −13.8% to −68.9%, the laser effect was relevant for all rats.

The experimental data of laser irradiation with low fluence (4.77 Joule/cm^2) (Table 4) indicated that parameters means and RV means for all RBCs and platelets are higher than those above (Table 3) for all parameters (RBC, HGB, HCT, MCH, MCHC, PLT, MPV, PCT, PDW) except the erythrocyte distribution width (RDW) parameter which is lower (Fig. 1).

As the laser irradiation fluence increases to (7.16) Joules/cm^2 (Table 5), experimental data indicated that erythrocyte count parameter (RBC) RV raised with a significant ($P \leq 0.05$) average increase (+15.3%) in all rats, HGB parameter RV was positive (2.1%–35.7%) with significant increase ($P \leq 0.05$) in all rats, HCT readings indicated significant positive increase ($P \leq 0.05$), RV ranged between 1.3% and 30.7%) with an average (+14.6%). MCV parameter RV almost stayed constant with zero change in 3 out of 5 rats and 2 rats RV (−3.4%, +1.7%) with no significant difference and likewise is the MCHC parameter with less than 1% RV non-significant decrease.

Laser radiation effect indicated a significant decrease ($P \leq 0.05$) seen as - sign in all rats in RDW parameter RV

Table 2. **Calibrated laser fluencies according to number of pulses**

LLLR		HLLR	
Number of laser pulses	Total laser fluence (J/cm^2)	Number of laser pulses	Total laser fluence (J/cm^2)
5	2.388	10	9.554
10	4.777	20	19.108
15	7.165	30	28.662
20	9.554	40	38.216
25	11.942	50	47.770

Table 3. **Nd:YAG laser fluence = 2.38 (J/cm^2)**

N = 5	RBC			HGB			HCT		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	6.628	7.188	0.104	11.66	12.56	0.092	36.26	39.22	0.102
± SD	1.084	0.524	–	1.514	1.155	–	6.107	3.413	-
P-value		0.183			0.19			0.192	
***N* = 5**	**MCV**			**MCH**			**MCHC**		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	54.8	54.6	–0.003	17.74	17.48	–0.012	32.36	32.02	–0.008
± SD	2.135	1.744	–	1.172	0.546	–	1.72	0.655	–
P-value		0.374			0.285			0.363	
***N* = 5**	**RDW**			**PLT**			**MPV**		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	17.38	16.8	–0.033	734	409.2	–0.443	9.48	9.56	0.008
± SD	0.826	1.251	–	706.6	384.9	–	2.895	4.078	–
P-value		0.077			0.156			0.467	
***N* = 5**	**PCT**			**PDW**					
	CONT.	IRR.	RV.	CONT.	IRR.	RV.			
Mean	0.575	0.279	–0.317	9.18	6.44	–0.269			
± SD	0.586	0.224	–	1.76	1.816	–			
P-value		0.15			0.058				

(*P*-values > 0.05 are not significan; **P*-values ≤ 0.05 are significant).

Table 4. **Nd:YAG laser fluence = 4.77 (J/cm²)**

N = 5	RBC			HGB			HCT		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	6.61	7.42	0.147	11.66	13.16	0.152	36.26	40.44	0.145
± SD	1.044	0.744	–	1.514	1.234	–	6.107	3.361	–
P-value		0.148			0.137			0.17	
N = 5	MCV			MCH			MCHC		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	54.8	54.6	−0.0003	17.74	17.8	0.005	32.36	32.58	0.01
± SD	2.135	1.744	–	1.172	1.266	–	1.72	1.788	–
P-value		0.374			0.46			0.436	
N = 5	RDW			PLT			MPV		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	17.38	16.72	−0.038	734	530.8	−0.241	9.48	10.42	0.109
± SD	0.826	1.496	–	706.6	452.7	–	2.895	3.327	–
P-value		0.124			0.125			0.189	
N = 5	PCT			PDW					
	CONT.	IRR.	RV.	CONT.	IRR.	RV.			
Mean	0.575	0.445	−0.123	9.18	8.24	−0.157			
± SD	0.586	0.355	–	1.767	5.286	–			
P-value		0.208			0.305				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

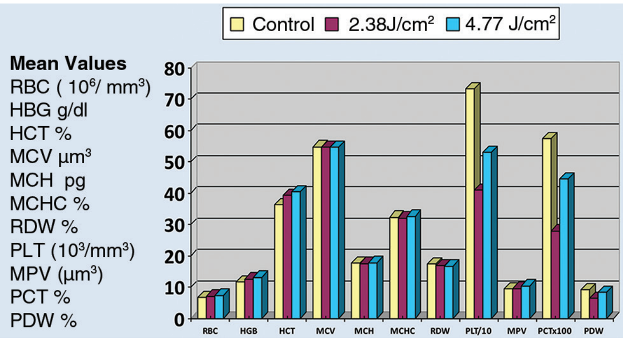

Fig. 1 **Means of red blood cells and platelet parameters with controls (using low laser fluence: 2.38 J/cm², 4.77 J/cm²).**

average = 4%, ranging (1.2–8.8%). PLT, MPV, PCT parameters indicated non-significant difference with an average RV of (−39.1%, −7.4%, −40.8%) respectively. PDW parameter significantly decreased ($P \leq 0.05$) by laser irradiation in all rats, RV ranged (11.8%-59.7%), mean RV = 33.7%.

The experimental data of laser irradiation with low fluence 9.55 J/cm² in (Table 6) showed a significant decrease ($P \leq 0.05$) in the RV of RDW (mean 4.4%) of all rats. Although lacks significance, a major trend appears that all other RBC and platelets parameters (RBC, HGB, HCT, MCV, MCH, PLT, MPV, PCT, PDW) means and RV means are higher than those corresponding to in the above (Table 5), except the parameter (MCHC) which is lower (Fig. 2).

As the laser irradiation fluence increases to (11.94) Joules/cm² (Table 7), experimental data indicated that in accordance to previous fluence (Table 6), the parameters: erythrocyte count RBC, HGB, HCT, MCV, MCH, MPV means decreased with no significance, except the parameter MCHC mean raised (29.6–32.3%) with fluctuating RV ranged −0.9% to +6.5%). While the parameters MCHC, PLT, PCT means in comparison to previous (Table 6) increased non-significantly, the parameter RDW significantly decreased ($P \leq 0.05$) with mean variation = 4.1% indicating RV ranging (1.2–7.1%). PDW parameter showed highly significant decrease ($P \leq 0.01$) due to laser fluence, with RV decrease ranged (31.9–42.1%), averaged (29.6%).

The Effects of High Level Laser Radiation on RBC and Platelets

As laser energy level precedes in growing (i.e. increased fluence) an impact appears to be significant only on specific parameter variation, especially the RBCs distribution width (RDW) parameter and platelets distribution width (PDW). Table 9 shows laser fluence = 19.1 J/cm² significantly decreased ($P \leq 0.05$) RDW parameter in all rats, RV ranged between 0.6–11.2% and a mean of 4.3%. In (Table 10), the laser fluence = 19.1 J/cm² significantly decreased ($P \leq 0.05$) PDW parameter in all rats with RV ranged 1.7%–28.9%, and mean = 14.5%. At laser fluence = 38.21J/cm² (Table 11), Only RDW parameter influenced by significantly increase ($P \leq 0.05$) by decreasing RV values ranging from 2.7% to 8.8% in 80% of rats.

At laser fluence = 47.77 J/cm² (Table 12), although only the PDW parameter showed significant decrease ($P \leq 0.05$), RV was relevant in all rats ranging between 1.1% and 40.8% with an average RV = 18.3%.

All RBCs and platelets average parameters will be represented in one figure to be in comparison with each other and in between for trends and relations (Fig. 3).

Table 5. **Nd:YAG laser fluence = 7.16 (J/cm²)**

N = 5	RBC			HGB			HCT		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	6.61	7.57	0.153	11.66	13.22	0.139	36.26	41.36	0.146
± SD	1.044	0.967	–	1.514	1.728	–	6.107	6.136	–
P-value		0.024*			0.04*			0.022*	
N = 5	MCV			MCH			MCHC		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	54.8	54.6	−0.003	17.74	17.46	−0.014	32.36	32.02	−0.008
± SD	2.135	1.854	–	1.1723	0.7228	–	1.72	0.865	–
P-value		0.352			0.188			0.314	
N = 5	RDW			PLT			MPV		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	17.38	16.7	−0.04	734	349.8	−0.39	9.48	9.12	−0.074
± SD	0.826	1.202	–	706.6	300.5	–	2.895	4.555	–
P-value		0.045*			0.111			0.369	
N = 5	PCT			PDW					
	CONT.	IRR.	RV.	CONT.	IRR.	RV.			
Mean	0.575	0.218	−0.40	9.18	5.8	−0.337			
± SD	0.586	0.15	–	9.18	5.8	–			
P-value		0.109			0.022*				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

Table 6. **Nd:YAG laser fluence = 9.55 (J/cm²)**

N = 5	RBC			HGB			HCT		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	6.628	7.856	0.215	11.66	13.68	0.2	36.26	43.08	0.146
± SD	1.084	2.204	–	1.514	3.422	–	6.107	6.136	–
P-value		0.182			0.177			0.187	
N = 5	MCV			MCH			MCHC		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	54.8	54.8	0.006	17.74	17.5	−0.012	32.36	29.6	−0.085
± SD	2.135	1.72	–	1.172	0.762	–	1.72	7.205	–
P-value		0.5			0.246			0.238	
N = 5	RDW			PLT			MPV		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	17.38	16.62	−0.044	734	537.8	−0.282	9.48	10.54	0.137
± SD	0.826	0.973	–	706.6	494.5	–	2.895	2.605	–
P-value		0.026*			0.093			0.073	
N = 5	PCT			PDW					
	CONT.	IRR.	RV.	CONT.	IRR.	RV.			
Mean	0.575	0.468	−0.15	9.18	8.6	−0.092			
± SD	0.586	0.393	–	1.767	3.417	–			
P-value		0.228			0.245				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

Discussion

Low-level laser therapy (LLLT) systems were firstly used with He–Ne (632.8 nm) red lasers, nowadays infrared and (532 nm.) green lasers are widely used for this purpose. Pulsed laser type utilizes a laser beam focused to a small spot carries an enormous amount of energy and allows an intense power density to be delivered to a given target in a short period of time. Q-switched Solid state pulsed Neodymium Yttrium Aluminum Garnet (Nd:YAG) laser has a wide range of application in medical and dental area.[9–11] Nd:YAG produces 5 to 10 nanoseconds (ns) high-intensity pulses at 532 nm. Nd:YAG is a promising new alternative method for causing biological effects: desensitizing, normalizing and revitalizing changes of the rheological factors of the blood. The hemodynamic effects of laser used opposes the physiologic destruction phenomenon (RBC aging), leading to a rising viability of the cells in the circulatory system.

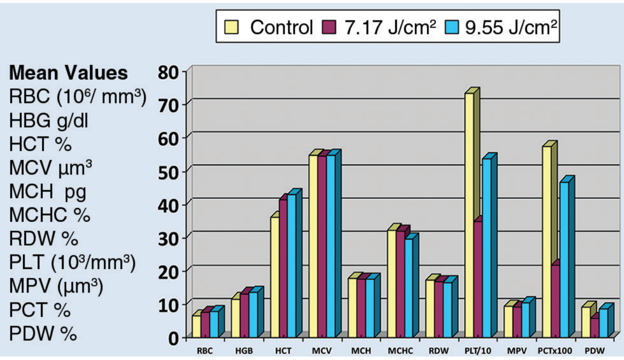

Fig. 2 **Means of red blood cells and platelet parameters with controls (using low laser fluence: 7.16 J/cm², 9.55 J/cm²).**

This study was undertaken to assess the effects of pulsed laser on rat blood *in vitro* and how it can induce modifications on some rheological indices of the RBCs and platelets and also to assess the significance of these modifications. The results of this study have effectively demonstrated that the irradiation of blood at low and high pulses and different energy densities (fluencies) leads to the following effects without causing any blood cell damage. Blood parameters like RBC, HGB and HCT were slightly influenced by low laser fluence 2.38–4.77 J/cm², its action is due to the strong absorption of irradiation laser light by Haemoglobin (HBG), but without damaging the RBCs membranes. RBC distribution width (RDW) is a measure of the variation of RBC volume, (RDW = (Standard deviation of MCV ÷ mean MCV) × 100). Higher RDW values indicate greater variation in size. HCT readings indicated highly significant positive increase with increasing laser fluence to 7.16 J/cm², the same result was obtained by researchers[1] but by using higher energy level to 12.2 J/cm² which may increase the risk of unwanted effects (elevating temperature = hyperthermia, over stimulation).

RDW test results are often used together with MCV results to determine the possible causes of the anaemia. Our study findings on low laser fluence (LLLT) indicated that RDW parameter was significantly decreased ($P < 0.05$). This fact inspires researchers to announce for irradiation of stored blood and for the clinical application of low power laser treatment for extracorporeal circulation since erythrocyte deformability and free haemoglobin levels were significantly lower than those in the control group.[12–14]

Table 7. **Nd:YAG laser fluence = 11.94 (J/cm²)**

N = 5	RBC			HGB			HCT		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	6.628	7.20	0.108	11.66	12.58	0.093	36.26	39.2	0.1
± SD	1.084	0.78	–	1.514	1.625	–	6.107	4.936	–
P–value		0.207			0.213			0.219	

N = 5	MCV			MCH			MCHC		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	54.8	54.4	–0.007	17.74	17.54	–0.01	32.36	32.3	–0.003
± SD	2.135	1.85	–	1.172	0.761	–	1.72	0.46	–
P-value		0.238			0.255			0.467	

N = 5	RDW			PLT			MPV		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	17.38	16.6	–0.041	734	650.4	–0.022	9.48	9.8	0.014
± SD	0.826	0.88	–	706.6	703	–	2.895	4.147	–
P-value		0.023*			0.083			0.375	

N = 5	PCT			PDW		
	CONT.	IRR.	RV.	CONT.	IRR.	RV.
Mean	0.575	0.47	–0.206	9.18	6.7	–0.296
± SD	0.586	0.50	–	1.767	3.074	–
P-value		0.056			0.008**	

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant; ***P*-values ≤ 0.01 are highly significant).

Table 8. **Nd:YAG laser fluence = 9.55 (J/cm^2)**

N = 5	RBC			HGB			HCT		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	6.628	6.57	0.008	11.66	11.74	0.018	36.26	35.7	0.001
± SD	1.084	0.17	–	1.514	0.658	–	6.107	1.996	–
P-value		0.454			0.456			0.417	
***N* = 5**	**MCV**			**MCH**			**MCHC**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	54.8	54.4	–0.007	17.74	17.84	0.007	32.36	32.96	0.021
± SD	2.135	1.85	–	1.172	0.72	–	1.72	0.734	–
P-value		0.238			0.37			0.209	
***N* = 5**	**RDW**			**PLT**			**MPV**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	17.38	14.86	–0.149	734	591	–0.251	9.48	10.2	0.068
± SD	0.826	4.873	–	706.6	517.3	–	2.895	3.504	–
P-value		0.14			0.164			0.115	
***N* = 5**	**PCT**			**PDW**					
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**			
Mean	0.575	0.58	0.846	9.18	9.9	0.056			
± SD	0.586	0.33	–	1.767	3.569	–			
P-value		0.474			0.257				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

Table 9. **Nd:YAG laser fluence = 19.10 (J/cm^2)**

N = 5	RBC			HGB			HCT		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	6.628	7.32	0.19	11.66	12.92	0.129	36.26	39.8	0.125
± SD	1.084	0.97	–	1.514	1.525	–	6.107	5.142	–
P-value		0.204			0.178			0.219	
***N* = 5**	**MCV**			**MCH**			**MCHC**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	54.8	54.2	–0.011	17.74	17.66	–0.002	32.36	32.5	0.007
± SD	2.135	1.6	–	1.172	0.781	–	1.72	0.994	–
P-value		0.104			0.427			0.44	
***N* = 5**	**RDW**			**PLT**			**MPV**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	17.38	16.6	–0.043	734	395	–0.335	9.48	10.38	0.098
± SD	0.826	1.19	–	706.6	388.9	–	2.895	3.217	–
P-value	0.04*				0.151			0.076	
***N* = 5**	**PCT**			**PDW**					
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**			
Mean	0.575	0.48	–0.113	9.18	7.86	–0.198			
± SD	0.586	0.42	–	1.767	5.08	–			
P-value		0.226		0.226					

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

Table 10. **Nd:YAG laser fluence = 28.66 (J/cm²)**

***N* = 5**	**RBC**			**HGB**			**HCT**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	6.628	6.82	0.047	11.66	12	0.041	36.26	37.04	0.04
± SD	1.084	0.33	–	1.514	0.788	–	6.107	2.528	–
P-value		0.352			0.33			0.394	
***N* = 5**	**MCV**			**MCH**			**MCHC**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	54.8	54.2	−0.01	17.74	17.62	−0.004	32.36	32.46	0.006
± SD	2.135	1.72	–	1.172	0.549	–	1.72	0.571	–
P-value		0.213			0.39			0.457	
***N* = 5**	**RDW**			**PLT**			**MPV**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	17.38	16.6	−0.041	734	519.2	−0.273	9.48	9.64	0.012
± SD	0.826	0.94	–	706.6	446.1	–	2.895	3.455	–
P-value		0.067			0.173			0.407	
***N* = 5**	**PCT**			**PDW**					
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**			
Mean	0.575	0.38	−0.222	9.18	8.02	−0.145			
± SD	0.586	0.31	–	1.767	2.669	–			
P-value		0.207			0.027*				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

Table 11. **Nd:YAG laser fluence = 38.21 (J/cm²)**

***N* = 5**	**RBC**			**HGB**			**HCT**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	6.628	6.73	0.039	11.66	12	0.046	36.26	36.52	0.031
± SD	1.084	0.27	–	1.514	0.718	–	6.107	1.377	–
P-value		0.43			0.366			0.47	
***N* = 5**	**MCV**			**MCH**			**MCHC**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	54.8	54.2	−0.011	17.74	17.78	0.004	32.36	32.82	0.016
± SD	2.135	1.6	–	1.172	0.631	–	1.72	0.73	–
P-value		0.104		0.449				0.248	
***N* = 5**	**RDW**			**PLT**			**MPV**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	17.38	16.7	−0.038	734	491	−0.284	9.48	9.9	0.03
± SD	0.826	1.09	–	706.6	428.5	–	2.895	4.089	–
P-value		0.04*		0.173				0.336	
***N* = 5**	**PCT**			**PDW**					
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**			
Mean	0.575	0.45	−0.202	9.18	7.92	−0.155			
± SD	0.586	0.29	–	1.767	2.64	–			
P-value		0.203			0.027*				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

Table 12. **Nd:YAG laser fluence = 47.77 (J/cm²)**

***N* = 5**	**RBC**			**HGB**			**HCT**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	6.628	6.676	0.024	11.66	11.98	0.041	36.26	36.84	0.035
± SD	1.084	0.515	–	1.514	0.638	–	6.107	2.077	–
P-value		0.465			0.352			0.422	
***N* = 5**	**MCV**			**MCH**			**MCHC**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	54.8	54.4	−0.007	17.74	15.66	−0.118	32.36	32.54	0.008
± SD	2.135	1.855	–	1.172	4.202	–	1.72	0.571	–
P-value		0.238			0.177			0.41	
***N* = 5**	**RDW**			**PLT**			**MPV**		
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**
Mean	17.38	16.8	−0.033	734	806.5	−0.2	9.48	6.951	−0.221
± SD	0.826	1.14	–	706.6	909.1	–	2.895	4.443	–
P-value		0.081			0.257			0.154	
***N* = 5**	**PCT**			**PDW**					
	CONT.	**IRR.**	**RV.**	**CONT.**	**IRR.**	**RV.**			
Mean	0.575	0.609	−0.082	9.18	7.72	−0.183			
± SD	0.586	0.649	–	1.767	3.02	–			
P-value		0.221			0.05*				

(*P*-values > 0.05 are not significant; **P*-values ≤ 0.05 are significant).

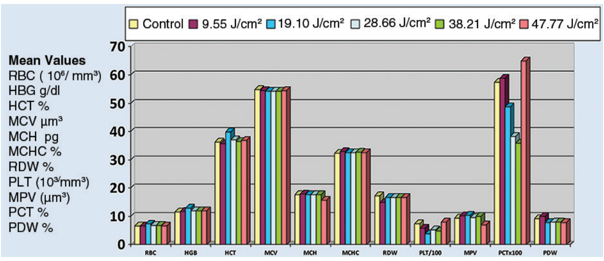

Fig. 3 **Means of red blood cells and platelets parameters with controls using (HLLR) scheme.**

Our study showed that increased fluence to high levels (HLLT) = 19.1 J/cm² significantly decreased ($P \leq 0.05$) RDW parameter in all rats which coincides with[7] findings, i.e. after irradiation 0–54 J a lowering of the erythrocytes aggreagability (viscosity) confirms the non-resonant mechanism of this bio-stimulating radiation effect by the changes in the cell membrane. Our findings at high laser fluencies reaching 38.21 J/cm² (Table 9) affects mainly RDW parameter which has been significantly decreased ($P \leq 0.05$) when using pulsed green laser, likewise[15] realized a rigid relationship between erythrocyte deformability and aggregation detected in patients' blood when applying photohemotherapy. Blood exposure to He-Ne laser and UV is a potent method for correcting the blood rheology.

The RBC depends on many factors: (1) its strongly negative surface charge (derived from surface glycoproteins) which permits it to repulse other circulating cells, thereby preventing "clumping", (2) its unique biconcave- shape which is rheologically highly efficient and permits rapid flow of the cells through narrow capillaries; and (3) its ability to prevent oxidative stress to the hemoglobin molecule *in vitro*, may undergo a variety of shape alterations, and these changes could be both reversible and irreversible.[16]

Platelet indices are potentially useful markers for the early diagnosis of thromboses and bleeding diseases, an increase in both MPV (resulting from platelet swelling and their shape changes from discoid to spherical) and PDW (due to platelet activation & pseudopodia formation). MPV and PDW are easily measured platelet indices and PCT is an effective screening tool for detecting platelet quantitative abnormalities.[13] In our study, LLLR effect on PDW parameter showed highly significant decrease ($P \leq 0.01$) due to laser fluence. Study sample was sufficient for revealing that PDW seems to be a more specific indicator of platelet activation than MPV, since it was not elevated during single platelet distention caused by platelet swelling. The combined use of MPV and PDW could predict activation of coagulation more efficiently.[17]

By continuing our study using laser irradiation fluence increases to 7.16 Joules/cm², PDW parameter significantly decreased ($P \leq 0.05$) in all rats, with high RV ranges (11.8–59.7%). It was indicated that low and medium laser light energies from 18 to 54 J increased platelet activation, but high-energy laser fluence at 108 J resulted in depressed platelet reactivity and attenuated platelet response to activators.[2] Ultra high laser light irradiation of blood platelets can trigger signal transduction, leading to platelet activation, as well as the gradual loss of natural platelet reactivity and platelets' ability to respond to activating agents.

As the laser irradiation fluence further increases to 11.94 J/cm², PDW parameter showed highly significant decrease ($P \leq 0.01$), with RV decrease ranged (31.9–42.1%) averaged (29.6%). At fluence = 19.1 J/cm² laser radiation significantly decreased PDW parameter in all rats ($P < 0.05$) with RV ranged 1.7–28.9% and mean = 14.5%. Similar findings to our results were obtained by researches using CW lasers, an examination of the *in vitro* effects of increasing doses of Infrared (CO_2) laser irradiation on platelet number, function, and surface ultrastructure and results indicated a progressive dose-response reduction of both platelet number and function following laser irradiation.[17]

High level laser radiation (HLLR) in our study didn't exceed the maximum clinical allowance readings (fluence 19 J/cm^2, 28.66 J/cm^2, 38.21 J/ cm^2, 47.77 J/ cm^2) to simulate the therapeutic laser doses applied. Results concerning the RBCs distribution width (RDW) parameter and PDW showed that (fluence = 19.11 J/cm^2) laser radiation effect significantly decreased ($P \leq 0.05$) RDW parameter in all rats, RV ranged between 0.6 and 11.2% and a mean of 4.3%. At laser fluence = 38.21 J/cm^2, Only RDW parameter influenced significantly ($P \leq 0.05$) by decreasing RV values ranging from 2.7 to 8.8% in 80% of rats. Interestingly, at laser fluence = 47.77 J/cm^2, instead only the PDW parameter showed a significant decrease ($P \leq 0.05$) which is relevant in all rats, ranging between 1.1 and 40.8% with an average RV = 18.3%.

Conclusion

RBC and platelets parameters measurement using a computerized HORIBA hemoanalyzer showed that low and high level of pulsed (Nd:YAG) laser radiation used at different pulse rate produced a non-damaging biostimulation and regenerating effect on RBC and platelets parameters. Nd:YAG laser becomes a promising easy handling new alternative method for causing revitalizing changes of the rheological factors of blood indices (by keeping unmodified MCV, MCH, and MCHC), and achieve effect on its membranes.

Conflict of Interest

None.

References

1. MostafaYM, Amin SN, Abdalwahab S, Elsherbini A. Effects of non-coherent and coherent light on complete blood picture and osmotic fragility of human blood. J Blood Disorders Transf. 2013;4:1.
2. Gresner P, Watala C, Sikurova' L. The effect of green laser light irradiation on whole blood platelets. J Photochem. Photobiol. B. 2005;79:43–50.
3. Maegawa Y, Itoh T, Hosokawa T, Yaegashi K, Nishi M. Effects of near-infrared low-level laser irradiation on microcirculation. Lasers Surg Med. 2000;27:427–437.
4. N'unez SC, Nogueira GE, Ribeiro MS, Garcez AS, Lage-Marques J. He-Ne laser effects on blood microcirculation during wound healing: a method of in vivo study through laser doppler flowmetry. Lasers Surg Med. 2004;35:363–368.
5. Baxter GD. Therapeutic lasers: Theory and practice. Edinburgh: Churchill Livingstone, 1994.
6. Stadler I, Evans R, Kolb B, Naim JO, Narayan V, Buehner N, et al. In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes. Lasers Surg Med. 2000;27:255–261.
7. Siposan DG, Lukacs A. Relative variation to received dose of some erythrocytic and leukocytic indices of human blood as a result of low-level laser radiation: an in vitro study. J Clin Laser Med Surg. 2001;19:89–103.
8. Huo XL, Zhang YD, Di Q, Wu XF, Fang Q, Hu ZR. Effect of the intravascular low level laser irradiation with different doses of different lasers on the blood biomarkers in patients with cerebral infarction. Chin J Laser Med Surg. 2000;9:80–84.
9. Goldberg D, Kasimova G. Treatment of infantile hemangiomas using a novel 650-microsecond pulsed Nd: yag 1064 nm laser. Lasers Surg Med. 2016;48:28.
10. Karsai S, Jäger M, Oesterhelt A, Weiss C, Schneider SW, Jünger M, et al. Treating onychomycosis with the short-pulsed 1064-nm-Nd:YAG laser: results of a prospective randomized controlled trial. J Eur Acad Dermatol Venereol. 2017;31:175–180.
12. Omrani LR, Taher A, Albujeer A, Parvin M, Daryakenari G, Gorgani-Firuzjaee S, et al. Penetration of hydrogen peroxide into the pulp chamber after conventional and laser-assisted bleaching. South African Dent J. 2016;71:58–61.
13. Itoh T, Murakami H, Orihashi K, Sueda T, Kusumoto Y, Kakehashi M, et al. Low power laser protects human erythrocytes in an in vitro model of artificial heart-lung machines. Artif Organs. 2000;24:870–873.
14. Anderson K. Broadening the spectrum of patient groups at risk for transfusion-associated GVHD: implications for universal irradiation of cellular blood components. Transfusion. 2003;43:1652–1654.
15. Iijima K, Shimoyama N, Shimoyama M, Mizuguchi T. Effects of low-power He-Ne laser on the deformability of stored human erythrocytes. J Clin Med Surg. 1993;11:185–189.
16. Katiukhin LN. The role of erythrocyte rheological determinants in the regulation of blood flow structure. Klin Lab Diagn. 2001;33:22–24.
17. Zalesskaya GA, Sambor EG, Kuchinskii AV. Effect of intravenous laser irradiation on the molecular structure of blood and blood components. J Appl Spectrosc. 2006;73:115–122.