The Effect of Low Level Laser Therapy on Some Plasma Ions Concentration

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Abstract

Low level laser therapy (LLLT) has various applications in clinical pathology. It has been found to be efficient in acceleration of wound healing, enhanced remodeling and bone repair, regeneration of neural cells following injury, pain attenuation, endorphin release stimulation and modulation of immune system. The possible intracellular sequence of events after treatment with LLLT, include induction of oxidative phosphorylation with increased enzymatic activity, increased expression of certain cytosolic enzymes and enhanced activity of intracellular organelles. A broad range of eukaryotic proteins require posttranslational modifications such as phosphorylation, glycosylation, or signal peptide cleavage to display full functional activity. Eukaryotic cell-free expression systems provide the possibility to synthesize eukaryotic proteins with posttranslational modifications and are especially useful for expression and analysis of human proteins with native structure and function. The stimulated mechanism involves protein- to-protein interaction, where two or more proteins bind together to facilitate molecular processes, including modification of proteins by members of small ubiquitin- related modifier proteins (SUMO) and also protein phosphorylation and tyrosination.

The consequence of laser application in treatment, can be seen as influencing the transmission of intracellular signals via an integrated and rapid modulation of ion channels, achieved through both direct action on photo-acceptors (such as cytochrome c-oxidase) and through indirect modulation via enzymes, including tyrosine hydroxylase (TH), tyrosine kinases and tyrosine kinase receptors. This exogenous action then facilitates an existing photonic biomodulation mechanism with the body, and initiates ion channel modulation both in the periphery and the central nervous system (CNS).

Evidence indicates that the ion channel modulation functions predominately through the potassium channels, including two pore leak channels (K2P), which act as signal integrators from the periphery to the cortex.

Objectives and Results

Fifteen blood samples were collected from healthy adult volunteers, subjected to low level laser therapy (LLLT) with different wavelengths (650, 532 and 405) nm, plasma concentration of Ca^{++}, Na^{+}, K^{+} and Cl^{-} were estimated after (30 min.) of incubation. The mean concentration of each ion before and after LLLT were as follows (Ca^{++}: 8.67 vs. 8.93, 8.79, 8.82 mg/dl), (Na^{+}: 147.4 vs. 146.73, 146.73, 146.13 mmol/L), (K^{+}: 3.94 vs. 3.94, 3.78, 3.92 mmol/L) and (Cl^{-}: 99.93 vs. 97.86, 102.73, 96.4 mmol/L). In the same manner, activated partial thromboplastin time (APTT) was estimated, the mean value of which were as follows (30.07 vs. 18.45, 21.72, 17.29 sec.).

Keywords: Biostimulation, low level laser therapy, ions (Ca^{++}, Na^{+}, K^{+}, Cl^{-}) and activated partial thromboplastin time .

الخلاصة

العلاج بالليزر ذي المستوي الواطئ له عدة تطبيقات فيما يخص علم الإمراض السريرية . فقد تبين أنه فعال في تعجيل التئام الجروح, تعزيز أعادة بناء وترميم العظام , تجديد الخلايا العصبية بعد حدوث الجرح, تسكين الألم , تحفيز أطلاق الاندورفين وتعديل نظام المناعة. ويمكن تعاقب التغيرات المحتملة الحاصلة في نطاق الخلايا بعد العلاج بالليزر ذي المستوي الواطئ, حيث يتضمن البدء في تحريض الفسفرة التأكسدية مع زيادة النشاط الأنزيمي, وزيادة مقدار أنزيمات العصارة الخلوية المحددة وكذلك تعزيز فعالية الخلايا العضوية. أن المدى الواسع من البروتينات حقيقية النواة يتطلب تعديلات متعدية سابقة مثل الفسفرة, الغليكوزيل, أو أشارة الببتيد الأنقسام لإظهار النشاط الوظيفي الكامل. أن نظم التعبير خالية من الخلايا حقيقية النواة فهي تهيئ لإمكانية تركيب البروتينات حقيقية النواة مع تعديلات متعدية سابقة وتكون مفيدة خصوصا في التوصيف والتحليل للبروتينات البشرية. فالميكانيكية المحفزة تشمل بروتين – بروتين, حيث أن ربط اثنين أو أكثر من البروتينات سوية سوف يسهل العمليات الجزيئية وبضمنها التعديلات المتحدة للبروتينات من خلال أفراد من بروتينات صغيرة والمتعلقة بالبروتينات المعدلة(سومو) . أن نتيجة تطبيق العلاج بالليزر يمكن لمسها في التأثير على انتقال الإشارات الواقعة ضمن الخلايا بواسطة أقنية أيونية ذات تعديل سريع ومتكامل ,والتي تتكون بوجود العملية المباشرة للمستقبلات الضوئية مثل (cytochrome c–oxidase) وكذلك تعديل غير مباشر عن طريق الأنزيمات , والتي تحوي على (TH) Tyrosination, كمتقبل , ومن ثم فأن هذا السلوك الجيني الخارجي سوف يسهل تكوين تقنية التغيير الثنائي الضوئي في الجسم , حيث يبدأ تغير في الأقنية الأيونية في كل من المحيط الخارجي, والمركزي للجهاز العصبي. النتائج تشير إلى أن وظائف تغير القناة الأيونية سيكون هو السائد من خلال أقنية البوتاسيوم متضمنة اثنين من الأقنية المسامية (K2P) والتي تعمل مجموع أشارة متكاملة من السطح الخارجي إلى الطبقة الخارجية للعضو الداخلي.

الأهداف والنتائج

تم أخذ خمسة عشر عينة من الدم من متطوعين بالغين أصحاء. وقد استخدمت تقنية العلاج بالليزر ذي المستوى الواطئ مع أطوال موجية مختلفة (650,532,405) نانومتر. تم تحديد تراكيز البلازما لكل من أيونات (الكالسيوم , الصوديوم, البوتاسيوم, والكلوريد بعد فترة (30) دقيقة من الحضانة. وأظهرت النتائج أن متوسط تركيز كل أيون قبل وبعد العلاج بالليزر ذي المستوى الواطئ عند الأطوال الموجية الثلاثة المذكورة آنفا كانت على التوالي:

(Ca^{++}: 8.67) تقابلها القيم (8.93, 8.79, 8.82) ملي غرام/ديسيليتر

(Na^{+}: 147.4) تقابلها القيم (146.73, 146.73, 146.13) ملي مول/لتر

(K^{+}: 3.94) تقابلها القيم (3.94, 3.78, 3.92) ملي مول/لتر

(Cl^{-}: 99.93) تقابلها القيم (97.86, 102.73, 96.4) ملي مول/لتر

وبنفس الأسلوب تم تحديد تنشيط وقت تجلط الدم الجزئي وكانت نتائج القيمة المتوسطة على النحو التالي:

(30.07) تقابلها القيم (18.45, 21.72, 17.29) دقيقة .

الكلمات المفتاحية: تحضير، العلاج بالليزر ذي المستوى الواطئ، ايونات الكالسيوم، الصوديوم، البوتاسيوم، الكلوريد، ومنشط تجلط الدم، أنزيم الثرموبلاستين.

Introduction

Four dental lasers emit visible light: the argon ion laser has a 488- nm blue color and a 514-nm blue-green color; the frequency doubled Nd: YAG, which has a green color of 532 nm; a low level (nonsurgical power) therapeutic device with a 635 nm red light; and another low-level detector with a similar red color at 655 nm (Duan *et al*., 2001). All other laser devices emit invisible laser light in the near, middle, and far infrared portion of the electromagnetic spectrum. During the interaction of laser radiation in vitro, with biological tissue, the light rays get scattered and at the same time an amount of light can get absorbed by the tissue. To understand the process of scattering and absorption it is necessary to understand the microscopic behavior of blood as blood is a suspension of cells of different shapes and sizes. A research discusses the structure and composition of

blood and effect of these components after exposure of laser radiation with different wavelengths (Watson *et al.*, 1997).

A kind of membrane potential of blood is essential parameter for determination of interfacial potentials during the flow of blood (Polla *et al*., 2001). A thorough study of electrical of human blood has been done with respect to the effect of laser radiation. Literature shows that the erythrocytes almost behave like a perfect nonconductor of direct current. Later studies showed that only the surface layer of the erythrocytes is nonconductive which offers the resistance to flow of blood while the inner structure of the erythrocyte had a conducting medium (Chen *et al*., 1994). Last literature also reveals the fact that erythrocyte does not conduct direct current and acts as a dielectric medium at sufficiently high frequency. Subsequent investigations in last two decades further explain the electro physical characteristics of red blood cells (Sammak *et al*., 1998). It should also be noted here that resistance offered by the blood is proportional to the hematocrit volume which means that red blood cell count has direct impact on the electrophoresis of plasma after exposure of laser radiation, depends upon electrolyte contents and concentrations of ions (Na^+ , K^+ , Cl^- , Ca^{+2}) and proteins (Akgul *et al*., 2014).

There are several lines of evidence that ion channels are involved in the subcellular effects of LLLT. Some channels permit the passage of ions based solely on their charge of positive (cationic) or negative (anionic) while others are selective for specific species of ion, such as sodium or potassium. These ions move through the channel single file nearly as quickly as the ions move through free fluid (Calame *et al*., 2003). In some ion channels, passage through the pore is governed by a “gate,” which may be opened or closed by chemical or electrical signals, temperature, or mechanical force, depending on the variety of channel. Ion channels are especially prominent components of the nervous system. Voltage-activated ion channels underlie the nerve impulse and while transmitter-activated or ligand-gated channels mediate conduction across the synapses (Miller, 2003).

Aim of Study

Determination of major ions concentrations in plasma of blood before and after exposure to LLLT in vitro

Materials and Methods

Fifteen blood samples were collected from healthy adult volunteers in 3.8% sodium citrate in a ratio of 9:1 and lithium heparin. Each sample was fractionated into three fractions, each of (1) ml, and treated with LLLT with three wavelengths (405, 532, and 650) nm for (30 sec.) with energy density of (13.2, 14.4 and 15.6) J/cm^2 respectively. Plasma was separated and calcium, sodium, potassium and chloride were estimated. Activated partial thromboplastin time was also estimated before and after (30, 60 and 90 min) of LLLT.

Results

The mean concentration of each ion (Na^+, K^+, Cl^-, Ca^{++}) before and after LLLT (650, 532 and 405 nm), were shown in table follows (1, 2, 3, 4). In the same manner, activated partial thromboplastin time, was shown in table (5, 6, 7).

The percent of increment or decrement in sodium, potassium, chloride and calcium concentration for each individual sample, as well for PTT was calculated according to the following equation:

% increment or decrement = {Concentration after LLLT - Concentration before LLLT}/Concentration before LLLT x100%

The mean rank on some plasma ions (Na^+, K^+, Cl^-, Ca^{++}) and PTT in (30, 60, 90) min. in test samples after low level laser therapy with different wavelengths (650, 532, and 405) nm, were calculated via Wilcoxon test.

Table (1): The mean concentration, % increment or decrement and mean ranks of Na^+ in test samples before and after LLLT.

Wavelength	Before (mmol/L)	After (mmol/L)	Na^+	Na^+	Z-value
	Mean ± SD	Mean ± SD	P- value	%	
650 nm	147.40 ± 2.06	146.73 ± 2.65	0.259	-0.44	-3.422^{-b}
532 nm	147.40 ± 2.06	146.73 ±2.63	0.302	-0.44	-3.433^{-b}
405 nm	147.40 ± 2.06	146.13 ± 3.15	0.172	-0.84	-3.433^{-b}

P-value was calculated with paired-T test. (-b) Based on positive ranks.

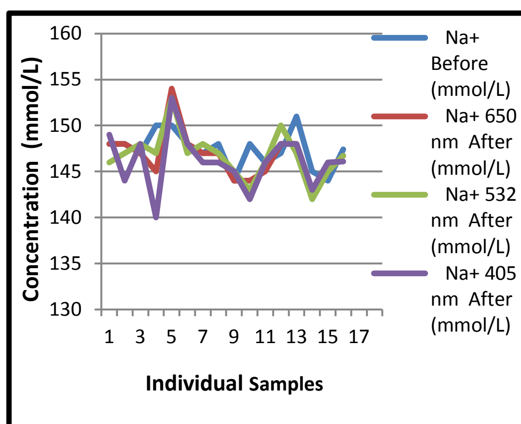

Figure (1): The concentration of Na^+ before and after LLLT for individual samples

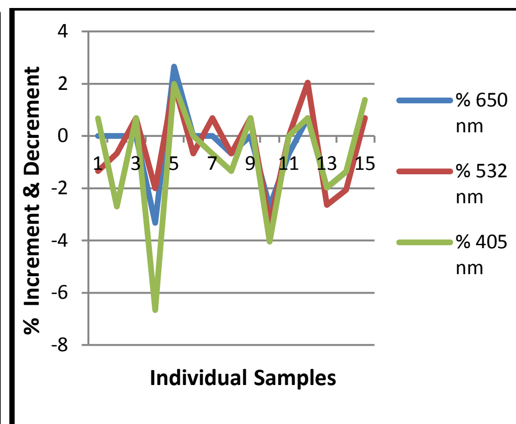

Figure (2): The percent of increment or decrement in Na^+ concentration for individual samples.

As illustrated in figure (1), it is obvious that a decrease in concentration of sodium plasma ion for wavelength (405nm) comparing with the other concentration of wavelengths (532, 650 nm) after LLLT for each individual sample.

Figure (2) shows an obvious positive and negative percent change with sodium after LLLT with three applied wavelengths for each individual sample, in addition it is noticed that the greatest positive percent change with the wavelength (650 nm), also a greatest negative percent change with the wavelength (405nm).

Table (2): The mean concentration, % increment or decrement and mean ranks of K^+ in test samples before and after LLLT.

Wavelength	Before (mmol/L)	After (mmol/L)	K^+	K+ %	Z-value
	Mean ± SD	Mean ± SD	P-value		
650 nm	**4.26 ± 1.16**	**3.94± 0.91**	**0.125**	**-4.99**	**-0.398^{-b}**
532 nm	**4.26 ± 1.16**	**3.87± 0.75**	**0.099**	**-6.44**	**-1.137^{-b}**
405 nm	**4.26 ± 1.16**	**3.92 ± 0.93**	**0.336**	**-3.71**	**-0.284^{-b}**

P-value was calculated with paired-T test, (-b) Based on positive ranks.

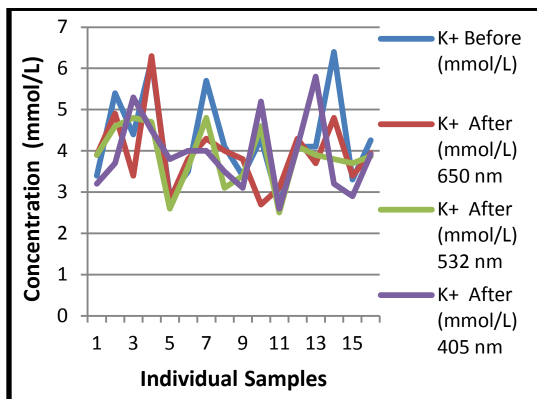

Figure (3): The concentration of K^+ before and after LLLT for individual samples

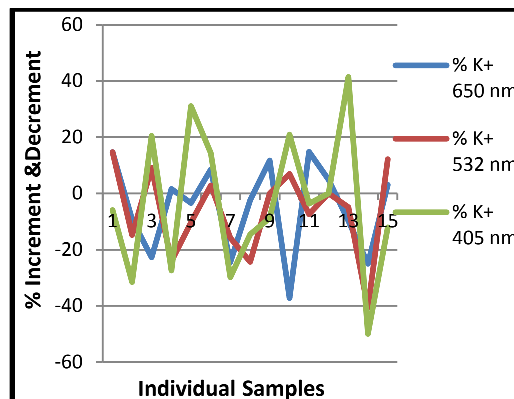

Figure (4): The percent of increment or decrement in K^+ concentration for individual samples

As demonstrated in figure (3), it is clear that a decrease in all concentration for the wavelengths with (650, 532, and 405) nm after LLLT for each individual sample.

Figure (4) shows an obvious positive and negative percent change with K^+ after LLLT in three applied wavelengths for each individual sample, also it is noticed that the wavelength with (405nm) has the greatest positive and negative percent change comparing with the other wavelengths (650, 532 nm.)

Table (3): The mean concentration, % increment or decrement and mean ranks of Cl^- in test samples before and after LLLT.

Wavelength	Before (mmol/L)	After (mmol/L)	Cl^-	Cl^- %	Z- value
	Mean ± SD	Mean ± SD	P-value		
650 nm	**99.93± 9.90**	**97.86 ±10.55**	**0.295**	**-1.93**	**-3.411^{-b}**
532 nm	**99.93± 9.90**	**102.73± 10.45**	**0.437**	**+3.86**	**-3.408^{-b}**
405 nm	**99.93± 9.90**	**96.40 ± 8.78**	**0.313**	**-2.42**	**-3.411^{-b}**

P-value was calculated with paired-T test, (-b) Based on positive ranks.

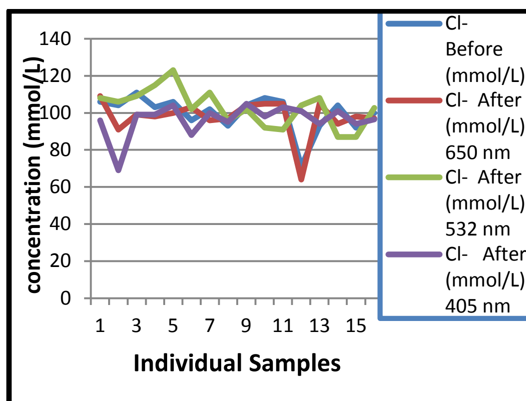

Figure (5): The concentration of Cl^- before and after LLLT for individual samples

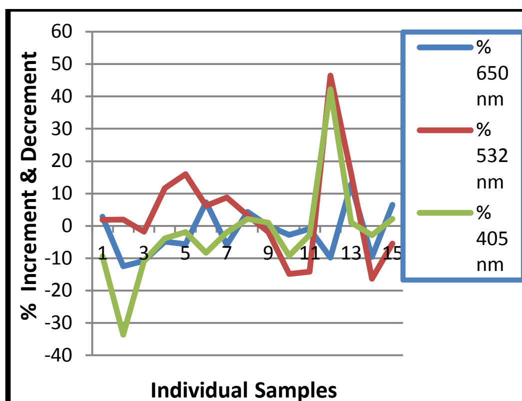

Figure (6): The percent of increment or decrement in Cl^- concentration for individual samples

As demonstrated in figure (5), it is obvious that an increase in concentration of Cl^- with wavelength (532 nm) after LLLT for each individual sample.

Figure (6) shows an obvious positive and negative percent change of Cl^- after LLLT in three applied wavelengths for each individual sample, as well it is noticed that the wavelength with (532, 405) nm have the greatest positive percent change, and it is apparent that the greatest negative percent change of Cl^- with wavelength (405 nm) after LLLT for each individual sample.

Table (4): The mean concentration, percentage increment or decrement and mean ranks of Ca^{++} in test samples before and after LLLT.

Wavelength	Before (mg/dl)	After (mg/dl)	Ca^{++}	Ca^{++} %	Z-value
	Mean ± SD	Mean ± SD	P-value		
650 nm	**8.67± 0.37**	**8.93 ± 0.34**	**0.011***	**+2.94**	**-3.296^{-b}**
532 nm	**8.67± 0.37**	**8.79± 0.38**	**0.001***	**+1.37**	**-3.297^{-b}**
405 nm	**8.67± 0.37**	**8.82± 0.34**	**0.016***	**+1.78**	**-3.233^{-b}**

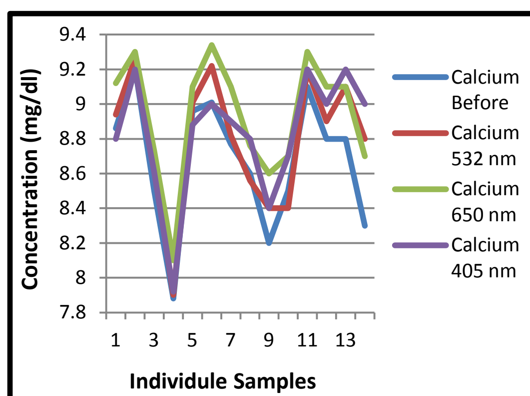

Figure (7): The concentration of Ca^{++} before and after LLLT for individual samples

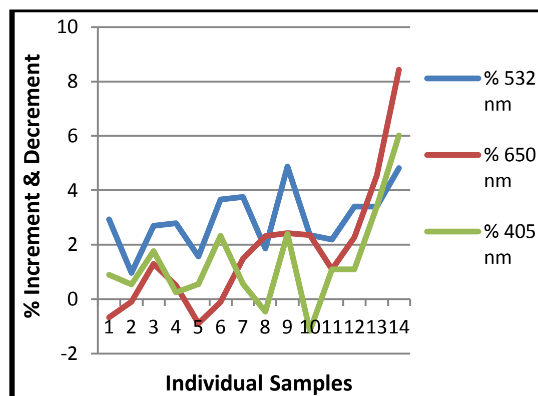

Figure (8): The percent of increment or decrement in (Ca^{++}) concentration for individual samples

As demonstrated in figure (7), it is obvious that an increase in concentration of Ca^{++} in three wavelengths, especially the wavelength with (650 nm) after LLLT for each individual sample.

Figure (8) shows an obvious increment in the percent change of calcium ion concentration after LLLT with 532 nm in all samples, while some of the samples showed a negative change with 650 and 405 nm LLLT, with a net increment in the mean change of all samples

Table (5): The mean value and % increment or decrement of PTT in (30) min. for test samples before and after LLLT.

Wavelength	Before	After	PTT	PTT %	Z-value
	Mean ± SD	Mean ± SD	P-value		
650nm	30.07± 5.25	18.45± 3.55	0.0001*	-36.91	-3.182^{-b}
532nm	30.07± 5.25	21.72± 4.97	0.0001*	-27.43	-3.182^{-b}
405 nm	30.07± 5.25	17.29± 3.66	0.0001*	-41.34	-3.182^{-b}

P- value was calculated with paired-T test, (-b) Based on positive ranks.

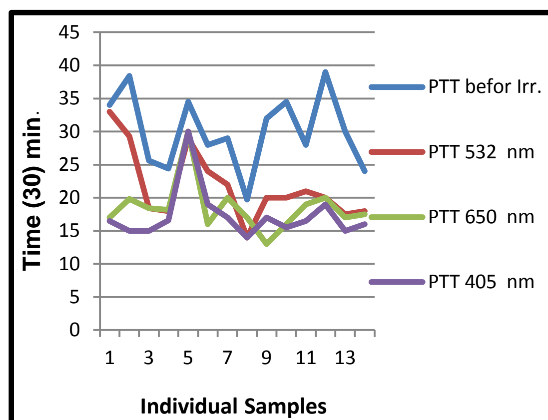

Figure (9): Time of (PTT) before and after LLLT for individual samples samples

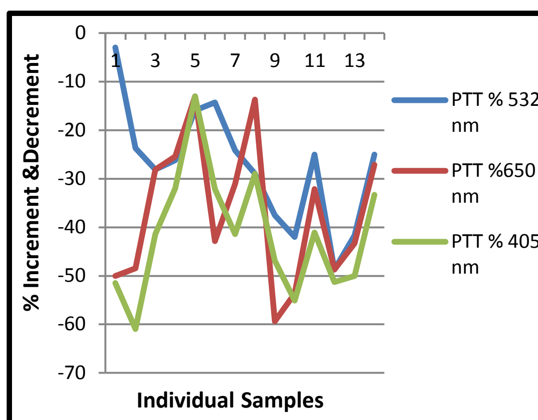

Figure (10): The percent of increment or decrement in (PTT) for individual

As illustrated in figure (9), it is clear that there is a decrease in PTT after (30 min.) of LLLT for each individual sample.

Figure (10) shows an obvious negative percent change of PTT after (30 min) of LLLT with three applied wavelengths for each individual sample, also, it is clear that the wavelengths with (650, 405 nm) have the greatest negative values compared with a wavelength (532 nm).

Table (6): The mean value and % increment or decrement of PTT in (60) min. for test samples before and after LLLT.

Wavelength	Before	After	PTT	PTT %	Z-value
	Mean ± SD	Mean ± SD	P-value		
650 nm	31.54 ± 5.20	18.6± 5.09	0.0001*	-40.61	-2.418 -b
532 nm	31.54 ± 5.20	21.07± 8.14	0.0001*	-32.92	-3.233-b
405 nm	31.54 ± 5.20	16.42 ± 3.88	0.0001*	-43.07	-3.297 -b

Table (7): The mean value and % increment or decrement of PTT in (90) min. for test samples before and after LLLT.

Wavelength	Before	After	PTT	PTT %	Z-value
	Mean ± SD	Mean ± SD	P-value		
650 nm	30.09± 5.62	27.21 ± 6.31	0.132 *	-7.42	-3.233 -b
532 nm	30.09± 5.62	28.53 ± 6.68	0. 180*	-5.18	-2.418-b
405 nm	30.09± 5.62	25.46 ±5.44	0.004*	-14.29	-3.297 -b

P-value was calculated with paired-T test, (-b) Based on positive ranks

Discussion

The above mentioned results listed in the results section obviously reveal that there is no significant change in $Na^{+,}$ K^{+} and Cl^{-} concentration after LLLT with the three wavelengths used (650, 532 and 405 nm), supported by the statistical analysis, with insignificant p-value for all, while the only ion that has revealed a significant change is calcium with a mean increment in all samples and a significant p-value. These provide a partial explanation to the general decrease in activated partial thromboplastin time (APTT) results in all samples, especially noted after 30 minutes of incubation. The possible explanation of increased plasma calcium ion concentration is expulsion of calcium ion from the intracellular compartment to the extracellular compartment. In general, calcium ion concentration is kept at a very narrow limit of concentration intracellularly by trans-membrane transport enzymes (Adenosine Triphosphate ATP dependant calcium transporters), that expel calcium ion and transport potassium to the intracellular compartment. The above results obviously show that there is a net decrease of potassium ion concentration in all three used wavelengths of LLLT versus a net increase of calcium ion concentration; this observation suggests that there is a possible enhancement of the activity of calcium trans-membrane transporters, maximally observed on 650 nm laser therapy.

Because that the concentration of sodium ion in blood plasma is relatively high (the normal value ranges from 135-150 mmol/L) in addition to the fact that sodium ion is freely diffusing across cell membrane following concentration gradient, the variation in sodium ion concentration is relatively narrow after LLLT, compared with other plasma ions. Similarly, chloride ion concentration showed no significant variation in plasma after LLLT, and probably this can be attributed to the same reasons above.

The alteration in activated partial thromboplastin time after LLLT is towards the reduction of APTT, this can be attributed to a great extent to the increase of calcium ion

concentration, since this test relies completely on calcium ion concentration added to the test reagent as an activator or a cofactor. Looking back to the results, the maximum reduction in APTT after 30 minutes of incubation was after LLLT with 405 nm, while the maximum increase of calcium ion concentration was noted with 650 nm. This discrepancy between these results suggests that there is another factor playing role in the decrease of APTT, probably the increased concentration in one or more of the coagulation factors or the accumulation of ADP as a result of increased consumption of ATP or many other cofactors. In general the reduction in APTT was clearer after 30 minutes of incubation after LLLT, remained lower than the initial time after 60 and 90 minutes of incubation of the same samples, but was higher than those of 30 minutes incubation. The possible explanation is that there is a gradual re-incorporation of calcium ion into the intracellular compartment by diffusion after exhaustion of energy stores and after recovery of the activated membrane transport enzymes.

There is a lot of literatures on the kinetics of various classes of ion channels but in broad summary it can be claimed that the time scale or kinetics for opening and closing of ion channels is of the order of a few milliseconds. For instance (Gilboa *et al*., 2005) used pulses having a width 10 milliseconds and a period of 40 milliseconds (25 Hz). Other reports on diverse types of ion channels have given kinetics with timescales of 160 milliseconds (Priestley & Kemp, 1994) 3 milliseconds (Schneggenburger & Neher, 2000) and one paper giving three values of 0.1, 4 and 100 milliseconds (Kampa *et al*., 2004). Potassium and calcium ion channels in the mitochondria and the sarcolemma may be involved in the cellular response to LLLT (Chow *et al*., 2007, Karu, 2008).

At last, there is a possibility that one mechanism of action of LLLT on a cellular level is the photo dissociation of nitric oxide (NO) from a protein binding site (heme or copper center) such as those found in cytochrome *c* oxidase (Karu, 2004). If this process occurs, it is likely that the NO would rebind to the same site even in the presence of continuous light. Therefore if the light was pulsed multiple photo dissociation events could occur, while in continuous wave (CW) mode the number of dissociations may be much smaller.

Conclusions

1. Low level laser therapy (LLLT) with the three used wavelengths (650, 532, 405) nm showed no significant effect on plasma levels of Na^+, K^+ and Cl^- ion concentration, but in general there is a net decrease in the concentration of all ions, especially noted on K^+ concentration.
2. LLLT has resulted in a significant net increase in calcium ion concentration especially noted with (650 nm) wavelength LLLT (an increment rate of 2.49 %).
3. There is a general decrement in activated partial thromboplastin time (APTT) after (30, 60 and 90) minutes of incubation following LLLT, which was more significant after (30) minutes in all used three wavelengths, possibly explained by the net increase in calcium ion concentration.

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