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ESTIMATION OF ATPASE ACTIVITIES AND EXTRACELLULAR ION CONCENTRATIONS OF (NA^+, K^+, CL^-AND CA^{+2}) AFTER IRRADIATION OF BLOOD SAMPLES WITH LASER 532NM AND LASER 650NM

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SUMMARY

The present study was conducted to explain how light energy of laser can be affected of ion pumps (ATPase) and concentrations of available ions across cellular membranes.

A whole blood samples were collected and divided into three groups, the first group was remained without irradiation to serve as a control group and to perform comparisons between irradiated and non-irradiated samples, The second group was subdivided into two subgroups. The first subgroup was irradiated with laser 532nm, 4mw for 10 minutes and the second subgroup was irradiated with 650nm, 135mw for 10 minutes , there after these samples were used to determine the activities of ATPase and the obtained results showed a remarkable increase in the ATPase activities of irradiated blood samples when compared with non-irradiated blood samples .The results showed increased activities of ATPase in laser 650nm via increase concentrations of in organic phosphate (pi) resulted from hydrolysis of ATP to ADP.

Three group of samples was also subdivided into two subgroups. The first subgroup was consequently irradiated with laser 532nm, 4mw for 5 and 10 minutes. The second subgroups was also consequently irradiated with laser 650nm, 4mw for 5 and 10 minutes. After irradiation, the samples were used to determine the common available ions (Na^+, K^+, Cl^- and Ca^{+2}). Results of Na^+ showed a progressive decrease in both irradiated blood sample (532nm, 650nm) when compared with non-irradiated samples. On the other hand, results of the other ions (K^+, Cl^-, Ca^{+2}) showed a remarkable increase in all irradiated of blood- samples in a comparison with control group. The low levels of Na^+ were found to be associated inversely with increase wave length of laser especially at 650nm.

On the other hand, the decrease in concentration of ion (Ca^+, K^+, Cl^-) were directly proportional with wave length of laser beam.

In conclusion, the data obtained from this study indicated that light energy can be exert photo-biostimulation, in particular, on ATPases enzymes and increases their activities through increase the final product of inorganic phosphate (pi). At the same time, the laser energy can be affect ion channels responsible for distribution of ions across cell membranes and enhances the movement of ions across cellular membranes too much when blood samples become irradiated with laser energy.

The aim of this study involved to determine the effects of two types of laser (532nm and 650nm) with 4mw and 135mw on adenosine triphosphatase enzymes (ATPases) activities and then after estimation of some available ions to explain the effects of light energy on membrane potential (ion distributions).

Keywords: ATPase, laser, photobiostimulation, ions

INTRODUCTION

Laser has ability to interact with living tissues by three reactions including photochemical, photothermal, photoplasmal pathways. The photochemical interactions involve that the very low energy irradiated acts to inactivate cellular functions by production harmful toxic compounds. Converting it to heat. The pohotoplasmal reaction occurs when irradiance located on the order of 10^8 or order 10^9 w/cm^2, so plasma formation happens in this reaction (Welch *et al*., 1989).

It has been showed that laser light can be induced activation of many changes in protein energy. These observations were supported by many evidences such as activation of the rhodopsin/ bacterio rhodopsin pigment by light induce excitation leading to hyper polarization states of cell membranes (Blank and Soo, 2001). It is well documented that laser represents a high stable origin for light that have coherent and mono chromatic light and so that they have been widely applied in different technical and medical applications. Studies were focused on the effects of laser light on cellular membrane functions especially changes occurring in enzymatic activities and ion channels across cell membrane (Kassak *et al*., 2006).

Erythrocytes are unique cells in studying of laser light effect because they are more prominent and simplicity. The red blood cells undergo many changes during exposure to He-Ne laser irradiation such as hemolysis and osmotic fragility (Zavodnik *et al*., 2002). Erythrocyte membranes have three ATPase enzymes particularly Ca^{+2}-ATPase, Na^+-K^+ATPase and Mg^{+2}ATPase in different regions of cell membrane (Drickamer, 1975).

The effect of light irradiation on living tissues can be classified into many patterns according to wave length and power intensity of the radiation.

The nanometer unites used to express wave length and power intensity means energy delivered by area of exposure (Basford, 1995).

It is well documented that absorption and scattering level of laser light in living tissues are highly rates in blue region than of red and this is because the tissue chromopheres particularly hemoglobin and melanin can to absorb bands of light at short region of wavelength (Karu and Afansa, 1995). Irradiated of isolated mitochondria with He-Ne laser showed accelerated ATP-ADP metabolism, increased ATP output and propagation of the electrical field across mitochondrial membrane (Passarella *et al*., 1988).

Laser has potobiological effect on many cells and tissues. These effects a ris as a result depend essentially on the wave length of laser and cell type (Anwer *et al*., 2012).

MATERIALS AND METHODS

Collection and irradiation of blood samples: The blood samples were drawn from healthy subjects, their ages ranged between 17 to 22 years old. The blood samples were collected at 8 to 10 A.M. The anticubital vein was employed and before collection the skin was cleaned with 70% alcohol solution and wormed to improve blood circulation. The torniquate was applied around arm at 7 cm above the site of collection.

A series of heparin containing tubes were prepare and arranged into several groups. The first group was irradiated with He-Ne laser (532nm, 4mw) for 5 and 10 minutes. The second group was also irradiated with red laser (650nm, 135 mw) for 5 and 10 minutes. The two groups were immediately transferred to centrifuge at 3000 rpm for 15 minutes to obtain the plasma that used to determine of available ions (Na^+, K^+, Cl^-, Ca^{+2}) and comparing their levels with non-irradiated blood samples (control group). Also, blood samples were used to estimate the activities of ATPase enzyme activities. Two groups of heparins containing tubes were also prepared. The first group was irradiated with He-Ne laser (650nm, 135mw) for 10 minutes. The second group was exposed to laser 532nm, 4mw for 10 minutes and then immediately used to determine ATPase activities and these activities were compared with non-irradiated blood samples (control group).

Determination of ATPase activities: This method was based on determination of the inorganic phosphate amount (pi) that liberated during hydrolysis of ATP compound by Adenosine tirphosphosphatase enzymes (ATPase) and expressed as nmol phosphate per mg of protein liberated during 30 minutes of incubation. Briefly, 15µL of suspension was incubated with 55µl of medium containing 100mMtris-HCl, 10Mm $MgCl_2$, 15Mm KCl, 85Mm NaCl, 1Mm EDTA, 2Mm ATP PH 7.4.

This mixture was incubated at 37°C for 30 minutes to observe enzymatic and non-enzymatic and hydrolysis of ATP molecules. Then, the reaction was stopped with addition of trichloro acetic acid (15%). The amount of inorganic phosphate was with malachite green and measured at 640nm of spectrophotometer.

Measurement of sodium: Sodium ion are precipitated with Mg-Uranl acetate. The uranyl ion form dark yellowish with thioglycolic and the absorbance read at 410nm (according to kit of spin react company)

Measurement of potassium: In alkaline medium and protein, potassium ions are reacting with sodium tetra phenyl boron to form potassium tetra phenyl boron with turbid color.

The intensity of color is proportional directly with concentration of potassium (according to kit of spin react). The absorbance was read at 578nm.

Measurement of chloride ion concentrations: In acidic medium, chloride ions and mercury 11-thiocynate react to form thiocynate ions. These ions then react with both HNO_3 and Fe^{+3} forming red color. The intensity of color proportional directly with chloride ion concentrations. The absorbance read at 505nm. (according to AGAPPe Diagnostic Switzerland).

Measurement of calcium ions: In alkaline medium, calcium ions tend to react with cresolftalein complex to form complex colour that proportional directly with calcium concentration in sample. The intensity of color is measured spectrophotometrically at 570nm (Randox Company).

Statistical analysis: The results of this study were expressed as Means± standard deviation.

RESULT AND DISCUSSION

The effects of laser (352nm, 4mw) and (650nm, 135mw) on ATPase activities (Table 1): The results of ATPase activities a explained remarkable increase when exposed to laser 532nm, 4mw for 10min and laser 650nm, 135mw for 10 minutes.

Table 1: The effects of laser 532nm, 4mw and laser 650nm, 135mw for 10 minutes on ATPase activities of irradiated and non-irradiated blood sample

No.	Native (mmol/L Pi)	ATPase (mmol/L Pi)	Protein (g/L)	ATPase/Protein ratio (µmol Pi/g Protein)	
1	1.88	0.73	174.4	4.2	Without laser
2	2.32	1.002	196.6	5.1	Green laser
3	2.79	1.57	216.0	7.3	Red laser
4	0.96	0.63	203.3	3.1	Without laser
5	1.3	0.86	205.1	4.2	Green laser
6	1.92	1.58	184.3	8.6	Red laser
7	1.16	0.67	192.6	3.5	Without laser
8	1.28	1.16	212.3	5.5	Green laser
9	1.77	1.6	205.3	7.8	Red laser
10	3.25	0.33	139.1	2.3	Without laser
11	2.66	0.57	135.6	4.2	Green laser
12	2.3	1.04	130.8	7.9	Red laser
13	1.75	0.58	181.4	3.2	Without laser
14	2.57	0.69	192.4	3.5	Green laser
15	2.95	1.45	187.5	7.7	Red laser

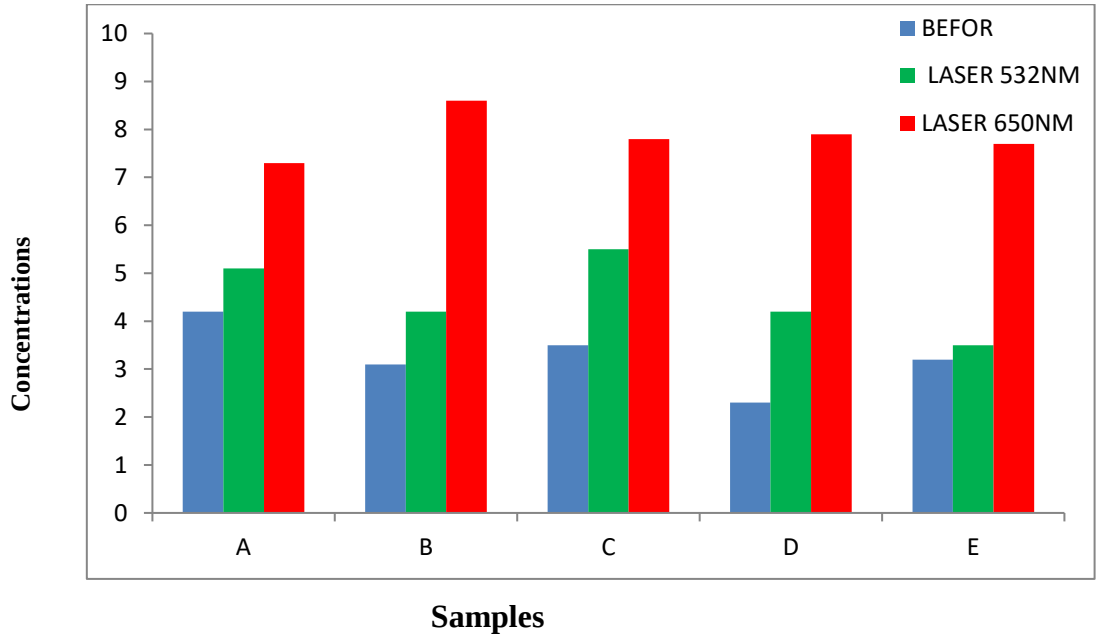


Figure 1: Shows the effects of laser 532nm and laser 650nm for 10 minutes on ATPase activities of irradiated and non-irradiated blood samples

Results of Na^+ ions of blood samples irradiated with laser 532nm, 4mw for 5 minutes and 10 minutes (142.3±6.75, 140.7±5.53 mmol/l, respectively) were decreased. Also, The Na^+ of irradiated blood samples with laser 650nm, 135mw for 5 min and 10 min (139.9±6.00, 138.9±12.30 mmol/l , respectively) recorded more decreased when compared with non-irradiated blood samples (control group).

Table 2: Na^+ ion concentrations of irradiated blood sampels with both la sers (532nm, 650nm) for 5 and 10 minutes.

		(laser) 650nm		(laser) 532nm	
No.	**Wit out laser**	**10min**	**5min**	**10min**	**5 min**
1	140	111	130	137	139
2	135	133	134	130	130
3	144	143	133	142	141
4	150	144	145	145	147

5	150	148	147	148	154
6	148	149	140	145	147
7	147	145	147	146	148
8	150	147	140	136	139
9	145	144	144	139	139
10	140	125	139	139	139
Means	**144.9±5.15**	**138.9±12.30**	**139.9±6.00**	**140.7±5.53**	**142.3±6.75**

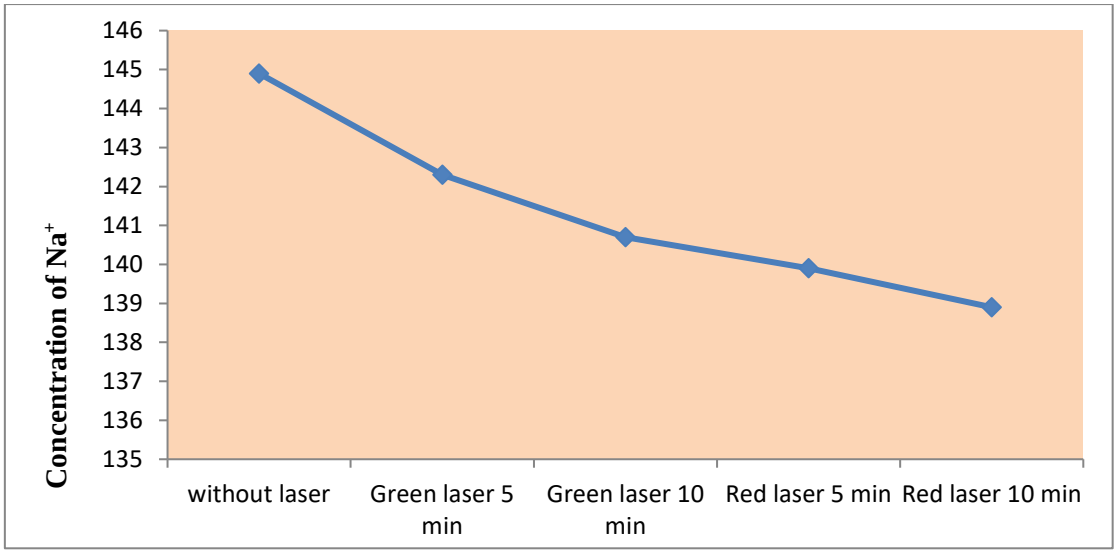


Figure 2: Concentration of Na^+ ions of irradiated blood samples with laser 532nm, 4mw and laser 650nm, 135mw for 5 and 10 minutes

Results of K^+ ion concentrations (Tab 3, Fig 3): Data of K^+ ions obtained from irradiated blood samples with laser 532nm, 4 mw for 5 and 10 minutes (4.39±0.30, 4.44±0.29 mmol/L, respectively) were markedly elevated in a comparison with control samples. Moreover, blood samples irradiated with laser 650 nm, 135mw for 5 and 10 minutes (4.48±0.32, 4.48±0.39 mmol/L respectively) were increased when compared to control samples

Table 3: Result of K^+ ions irradiated blood samples with laser 532nm, 4 mw and laser 650nm, 135mw for 5 and 10 minutes

		(laser) 650nm		(laser) 532nm	
NO.	**Without laser**	**10 min**	**5min**	**10 min**	**5 min**
1	4	4	4.1	4.2	4.2
2	3.8	4.2	4	4.1	4
3	4.5	4.8	4.8	4.6	4.6
4	4	4	4.6	4.2	4.1
5	4	4.3	4.2	4.1	4.1
6	4.3	4.8	4.4	4.7	4.6
7	5.7	4.5	4.3	4.9	4.8
8	4.4	4.2	4.9	4.8	4.8
9	4.5	5	4.9	4.5	4.5
10	4	5	4.4	4.3	4.2
Means	**4.32±0.54**	**4.48±0.39**	**4.46±0.32**	**4.44±0.29**	**4.39±0.30**

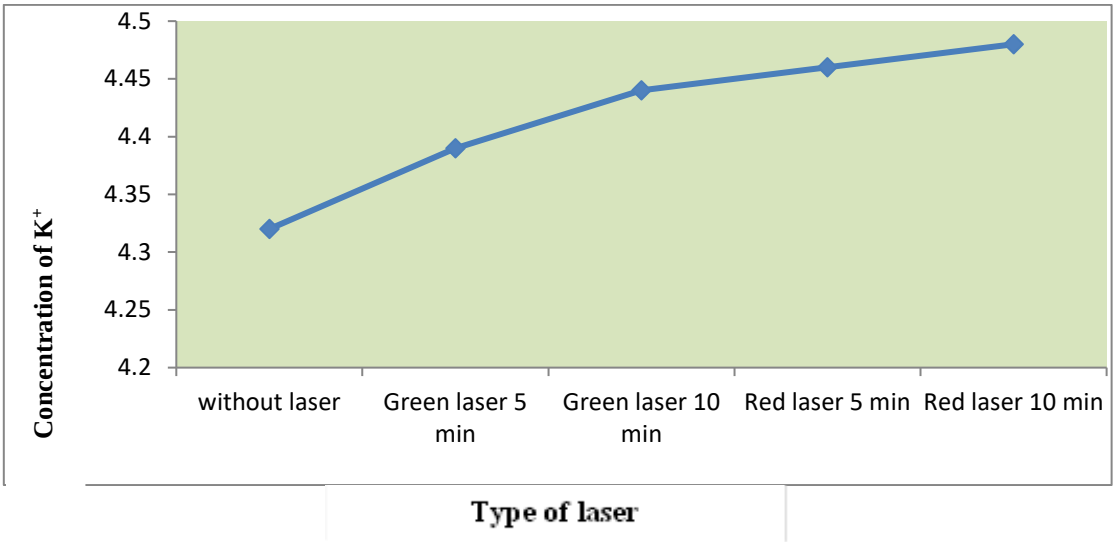


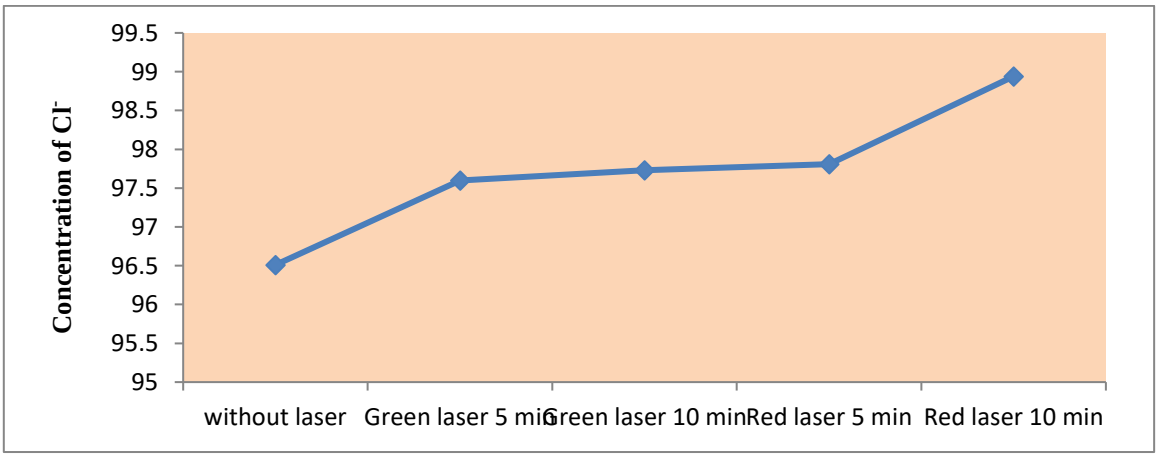
Figure 3: Concentrations of K^+ ions of irradiated blood samples with laser 532nm, 4mw and laser 650nm, 135mw for 5 and 10 minutes

control samples. Concerning blood samples irradiated with laser 650nm, 135mw for 5 and 10 minutes (97.81±5.57, 98.94±6.08 mmol/L, respectively) appeared more increased in a comparison with non-radiated blood sample.

Levels of chloride (Cl^-) ions (Table 4, Fig 4): Levels of chlorid ions (Cl^-) of irradiated blood samples with laser 532nm, 4mw for 5 and 10 minutes (97.6±5.29, 97.7±3.50 mmol/L, respectively) pointed out an elevation compared to

Table 3: Results of K^+ ions of irradiated blood samples with laser 532nm, 4 mw and laser 650nm, 135mw for 5 and 10 minutes

		(Laser) 650nm		(Laser) 532nm	
No.	**Without laser**	**10min**	**5min**	**10min**	**5min**
1	91.6	92.1	94.3	93.4	92.6
2	98.1	106.1	100.2	99.3	98.1
3	98.2	92.1	95.5	95.3	97.1
4	90.9	101.2	91.4	91.3	91.3
5	95.1	100.1	96.3	97.1	96.5
6	97.7	99.1	98.5	99.1	97.3
7	90.9	94.5	96.3	96.1	97.1
8	97.7	96.4	98.2	98.3	98
9	97.8	96.6	95.3	97.3	96.8
10	107.1	111.2	112.1	110.1	111.2
Means	**96.51±4.84**	**98.94±6.08**	**97.81±5.57**	**97.73±5.02**	**97.6±5.29**



Type of laser

Figure 4: Concentrations of Cl^- ions of irradiated blood samples with laser 532nm, 4mw and laser 650nm , 135mw for 5 and 10 minutes

Levels of Ca^{+2} ions in irradiated and non-irradiated blood samples (Table 5, Fig 5): Results of calcium ions Ca^{+2} of irradiated samples with laser 532nm, 4mw for 5 and 10 minutes (9.43 ± 1.06, 9.68±1.06 mg/dl, respectively) are increased when compared to control group (non-irradiated). Also, irradiated blood samples with laser 650nm, 135mw for 5 and 10 minutes (10.26±1.85, 10.34 ± 1.87 mg/dl, respectively) are heightened levels in a comparison with control groups.

Table 4: Levels of Ca^{+2} ions of irradiated blood samples with laser 532nm, 4 mw and laser 650nm, 135mw for 5 and 10 minutes

		(Laser) 650nm		(Laser) 532nm	
No.	**Without laser**	**10min**	**5min**	**10min**	**5min**
1	9.17	10.9	10	9.9	9.9
2	9	10.4	10.2	9.2	9.1
3	8.8	10.4	8.9	9	9.4
4	8.3	8.9	8.5	8.1	8.5
5	8.2	8	8.8	8.8	8.4
6	8.5	9.5	12.5	9.9	8.5
7	9.3	9.9	9.4	9.9	8.8
8	9	9.8	9.4	9.5	9.2
9	9.7	10.5	10.5	10.5	11.3
10	10.8	15.1	14.4	12	11.2
Means	**9.077±0.76**	**10.34±1.87**	**10.26±1.85**	**9.68±1.06**	**9.43±1.06**

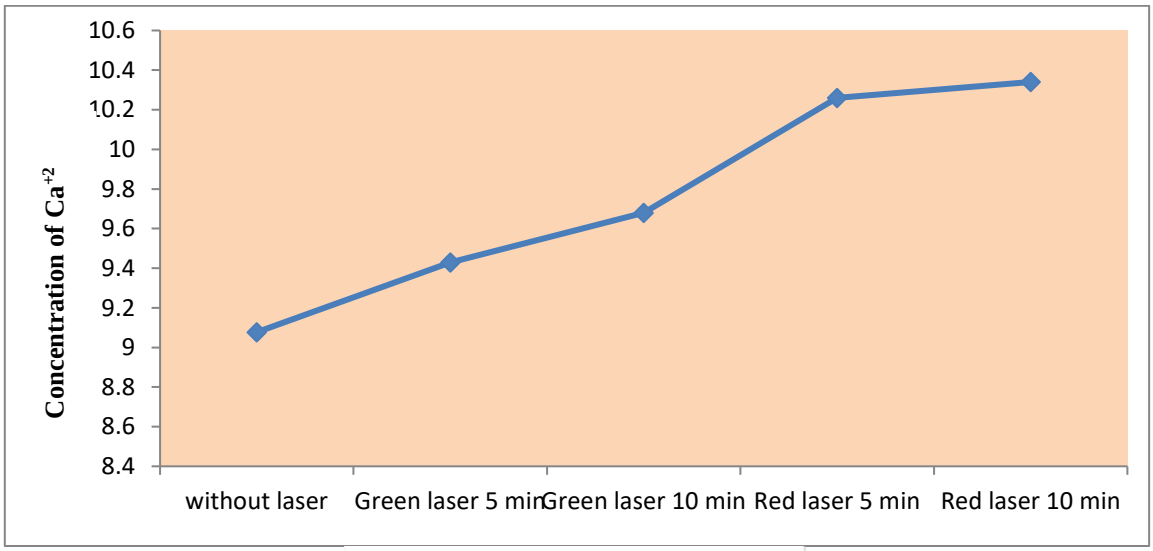


Figure 5: Concentrations of Ca^{+2} ions of irradiated blood samples with laser 532nm, 4mw and laser 650nm, 135mw for 5 and 10 minutes

Membranes of cells are primarily surrounded with aqueous buffer having many electrolytes such as Na^+, K^+, Mg^{+2}, Ca^{+2}, and Cl^-. These ions are not distributed in equall manner, since, the intracellular and extra cellular enviroments have variant concentrations of these ions. The distribution of ions across cell membrane depends on many factors including channel of ions, ATPase (pump), electrochemical gradient and concentration gradient (Bokmann *etal*, 2003).

Previous study indicated that elevated levels of intracellular H^+ ions affects the Na^+-H^+ antiport activities within plasma membrane (Pouyssegur, 1985). Experimental studies that are carried out on irradiated cells with laser invitro give an evidence that a high amounts of hydrogen ions and ATP can activated of other channels and ions carriers of the cell membranes such as Na^+. Also, the activation of essential ion pumps , particularly Na^+-K^+ pump causes to increase levels of intracellular K^+

and decrease intra cellular Na^+ (Rozengurt and Mendoza, 1980).

It is well documented by previous research that changes occurring in respiratory chain as a result of photobiostimulation for enzymes can shift the flux of calcium ions between mitochondria and cytoplasmic compartments (Karu, 1988). Our suggestion about increase extracellular ions (Cl^-, K^+, Ca^{+2}) in extracellular fluids (plasma) can be return to increase motion of intracellular molecules and ions that resulted because of light absorption that resulted because of light absorption by intracellular chromophores and these alterations can increase movment of ion out of cells.

Following many reseach, there is growing evidence that UV light have many possible pathways implicated in the breakage of disulphid bonds within molecule of proteins leading to produce many conformational changes of affected protein molecules (Petersen *et. al*, 2009). There are many alterations can be appeared in cells especially their membranes during irradiation by laser light . It well mentioned that laser can affected many enzyme activities of cell membranes including acetyl cholinesterase (AChE) and Na^+-K^+ ATPase (Piasceka *etal*, 2000, Kujawa *etal*, 2003). Membranes of red blood cells have not only lipid but als contain other components including protein molecules, glyco protein and elements either embedded within membrane or localized on its surface. The variation of membrane structures appears responsible for the differences in extent of absorption for laser radiation (Pasternak *et al*., 2014). Study of Kassak *et al.,* (2006) shwed that there is a positive effects (biostimulation) of Na^+/K^+-ATPase of red blood cells activities when irradiated with green light laser at fluences ranged between 9-63J/cm^{-2}.

Photon is absorbed and intiates many electrically event by excitation of atoms and then energy is taransferred and causes cascad of many chemical and signaling pathway within the cells or enzymatic activity (Karu, 1998). When laser irradiation is focused on living cells, many of energy are transferred to transient temperature that in turn causes many conformational changes and initiate several biochemical reactions, in particular, membrane ATPase affecting active site of enzyme molecules (Lumry, 1959) and thus functions of enzymes can be increased due to absorbs of light by surrounding lipid and enzymes itself. The decrease of Na^{+2} concentrations in this study may be attributed to increase photobiostimulation of enzyme Na^+-K^+ ATPase that exceed the transport of Na^+ ions by Na^+ channels and this enhancement appear overwhelming to Na^+-K^+ ATPases.

Concerning IR effects on lipid bilayer of cellular membrane, it is enabling to damage lipid peroxidation especially polyunsaturated fatty acids causing to increase membrane pereambility (Reisz *et al*., 2014).

It is well known that photobiostimulation showed laser with low power has marked effects on living cells of biological system, the photons of the light can be absorbed by specific molecules called chromophores (Sutheland, 2002). Low intensity of laser increases reduction-oxidation potential of cells lead to increase oxidation rate and reactive oxygen species (Karu, 1999, Alexandratou *et al*., 2002).

The light of laser enhances ATP production with elevated gradient of proton across mitochondrial membrane. Activities of many antiport ion exchange also increased such as Na^+/H^+ and Ca^{+2}/Na^+ and accompanied with increase many ion carriers dependent ATP including Na^+/K^+ ATPase and Ca^{+2} ATPase (Hamblin, 2009).

Irradiation of cellular components with laser can be produced many harmful compounds called lipid peroxides that they orginate from unsaturated fatty acids of cell membrane and the final product of lipid peroxide essentially called MDA (Stadler *et al*., 2000). It is well suggested that photobiostimulation of laser is focused on respiratory chain of mitochondria and lead to enhance electron transport activity and finally associated with increase ATP production (Yu *et al.*, 1999).

Previous study of Kilanzyk *et al*., (2002) indicated that irradiation of cells with many doses of radiation lead to increase enzymatic activities of red cell membranes in particular Na^+/K^+-ATPase and this study used 670nm wavelength on red cell membrane of human to explain the activity of Na^+/K^+-ATPase our results can be consistent with the purpose of this study, since, we recognized increase activities of ATPase enzymes when irradiated with red laser.

There are many alterations of ATPase activities are found when red blood cells irradiated with near infrared low intensity laser. These changes that occurring in pumps of ions are found not associated with other parameters such as oxyhemoglobin, antioxidants, and membrane lipid. When laser power of 10mw applied on erythrocytes, it is accompanied with increase ATPase activities but when laser intensity reaches to 400 mv led to inhibition of ATPase activities (Kujawa *et al.*, 2004).

Study of Ando *et al*., (2009) revealed increase membrane potential hyper-polarization when non-excitable cell exposed to the laser with 780nm. It

is well noted increase intracellular calcium ion (Ca^{+2}) and this study confirmed that cellular interactions with laser occur as a result of release of Ca^{+2} ions which in turns activate potassium ion channels causing hyperpolarization to occur.

CONCLUSIONS

From data mentioned above, Laser energy exerts photobiostimulation of enzymes located within cellular membranes and increase their activities to transport more ions and increase enzymatic hydrolysis of ATP to ADP Increase flow of major ions across cell membranes and mediated this pocess by induction of conformational changes in ion channels that might be increase ion redistribution across cell membrane. It is appeared more obviously that red laser (650nm) has more effects on studied parameters than of green laser (532 nm). Increase light absorption via intracellular chromophores can lead to increase motion of ions and other intracellular molecules.

REFERENCES

Alexandratou, E., Yova, D.; Handris, P. Kletsas, D. and S. loukas, Human fibroblast alterations induced by low power laser irradiation at the single cell level using confocal microscopy. Photochem. Photobiol. Sci. 1(8): 547-552 (2002)

Ando, J., Smith, N., Fujita, K. and S. Kawata, Photogeneration of membrane potential hyperpolarization and depolarization in non-excitable cells. Eur. Biophys. 38(2): 2255-262 (2009)

Anwer, A.G., Gosnell, M.E., Perinchery, S.M. Inglis, D.W. and E.M. Goldys, Visible 532nm laser irradiated of human adipose tissues derived stem cells: effect on proliferation rates mitochondrial membrane potential and Auto fluorescence. Laser in Surgery and Medicine 44: 769-778 (2012).

Basford, J.R., Low intensity laser therapy still not an established clinical tool. Laser in Surgery and Medicine. 6: 331-342 (1995).

Blank, M. and L. Soo, Optimal frequencies for magnetic acceleration of cytochrome oxidase and Na^+/K^+ ATPase reactions. Bioelectrochemisty and Bioenergetics 53: 171-174 (2001).

Bokmann, R.A., Hac, A., Heimburg, T. and H. Grubmuller, Effect of sodium chlorid on a lipid bilayer. Biophs. J. 85(3): 1647-1655 (2003).

Drikamer, L.K., The red cell membrane contains three different adenosine triphosphatases. J. Biol. Chem. 250: 1952-1954 (1975)

Hamblin, M.R., Mechanism of low level light therapy. Proc. Of SPIE 6140:61400-10 (2009)

Karu, T., Primary and secondary mechanisms of action of visible to near – IR radiation on cells. J. Photochem. Photobiol. 49(1): 1-17 (1999)

Karu, T.I., Molecular mechanism of the therapeutic effect of low – intensity laser radiation. Laser in the Life Sciences 2(1): 53-74 (1998).

Karu, T.I., The science of low power laser therapy. Gordon and Breach Sci. Publ., London (1998).

Karu, T.I. and N.I. Afanas, Cytochrome C oxidase as the primary photoacceptor upon laser exposure of cultured cell to visible and nesr IR-rang light. Dokl. Akad Nauk 342(4): 355-361 (1995)

Kassak, P., SikuRova, L., Kvasnicka, P. and M. Bryszewska, The response of Na^+/K^+-ATPase of human erythrocytes to green laser light treatment. Physiol. Res.55: 189-194 (2006).

Kilanczyk, E., Palecz, D. and M. Bryszewska, Effect of red laser light on Na^+,K^+(+)-ATPase activity in human erythrocyte membranes sensitized with Zn-phthalocyanin. J. Clin. Laser Med. Surg. 20(2): 71-75 (2002).

Kujawa, J., Zavodink, L., Zavodink, I. and M. Bryszewska, Low intensity near infrared laser radiation induced changes of acetyl cholinesterase activity of human erythrocytes. J. Clin. Laser Med. Surg. 21(6): 351-355 (2003.)

Kujawa, J., Zavondink, I., Buko, V., Zavondik, L. and M. Bryszewska, Effect of low-intensity ($3075\text{-}25Jcm^2$) near infrared(810nm) laser radiation on red blood cell ATPase activities and membrane structure. J. Clin. Laser Med. Surg. 22(2): 111-117 (2004)

Lumry, R., Some aspect of thermodynamics and mechanism of enzyme catalysis. The Enzymes, Vol. I. Boyer P.D., Lardy, I.T., MyrBack, K. (eds) Academic Press, Newyork, Pp. 157 (1959).

Pasecka, A., Leyko, W., Krajewska, E. and M. Bryszewska, Effect of combined treatment with perindoprilat and low – power red light laser irradiation on human erythrocyte membrane fluidity, membrane potential and energy dosage. Fizjoter Pol. 4(2): 136-142 (2000).

Passarella, S., Ostuni, A., Atlante, A. and E. Quagliariello, Biochem. Biophys. Res. Commun. 156: 978-987 (1998).

Pasternak, K., Nowacka, O., Wrobel, D. and I. Pieszynski, Influence of MLS laser radiation on erythrocyte membrane fluidity and second-

ary structure of human serum albumin. Mol. Cell. Biochem. 388(1-2):261-267 (2014)

Petersen, M.T.N., Jonson, P.H. and S.B. Petersen, Amino acid neighbours and detailed conformational analysis of cysteins in proteins. Protein Engineering Design and Selection 12(7):535-548 (1999)

Pouyssegur, The growth factor activabable Na^+/H^+ exchange system; a genetic approach. Trends. Biochem. Sci. 10(10): 453-455 (1985).

Reisz, J.R., Bansal, N., Qian, J., Zhao, W. and C. M. Furdui. Effects of Lionizing on biological molecules–mechanism of damage and emerging method of detection. Antioxd. Redox Signal. 21(2): 260-292 (2014).

Rozengurt, E. and S. Mendoza, Monovalent ion fluxes and control of cell proliferation in cultured fibroblasts. Am. N. Y. Acad. Sci. 339: 175-190 (1980)

Stadler, I., Evans, R., Kolb, B., Naim, I.O., Narayan, V., Buehner, N. and I. Lanzafame, In-vitro effects of low – lever laser irradiation at 660nm on peripheral blood lymphocytes. Laser Surg. Med. 27: 255-261 (2000).

Sutheriand, J.C., Biological effects of polychrometic light. Photochem. Photobiol. 76(2): 164-170 (2002)

Welch, A.J., Torres, J.H. and W.F. Cheong, Laser physics and laser tissue interaction. Texas Heart Institute J. 16: 141-149 (1989)

Yu, W., Naim, J.Q., McGowan, M., Ippolito, K., and R.J. Lazafame, Photomodulation of oxidative metabolism and electron chain enzymes in rat liver mitochondria. Photochem. Photobiol. 66: 866-871 (1999)

Zavodnik, I.B., Zavodink, L.B. and M. Bryszewska, The mechanism of Zn-phtalocyanine photosensitized lysis of human erythrocytes. J. Photochem. Photobio. B. Biol. 67: 1-10 (2002)