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Effect of diode laser (532 nm) and Helium – Neon laser (632.8 nm) on the Activity of White Blood Cell

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Abstract

It is widely recognized that low-level laser irradiation affects immune system cells in various ways, one of which is enhancing the phagocytic activity of the treated cells. This study has been established to shed light on the possible effect of laser irradiation on phagocytic activity of W.B.Cs. Fresh blood samples have been obtained from 50 healthy adults volunteers for phagocytosis assay. The activity of W.B.C for whole blood have been measured at (5, 15, 30, 60, 90 and 120) min before and after exposure to laser He-Ne laser (632.8nm), Diode laser (532nm) irradiation. The present study revealed a significant increase in the mean activity of phagocytosis after exposure to 632.8 nm and 532nm, this activity further increased by increasing exposure time. The laser irradiation by 532 nm has been much more efficient; 30 minutes irradiation with 532 nm reached comparable and produced even better effects on the improvement of blood activity than the 60 min irradiation with 632.8 nm at significant level ($P<0.05$). The W.B. Cs count has been significantly increased by using 632.8 nm and 532 nm irradiation compared with non-irradiated group. ($P<0.05$). In conclusion, the current study suggests that low level laser can increase the phagocytic activity of W.B. Cs, and this increase is proportional to increasing the exposure time.

Keywords: He-Ne Laser, Diode Laser, Chemiluminescence, Granulocytes.

Introduction

Low Level Laser Therapy (LLLT) was used in Eastern Europe and Asia for the treatment of a wide range of conditions for many years. Its continued acceptance in these populations reflects the efficacy with which it is regarded both by clinicians and their patients. There are many applications of laser in various medical fields such as wound healing, repairing damaged tissues, as well as in studying the effect of lasers on the effectiveness of leukocytes as well as its effect on red blood cells, where studies have proven the deformity of red blood cells when exposed to lasers. [1, 2, 3, 4]. Aortic ring assay was used to assess the effect of irradiation on aortic sprout outgrowth. And experimental medicine requires detailed information on the mechanisms of their biological effects One objective of Sixty-

eight studies in vitro and in animals were analyzed. LED and LASER promote similar biological effects, such as decrease of inflammatory cells, increased fibroblast proliferation, stimulation of angiogenesis. Low-level laser therapy may be helpful in lesions with a protracted healing course, but evidence for its use is still limited. The protocol of a randomized controlled trial designed to compare the effect of adjuvant low-level laser therapy versus conventional venous ulcer tissue repair [5, 6]. The emitted laser light is polarized and coherent and may be absorbed by different tissues[7]. Tissue bio stimulation is only possible if irradiated cells passess molecular photo acceptors that absorb the light and enter into state of excitation triggering intracellular cascade of signals leading to a measurable biological effect [7, 8]. It is generally accepted that the mechanism of laser biostimulation is based on the absorption of monochromatic light by components of the cellular respiratory chain [7, 8]. NADPH-oxidase is responsible for non-mitochondrial respiratory burst of phagocytic cells. This enzyme constitutes a redox chain that generates reactive oxygen species in response to activation and can react to laser irradiation [7, 8]. Study on homogenates granulocytes show that catalyzed reaction between NADPH and oxygen producing superoxide according to the following formula [9, 10]:

$$2O_2 + \text{NADPH} \longrightarrow 2O_2^- + \text{NADP}^+ + \text{H}^+$$

$$2O_2^- + \text{H}^+ \longrightarrow O_2 + H_2O_2$$

Chemiluminescence (CL) is a phenomenon that is related to the respiratory burst. The emission of light by stimulated granulocytes the effects of silica MPs on inflammation, we examined silica MP-induced changes in a human promyelocytic cell model in vitro [10]. Was confirmed by several groups [11, 12, 13]. Observed that granulocyte cells emit a substantial burst of CL that could be quantities with a liquid scintillation spectrometer. Although, singlet oxygen may be the initial excited species, it does not appear to be the ultimate source of the CL [14].

The aim of the present study is to assess the influence of light emitted by a low energy He-Ne laser (λ = 632.8 nm) and diode laser (λ = 532 nm) with irradiation time (5, 15, 30 and 60) min on kinetics of spontaneous-stimulated chemiluminescence of white blood cells activity in normal adults.

Materials and Methods

1. Tris - HCl Stock Solution:

Has been prepared using the following procedure:

24.2 g of Tris (FLUKA - GARANTEE) dissolved in 1000 ml distilled water, 50 ml of Tris solution has been added to 41.4 ml of 0.2 M HCl (FLUKA - GARANTEE), and diluted to 200 ml by using distilled water. The final Tris – HCL solution is of morality equals to 0.015 M at pH=7.4.

2. CL Inducer:

In order to activate granulocytes to burst luminol-dependent CL, a medium of 165 Mm NaCl,

15 mM Tris - HCl solution, 2.25 mM CaCl and 25 mM $BaS0_4$ solution have been used $BaSo_4$ has been used in this medium as a suspended agent.

3. Luminol Solution:

Luminol solution has been prepared by dissolving 1.13 $\times 10^{-2}$M of luminol (5-amino -2,3-dihydro -1,4-phthalaziuedione) in 2 ml of 0.2 M NaOH, this stock solution has been diluted up to 100 ml with deionized water and has been kept prior to use.

Chemiluminescence of Granulocytes:

Chemiluminescence of granulocytes has been studied in full blood according to the method described earlier[10]. Luminol -dependent chemiluminescence has been assessed before and after chemotherapy. Light emission has been measured in multipurpose photon counting with an option for chemiluminescence. Results are shown as a (peak high per W.B.C counts) for 100 cell.

Laser Irradiation:

Here under is specification of the lasers used in irradiation:

Laser	Wavelength (nm)	Power(mw)	Company
Green diode	532	29.5	Shanghai Dream Laser Technology
Helium – Neon	532	30	LJL40-HA, Shanghai Research Institute of Laser Technology

These lasers have been used as power sources for a continues wave mode at distance of 6 cm from tube contains the tested blood. During the experiments, the laser beam has been delivered to the tubes of blood samples with an irradiation spot of 5 mm diameter. Samples have been irradiated through different periods time (5, 15, 30, 60, 90, 120) minute.

Preparation of Blood Samples:

Venous blood samples have been obtained from 50 healthy adults using disposable syringe 5 ml. Blood (3ml) has been transferred into EDTA tubes (EDTA sed as anticoagulant) and then kept at 4 $^{\circ}$C until the start of the assay (usually CL is measured within 1 hr.), the blood divided into three groups, the first one irradiated with He-Ne laser (632.8nm), the second one has been irradiated with Diode laser (532nm) and the third has not irradiated. The activity of W.B.C for all groups has been measured at (5, 15, 30, 60, 90 and 120) min using Photon Counting System and the W.B.C has counted using hemocytometry method.

Statistical Analysis:

The results have been evaluated by the analysis of the variance (ANOVA), mean± stander deviation (SD), P-values at levels ($P \leq 0.05$) is considered to be statistically significant, these calculations have been carried out by Statistical Package for Social Science (SPSS version 19).

Results

The effect of He-Ne laser (λ = 632.8 nm) and diode laser (532 nm) with irradiation time of (5,15,30 and 60) min on kinetics of spontaneous -stimulated chemiluminescence of white blood cells activity in normal adults is significantly changed compared with non-irradiated group ($P<0.05$). CL of granulocytes (activity) from irradiated blood by 532 nm diode laser increased (0.336) in comparison with non-irradiated group (0.3267) and the group irradiated by 632.8 nm (0.3015) ($P<0.05$). The activity at irradiated time 60 min is greater than those of other times of irradiated by 532nm laser, while the activity has been decreased when irradiated with 632.8 nm compared with zero time of irradiated within the same group except at 30 min of irradiation, Figure (1) (A&B).

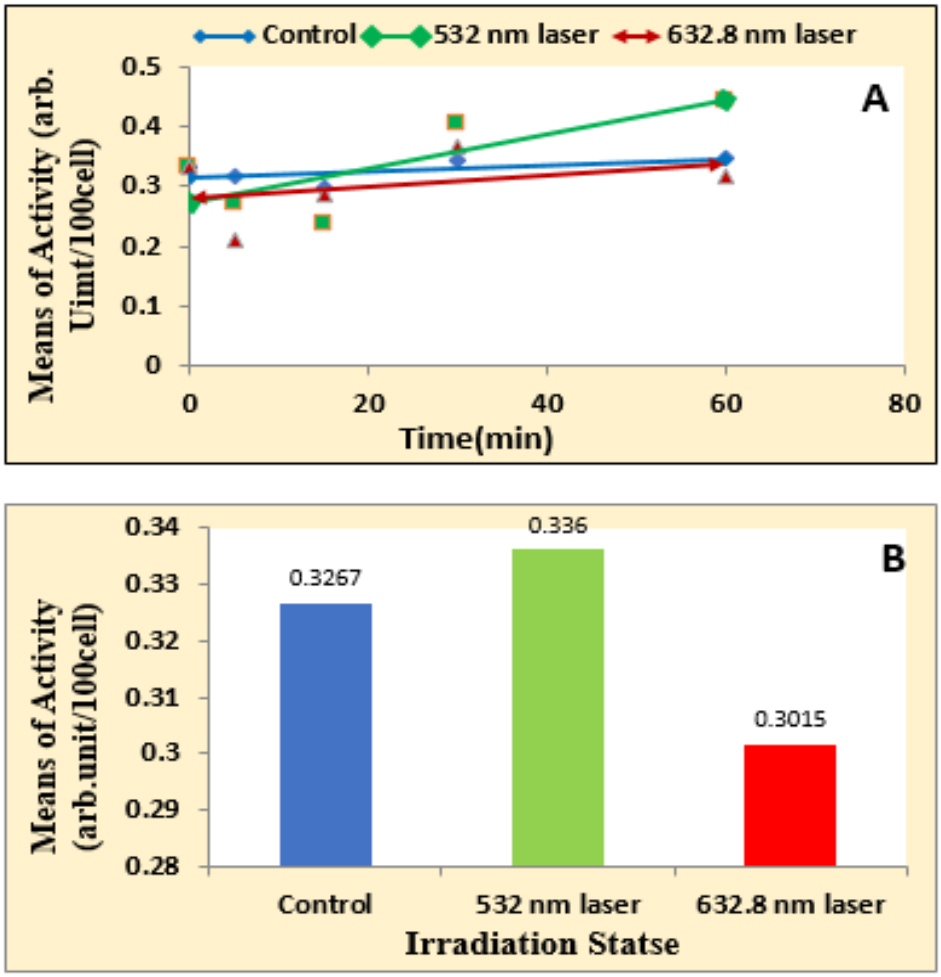


Figure (1). A: Effect of 532 nm and 632.8 nm laser irradiation on activity of W.B. Cs of normal adults. B: Comparisons mean of activity of white blood cell.

The significant increase in white blood cells has been seen when using 632.8 nm laser in irradiated (4465.83 cell/µl) compared with group irradiated by 532 nm laser (4282.5cell/µl) and non-irradiation group (4175 cell/µl) ($P<0.05$), Figure (2) (A & B).

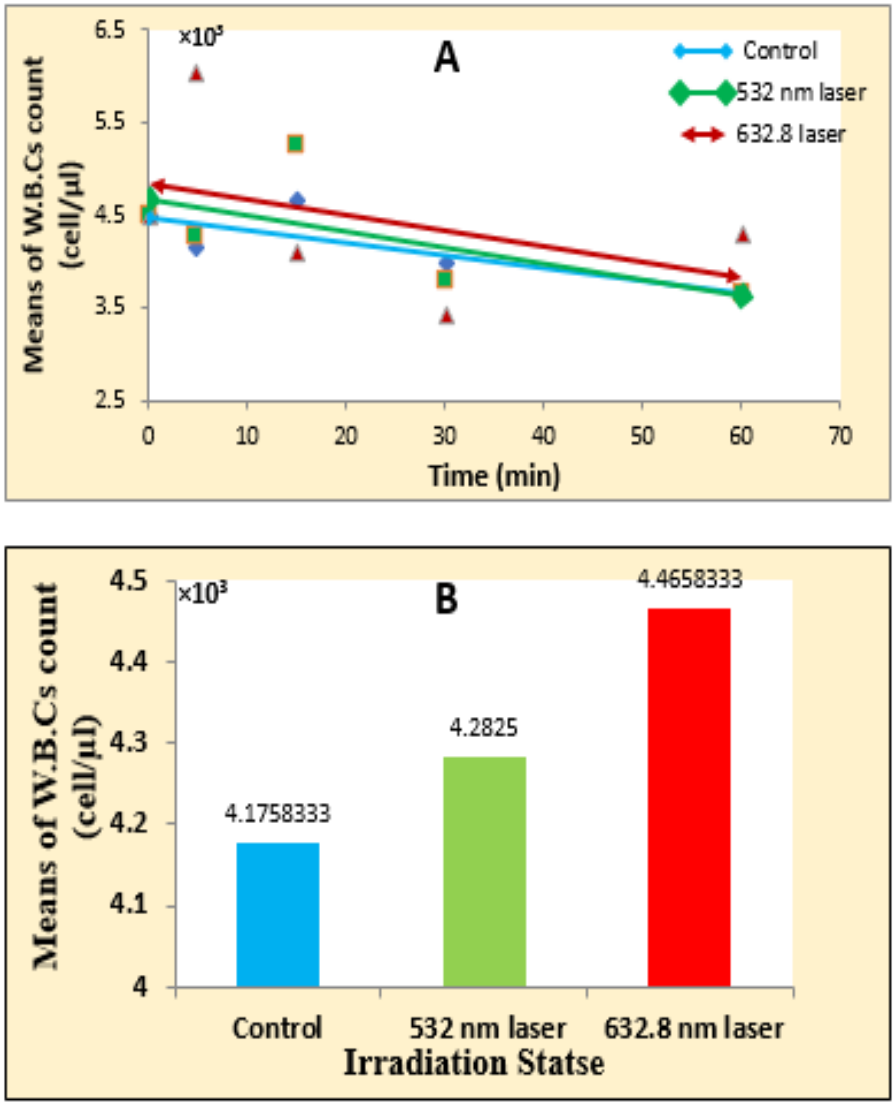


Figure (2). A: Effect of 532 nm and 632.8 nm laser irradiation on count of W.B. Cs of normal adults. B: Comparisons mean of count of white blood cell

The increase in peak height of Chemiluminescence of granulocytes has been seen in the group irradiated by 532nm laser (13.98) compared with non-irradiation group (13.59) and the group irradiated by 632.8 nm laser (13.05), figure (3) (A&B). All data have been listed in table (1).

Table (1) illustrated the effect of 532 nm and 632.8 nm laser compared with non-irradiated blood on peak height, W.B.Cs count and activity of white blood cells. (Activity=$\frac{\text{Peak Height}}{\text{W.B.Cs count}} \times 100$).

Irradiation* Stats	Time (min)*	Means of		
		Peak Height* (arb, unit)	W.B.Cs count* (cell/µl)	Activity* (arb .u/100 cell)
Control	0	14.75	4485.8333	0.3288
	5	13.1833	4152.5	0.3175
	15	13.85	4652.5	0.2977
	30	13.65	3969.1667	0.3439
	60	12.5167	3619.1667	0.3458
	Total	13.59	4175	0.3267
532 nm laser	0	14.75	4485.8333	0.3288
	5	11.4833	4269.1667	0.269
	15	12.3833	5252.5	0.2358
	30	15.3167	3785.8333	0.4046
	60	15.9833	3619.1667	0.4416
	Total	13.98	4282.5	0.336
632.8 nm laser	0	14.75	4485.8333	0.3288
	5	12.75	6052.5	0.2107
	15	11.55	4069.1667	0.2838
	30	12.65	3435.8333	0.3682
	60	13.55	4285.8333	0.3162
	Total	13.05	4465.83	0.3015
*The means is significant at the 0.05 level.				

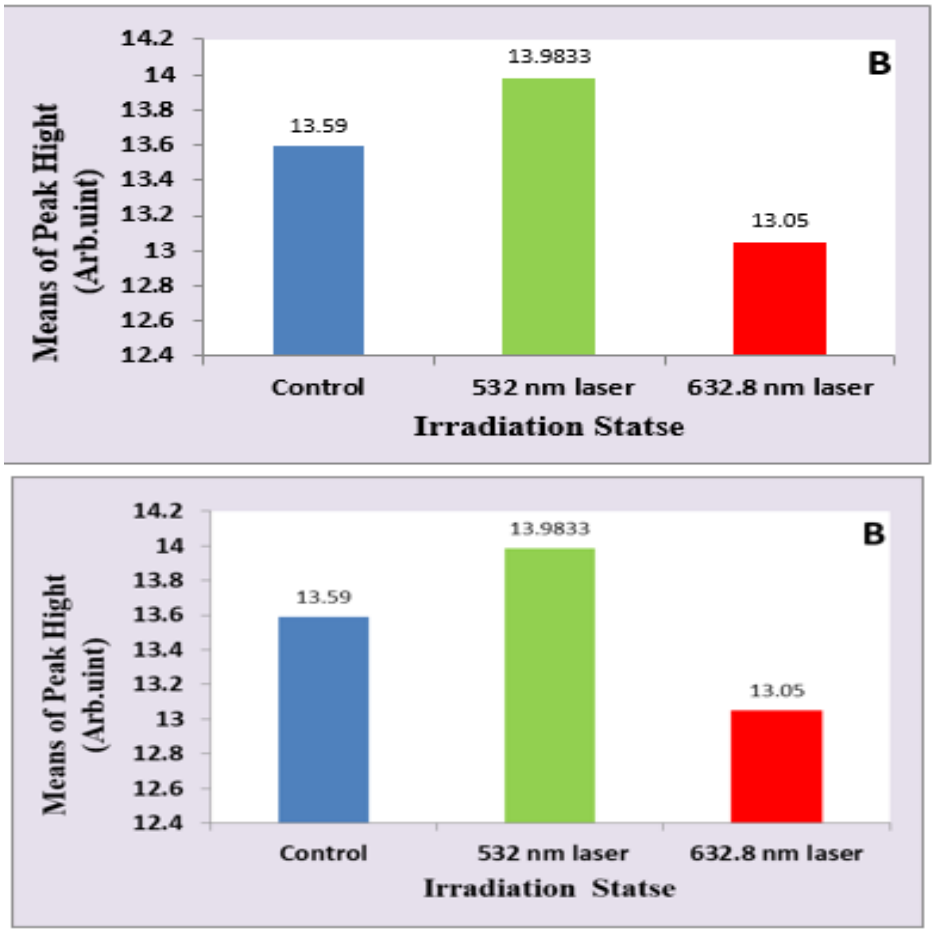


Figure (3): Effect of 532 nm and 632.8 nm laser irradiation on peak height of Chemiluminescence of granulocytes of W.B. Cs of normal adults. B: Comparisons means of peak height of Chemiluminescence of granulocytes of W.B.Cs.

Discussion

The effects of low-energy He-Ne (632.8nm) and diode (532 nm) laser irradiation on activity of granulocytes led to increase spontaneous stimulated CL reactions[15-18]., who examined the effects of low-level laser therapy using the infrared diode laser (GaAlAs), 830 nm on production of reactive oxygen species by human neutrophils [19].

The CL response of neutrophils has been increased by using (532nm) diode laser in irradiation at 60 min and by (632.8 nm) He-Ne laser in irradiation at 30 min. The stimulation with $BaSO_4$ and luminal is according to those of [14]. Who found that, the CL response of neutrophils was reduced by laser irradiation at 60 min prior to the stimulation with opsonized zymosan and calcium ionophore[19].Those differences may result from different models used.

Comparing the results of 632.8 nm and 532 nm irradiation, it's demonstrated that the irradiation from both lasers can increase the activity of White blood cell compare with non-irradiation. Comparing to 632.8 nm irradiation, the laser irradiation with 532 nm has been much more efficient, 30 minutes irradiation with 532 nm reached comparable and produced even better effects on the improvement of blood activity (0.4046) than the 60 min irradiation with 632.8 nm (0.3162).

W.B. Cs count has been significantly increased with 632.8 nm and 532 nm irradiation compared with non-irradiated group [20].

Conclusion

In this study, it was determined that both types of lasers have effects on the CL response of neutrophils. It has been increased by using (532nm) diode laser in irradiation at 60 min and by (632.8 nm) He-Ne laser in irradiation at 30 min. Comparing the results of 632.8 nm and 532 nm irradiation, it's demonstrated that the irradiation from both lasers can increase the activity of White blood cell compare with non-irradiation.

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