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The effect of different wavelengths laser on the antioxidant systems and product of lipid peroxidation in the blood

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ABSTRACT

The present study accompanied to evaluate the effects of laser with different wavelengths upon some physiological parameters of blood samples *in vitro.* In this study, some blood samples were taken from women and their ages ranged between 17-25 years. The blood samples were exposed to two types of laser, first laser was He-Ne laser with wavelength 632.8nm. With power 2mW. The second laser was Nd: YVO4 with wavelength 532nm and 4mW.The blood samples were exposed to laser for two period times, 5 and 10 minutes. The present study involved determination the effect of laser radiation on antioxidant system, the levels of catalase activity were progressively increase ($P>0.05$) in both radiated blood samples. On the other hand, level of malondialdehyde (product of lipid peroxidation) pointed out a significant fall ($P<0.05$) in both groups of radiated blood peroxidation) compare to non-radiated blood samples The benefits of laser effects are included increase the efficiency of antioxidant system against oxidative stress in a whole body tissues.

KEY WORDS: Laser, Antioxidant, Lipid Peroxidation, Wavelength, Blood.

1. INTRODUCTION

Free radicals discovered a long time ago (Commoner, 1954), and later the scientist Denham Harman assumed that oxygen free radicals is possible that free radicals composed as a side products of the interactions enzymatic reactions that occurred inside the body, and in 1956, free radicals was described as damage factor for the cells and generate mutagenesis, cancer, aging and many degenerative diseases (Harman, 1956). Generated oxidants during the course of the metabolic reactions in the blood cells and most other cells of the body, and this oxidized substance has high speed of interacting with proteins and lipids, nucleic acids and other molecules, causing of physiological chemical disturbances for cell and other organelles within cells. Lipid per oxidation is derived from poly-unsaturated fatty acid, which are often unstable and disintegrate into many compounds and these include reactive carbonyl group, which is abundantly malondialdehyde (MDA) compound, it represent factory to Lipid per oxidation which causes many acute and chronic physiological disturbances in humans and animals (Killic, 2003).The oxidative stress causes many disorders to metabolism of cells; it acts on a damage of DNA and cause high free calcium (Free Ca^{+2}) accumulated within the cytoplasm and the destruction of many ionic transporters of cellular membranes in addition to the oxidation of lipid (Orrenius, 2003; Hazim, 2016). Damage by oxidizing agents occur either directly when H_2O_2 oxidizing Thiol group (SH) as well as OH consists in very close to the DNA, causing fragmented it or if the damage occurs indirectly through higher levels of free calcium ions (Free Ca^{+2}), which in turn activates many protease enzymes which attacks the cytoskeleton and nuclease enzymes that attack DNA (Halliwell, 1991) and there are several types of oxidizing agents such as super oxide O_2^-, which is one of the most important types of free radicals of oxygen, that is made up by some of the enzymes such as NADPH and Oxidases or indirectly enzymatic reaction through redox reactions, especially in electron transport chain in mitochondria.

Super oxide dismutase (SOD) can be convert to O_2^-, H_2O_2 (Hydrogen peroxide), and it has been found that the presence of transition metals such as iron ions Fe^{+2} and copper ion Cu^{+2} it is possible to transform hydrogen peroxide (H_2O_2) to the reactive hydroxyl radical (OH) as well as possible to H_2O_2 transformed by the enzyme catalase and enzyme Glutathione peroxidase into the water (Deby, 1990). Glutathione peroxidase enzyme works to convert reduced glutathione (GSH) to oxidized glutathione (GSSG), which turns in back into reduced glutathione by glutathione reductase enzyme using the equivalent enzyme NADPH (Shelly, 1999).

2. MATERIAL AND METHODS

The blood samples collection: Blood samples have been taken from people (women aged 17-25 years) and put this samples in the tubes containing inti coagulant (EDTA) after that, divided into two equal parts in test tubes the first tube exposure to the (He-Ne) laser beam with a wavelength of 632.8 nm and power 2mW, for two period, the first period 5 minutes and the second period 10 minutes as well as the second tube is exposed to the laser Nd: YVO4 with wavelength of 532 nm and power 4 mW for the same periods. Then tubes were transferred to the centrifugation for separated plasma from the sample and a measurement of the activity of the catalase enzyme and the products of lipid peroxidation by malondialdehyde (MDA) by a spectronic system and all tests has been achieved within a period not to exceed than one hour.

Irradiation by He-Ne laser (2mW): Helium-neon Laser mixture of helium atoms (He) and neon (Ne) gas at a given, which is the most popular effective medium materials for lasers carbonated and non-expensive works of this type of laser at the wavelength of 632.8 nm in the red region of the electromagnetic spectrum (White, 2011).

Laser irradiation (Nd: YVO4) (4mW): It is a solid lasers sends wavelengths within the visible by wavelength 532 nm, One of the lasers second harmonic generation and which called doubled frequency laser, the wavelength of the laser 1064 nm has been doubling its frequency to obtain wavelength 532 nm which results when pass the laser beam 1064 nm, which is visible (pump laser diode 810 nm) through linear crystal (KTP) which are placed outside the resonator laser, that portion of the rays wavelength of 1064 nm turn to green rays with wavelength 532 nm. As for the other part come out of the system as it was. The wavelength of 532 nm output equivalent to half the original wavelength 1064 nm and have the multiple frequency, so that this laser called multiplier frequency (Jing, 1998; Zaied, 2016).

Measurement of Plasma malondialdehyde (MDA) Levels:

Preparation of solutions:

Solution 70% trichloroacetic acid (TCA): Added 70 gm from TAC to distilled water, and complete the volume to 100 ml by using distilled water

Solution 17.5% of TAC: Taken 5 ml from 70% TAC solution, and complete the volume to 20 ml by using distilled water.

Solution 0.6%TBA thiobarbituric acid: To make Solution 0.6%TBA thiobarbituric acid, added 60 mg from TBA to distilled water and complete the volume to 20 ml by utilizing distilled water. It was using water bath to complete melting of TBA.

Concentration of MDA was measured by using, the method, which has been described previously (Guidet, 1989). Where such examination depends on the interaction between (MDA) and TBA (Thiobarbituric acid) to form complex MDA-TBA2. It was measuring the absorbance at 532nm wavelength amount where the suit color intensity is directly proportionate to the concentration of (MDA).

Calculations:

Molndehid concentration (μmol / L) = D × (Ab / L × ξ) …….1

Where:-

Ab = absorbance

L = Light bath = 1 cm

ξ = deviation coefficient = $1.5 \times 105M^{-1}Cm^{-1}$

D = dilution coefficient = size of the using solution with unit ml /0.15=1+1+1+0.15/0.15=21.

Assay of Plasma catalase activity: The catalase convert the hydrogen peroxide H_2O_2 to give the water molecule and oxygen molecule, and can be determined enzymatic activity of the catalase by depending on the method used previously .The decomposed H_2O_2 can be directly determined and sequentially through winning decrease in absorbance at 240 nm wavelength. The activity of the enzyme is measured by the difference in absorbance at unit time.

Calculations:

The activity of the catalase [cat (K/ml)] = Vt / Vs × 2.3 / Δt ×logA_1/A_2× 60 …….. 2

Whereas:

V_t = total volume = 3ml

V_s = sample size = 2ml

$\Delta t = t_2-t_1$ = 15 seconds.

A_1 = absorbance at time 15 seconds

A2 = absorbance at time 30 seconds

In addition, it was calculating the effectiveness of the enzyme cat (K / ml), according to the following equation: -

The effectiveness of the enzyme cat (K / ml) = 13.8 × logA_1 / A_2…….3

3. RESULTS

Table.1. Means catalase activity (K/ml) and concentrations of malondialdehyde (μmol./ml) before and after exposure to two types of laser.

Criterions / Laser magnitude		Catalase(K/ml)	MDA(μ mol/ ml)
Before irradiation		1.521±3.621	2.23± 8.56
Irradiation with He-Ne laser 632nm and 2mW	After 5 min	1.272±3.776	1.72± 8.45
	After 10 min	1.281±3.957	0.91 ±8.47
Before irradiation		1.521 ±3.621	2.23± 8.56
Irradiation with He-Ne laser 532nm and 4mW	After 5 min	0.821± 4.923*	0.871± 7.85*
	After 10 min	1.023± 5.897*	0.97± 7.63*

Levels of catalase activity in plasma: The activity of the catalase values in the Table.1, not significant rising ($p > 0.05$) when exposed to He-Ne laser beam and for periods of time of 5 minutes (± 1.272 k / ml 3.776) and at the time

10 minutes (3.957 ± 1.281 k / ml) as well as when exposure to laser beam Nd: YVO4 observed significantly higher (P <0.05) and for periods of time of 5 minutes 4.923 ± 0.821 k / ml)) and at the time 10 minutes 5.897 ± 1.023 k / ml)) when compound the with non-irradiated samples (3.621 ± 1.521k / ml) as shown in Figure.1.

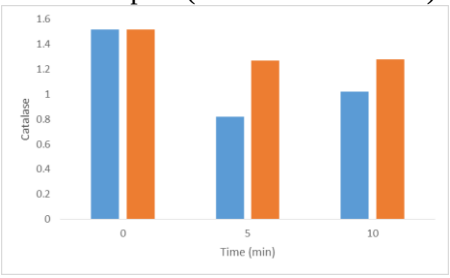

Figure.1. The effect of different irradiation periods on the level of the catalase enzyme in plasma

Table.1, achieved a concentration Molendehide values (MDA) decline was not significant (p> 0.05) when exposed to package laser He-Ne and for periods of time of 5 minutes (9.45 ± 1.72) and at the time 10 minutes 8.47 ± 0.91, as well as when exposed to package laser Nd : YVO4 note for a significant decrease (P <0.05) and for periods of time of 5 minutes (7.85 ± 0.871) and the time of 10 minutes 7.63 ± 0.97, when comparing the results with samples non-irradiated (8.56 ± 2.23). As well as note the decline in both two lasers in Figure. 2.

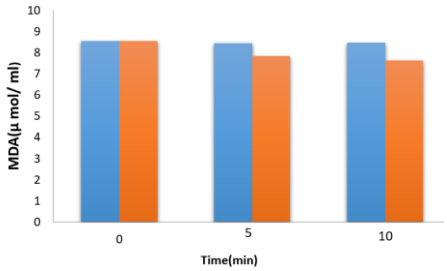

Figure.2. Different periods of irradiation on the concentration level of Molendehide in the plasma

DISCUSSION

Catalase activity: Data obtained from the present study indicated a significant elevation (p‹0.05) of catalase activity when exposed to laser Nd:voy with power 4 mW and wave length 532 nm, and showed non-significant increase (p›0.05) when exposed of blood samples to laser He-Ne (632.8 nm, 2 mW).

The present study agree with buki (Aebi, 1974), who showed raising of catalase activity, glutathione peroxidase GPX and superoxide dismutase SOD salivary gland of rates when exposed to laser beam 660 nm. Anti-oxidant enzyme's system are most important mechanism to protect the body from raising the free radicals. Anti-enzyme's system compose essentially from superoxide dismutase (SOD), catalase and glutathione peroxidase.

Catalase enzyme possesses an quad-symmetric form of formula (Homotetramer) with a small unit (small subunit), which contain Heme compound and ferriprotoporphyrin compound in effective site of enzyme which responsible for linking compound NADPLT and when linking the last compound with the enzyme makes the enzyme is capable of absorbing light (Ibuki, 2012). Study of Artyukhov (Halliwell, 2007) confirmed that irradiation with low power laser radiation can be inhibition some of metalloenzymes and enzymes contained coper and iron such that catalase and superoxide dismutase, these enzymes play essential role in absorbance of radiation.

Previous study described that using laser radiation with stable doses acts to improve plant metabolism through activation many enzymes 15.it have been found over production of reactive oxygen species (ROS) lead to increase toxicity to many metabolic functions practically when converted results of the Silveira and his group (Kirkman, 2007). Who showed increased in catalase and superoxide dismutase (SOD) activities with decrease of super oxide anion ($O_2^{\cdot-}$). Subsequently there is down regulation in mitochondria enzymes when irradiated wounds to know the effect low laser power in accelerated of wound healing. They concluded that laser can be to stimulate anti-oxidant agents and protect the cells by reducing free radical product ions. Other study, provided that the absorption laser acts to accelerate electron transport in respiratory chain and induce (ROS) productions, in particular, (O_2^-). The excessive production and higher of free radical oxygen (ROS), it characterized to destroy the cell components such as Lipids, proteins and nucleic acids as it was observed that the using laser for different doses, intensities and periods, can be induce body to prevent production of oxidants (Artyukhov, 2000).The results of the present study coincidence with the study of the Yu, Naim and his group (Qui , 2010) as they had noticed the effect of the low-level laser therapy (LLLT) with Different intensities on the effectiveness of mitochondria respiratory chain and oxidative stress guides. They proved a significant decrease in the effectiveness of the second complex II in the respiratory chain was observed a positive correlation between inhibition of complex II and intensity of radiation, leading to a drooping in the effectiveness of the mentioned complex due to the oxidized, but this inhibition did not affect the transmission speed electrons. It should be pointing that the effects of stimulating and inhibiting the activities of the cells using the laser

to be dependent on dose and wavelength as it was found that irradiation by low level laser (LLL) acted to adjustments to the vital functions of cells (Sairam, 2004).

White blood cells, in particular, monocytes and neutrophils consume large quantities of oxygen, this proses achieved by enzyme NADPH-oxidase which help transporting electrons from NADPH compound to oxygen for product superoxide (O_2^-) (Silveira, 2009 ; Hazim , 2016) . From the obtained results from the current results can be concluded that the laser (ND: YVO4, 532 nm, 2mW) has a higher stimulating effect in activation of catalase in a comparison with laser (He-Ne, 632.8 nm, 2mW). It is probably that laser beam act to stimulate antioxidant enzymes presented with white blood cells and red blood cells, these facts agree with previous report involved application of laser in stimulating of antioxidant enzymes including SOD and catalase in *in vitro* blood samples associated with decrease lipid peroxidation.

Concentration of malondehyde (MDA): The present results reported a significant drop $p<0.05$ in the levels of MDA of blood samples irradiated with laser ND: YOV_4 (432 nm, 4 mW), When compared with non-radioactive samples. Where there is no significant differences $p<0.05$ in MDA level when irradiated with He-Ne laser (632.2 nm, 2 mW). MDA is well known compound of lipid peroxidation resulting from reactive oxygen species (ROS). Moreover, ROS can be damage the cellular component. MDA using as clear index of oxidation lipid peroxidation, which causes several physiological disturbances. Previous study achievements by Volotoskaia (2003) show that when using therapeutic doses of laser appeared anti-oxidation effects of blood samples. Laser exerts essential role to stimulate SOD, which the first anti-oxidation enzyme. Radical roots have many physiological functions including cellular signal transduction, phagocytosis and apoptosis, when they remain with normal ranges. Excesses the radical roots cause many disturbance such as cytotoxicity, cell death, ageing and appearance of many chronic diseases, such as, mutations tumors, diabetes mellitus, and degenerative disease (Schaffer, 1997). Some studies hypothesized that the effected inhibitory and stimulatory of low-level laser in treating many diseases (Fujimaki, 2003; Hazim, 2016). As observed That the laser absorption urges the production of free radicals, which in turn stimulates certain cells, particularly white blood cells (Leukocytes) to produce the initial oxidizing agents (pro-oxidants), which includes in particular nitric oxide and the outputs of the active oxygen (ROS) (Volotovskaia, 2003).

The results of the present study is compatible with the findings of the Omran and his group, as they had noticed the high level of MDA when irradiation by argon laser that different doses, where, it was noted that the high level of MDA directly proportional to the higher dose of exposure laser which has been the increase in the level of MDA compatible with high enzyme anti-oxidants SOD.

But, the results of the current study coincided with the results of Silveira. as it proved the low level of lipid peroxidation in cells which has exposed to the laser beam with the low level. This lower, in the level of MDA coincided with rising of the effectiveness of anti-oxidants enzymes particularly SOD and catalase, while stadler (Vijayalaxmi, 2000)

can be proved that the exposure to the low level laser radiation, lead to a high level of lipid peroxidation and supposed that there is a rise in the generation of free radicals level (ROS) has been attributed to the fact that hemoglobin (Hb) which found in the red blood cells was an substance with intensity photoreaction, and therefore it considered as a source of ROS, when exposed to the laser beam. The bio-stimulation for the low level laser beam represented by modifying or correction to many forms of cellular proteins, low level laser radiation working to improve antioxidant enzymes by increasing the effectiveness of the SOD enzyme, which can break down free radicals from red blood cells with high efficiency and lower the level of lipid peroxidation and the results of its oxidation, particularly MDA (Shawrrn, 2002).

Also the results of the current study agreed with study Yanhong, 2007; Omran, 2011; Stadler, 2000; Hazim, 2016; Zhy, 2002) as it proved that exposing the red blood cells to the low level laser it has been working to repair the damage in membranes of red blood cells has been attributed to the fact that the laser works to stimulate anti-oxidants enzymes, and reduce the level of MDA and at the same time stimulating the enzyme ATPase located in the membranes of the red blood cells and in the cytoplasm of the white blood cells, as well as, in the experimental study was conducted on the seeds of some plants, especially wheat seeds, as used for the CO_2 laser in the treatment of some seeds led this use to the high level of effectiveness of the antioxidant enzymes, particularly catalase, peroxidase and SOD, decline the level of, O^{-2} and MDA (Yamhong, 2007; Alkaim, 2016). Moreover, a study carried out on the metabolism of the yeast using He-Ne laser showed, a rising of the effectiveness of the catalase enzyme, protein synthesis rate after 18 hours (Qiu, 2011; Karu, 1993). Catalase is the main enzyme responsible for regulating the level of H_2O_2. Therefore the catalase is an enzyme in the peroxisomes organelles or other organelles, but it does not exist in the organelle mitochondria if the compound H_2O_2 comes out of mitochondria toward the peroxisomes (Chance, 1979; Ruwaida, 2016). As it noted in some researches, there is a significant decrease in the level of oxidation of fat during their wounds treatment low level laser, it was concluded that the use of the laser stimulated to defend the body against damaging oxidative lipid membrane, as the high level of effectiveness of the catalase enzyme, was found when exposed to laser and this belong to the photo-stimulation catalase enzyme, and perhaps

other non-enzymatic antioxidants. The variance in the effectiveness of catalase after exposure to laser may be due to the generation of free radicals through rotational changes in the macromolecules, due to the photo-stimulation, and this stimulus will be necessary because of the anti-oxidants enzymes are governed by dose and the period of exposure to the laser has been observed that exposure to low laser power is sufficient to decrease oxidative damage in different situations (Berki, 1991). Based on these references, that the high effectiveness of antioxidant enzymes can be assumed was the main reason, which led to the decrease of free radicals directly and decrease lipid peroxidation through lower level of MDA compound.

4. CONCLUSION

It has been concluded that laser can be to stimulate anti-oxidant systems, and as showed from the results the values of the effectiveness of catalase enzyme had a sharp rising in all samples irradiated by laser. While there was a decrease in the concentrations of lipid peroxidation values (MDA) in all samples irradiated by laser when compared with non-irradiation results, this inffluance, may be caused mainly to get some positive effects of laser rays on blood samples, by increasing the efficiency of the anti- oxidants systems and reduce the effect of oxidizing agents, in general, on the cells of the body It has been concluded that laser can be to stimulate anti-oxidant systems, and as showed from the results the values of the effectiveness of catalase enzyme had a sharp rising in all samples irradiated by laser. While there was a decrease in the concentrations of lipid peroxidation values (MDA) in all samples irradiated by laser when compared with non-irradiation results, this influence, may be caused mainly to get some positive effects of laser rays on blood samples, by increasing the efficiency of the anti-oxidants systems and reduce the effect of oxidizing agents, in general, on the cells of the body. Has been concluded that laser can be to stimulate anti-oxidant systems, and as showed from the results the values of the effectiveness of catalase enzyme had a sharp rising in all samples irradiated by laser. While there was a decrease in the concentrations of lipid peroxidation values (MDA) in all samples irradiated by laser when compared with non-irradiation results, this influence, may be caused mainly to get some positive effects of laser rays on blood samples, by increasing the efficiency of the anti- oxidants systems and reduce the effect of oxidizing agents, in general, on the cells of the body

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