Iraqi J. Laser, Part B, Vol.10, No.1, pp. 15-24 (2011)

IRAQI JOURNAL OF LASER

The Effect of 532nm Nd: YAG Pulsed Laser on the Activity of Superoxide dismutase and Alcoholdehydrogenase of Saccharomyces Cerevisiae

Noor A. Jadah (1) **Ghazi M. Aziz** (2) **Ayad G. Anwer** (1)

(1) *Institute of Laser for Postgraduate Studies, University of Baghdad, Baghdad, Iraq.*
(2) *Department of Biotechnology, College Of Science, University of Baghdad, Baghdad, Iraq.*

(Received 5 January 2010; accepted 2 May 2011)

Abstract : In this study, the effect of Nd: YAG laser on the activity of superoxide dismutase (SOD) and alcoholdehydrogenase (ADH) was investigated. The Saccharomyces cells were irradiated using 532nm Q-Switched Nd: YAG laser with (1Hz) frequency. Different fluences 11.3, 22.6 and 33.9mJ/cm^2 and different number of pulses 15, 30 and 60 pulse were used. The irradiated cells were incubated in a liquid nutritive medium for 24 hours. After incubation, the cells were harvested and disrupted to extract the intracellular enzymes and their activities were assessed. In comparison with the control, the irradiated cells showed a significant increase in the activity and the specific activity of SOD at energy densities of 11.3 and 22.6mJ/cm^2 at 30 and 60 pulses respectively. Maximum activity and specific activity of ADH was observed using 11.3mJ/cm^2 and 22.6mJ/cm^2 at 15 pulses. While an inhibition in both activity and specific activity of two enzymes was observed at 33.9 mJ/cm^2. It is concluded that the fluence at 22.6mJ/cm^2 stimulate the activity and specific activity of the two enzymes to a maximum values.

Introduction

Lasers in the visible and near infrared (IR) have emerged for the treatment of various pathological conditions such as wound healing (Conlan, et al., 1996), nerve regeneration (Anders, et al., 1993; Rochkind, et al., 2001) and more. The stimulatory effects of laser irradiation on cell activation have been largely demonstrated in vitro in a variety of cell lines. For example, LEL were found to stimulate the release of transforming growth factor (TGF-) (Yu, et al., 1994) and platelet- derived growth factor (PDGF) from cultured fibroblasts (Grossman, et al., 1998). Nevertheless, the mechanism of photobiostimulative processes is still being debated. It is obvious that, in order to interact with the living cell, light has to be absorbed by intracellular chromophores (Lubart, et al., 2005). Chromophores responsible for photobiostimulation are endogenous porphyrins, mitochondrial and membranal cytochromes and flavoproteins were found to be suitable candidates. The above-mentioned chromophores are photosensitizers that generate reactive oxygen species (ROS) following irradiation. As the cellular redox state has a key role in maintaining the viability of the cell, changes in ROS may play a significant role in cell activation (Lubart, et al., 2005).

Such ROS as the superoxide radical anion, hydrogen peroxide and hydroxyl radicals can cause oxidative damage to proteins, lipids and DNA leading to impairment of cell growth and

even to cell death (Sigler, et al., 1999; Strutz, et al., 2002). Cell of S.cerevisiae contains two superoxide dismutase (SOD), cytosolic Cu,Zn-SOD and mitochondrial Mn-SOD, that play a role in the protection against the superoxide radical, whose main source is thought to be mitochondrial respiration (Longo, etal.,1996; Jamieson,1998) and laser illumination (Lubart, et al., 2005).

There is evidence in the literature showing that light-induced ROS (Reactive Oxygen Species) increases the activity of various antioxidants such as superoxide dismutase (SOD) and catalase (CAT), or stimulates their synthesis in the cell (Stadler, et al., 2000; Zhu, et al., 1997). Superoxide dismutase (superoxide oxidoreductase.1.15.1.1) (Huu, et al., 1984; Beck, et al., 1988) reduces the concentration of superoxide radical $O^{\cdot-}_2$ by catalyzing the reaction of dismutation(Bull, et al., 1991; Ludwig, et al., 1991):

$$O^{\cdot-}_2 + \text{Mn (III) SOD} \longrightarrow O_2 + \text{Mn (II) SOD}$$
(Oxidation reaction)

$$O^{\cdot-}_2 + 2H^+ + \text{Mn(II)SOD} \longrightarrow H_2O_2 + \text{Mn(III)SOD}$$
(Reduction reaction)

Several species of evidence show that mitochondria are sensitive to irradiation with monochromatic visible and near-infrared light. The illumination of isolated rat liver mitochondria increased adenosine triphosphate (ATP) synthesis and consumption of O_2 (Kato, et al., 1981; Gordon, et al., 1960). Oxygen consumption was activated by illumination with light at wavelengths of 365 and 436nm, but not at 313, 546 and 577nm (Vekshin, et al., 1982).

Also, irradiation with light at 633nm, increases the mitochondrial membrane potential ($\Delta\psi$) and proton gradient (ΔpH), caused changes in mitochondrial optical properties, modified some NADH-linked dehydrogenase reactions (NADH is a reduced form of nicotinamide adenine dinucleutide) (Passarrella, et al., 1983), and increased the rate of ADP/ATP exchange (ADP is adenosine diphosphate) (Passarrella, et al., 1988), as well as RNA and protein synthesis in the mitochondria (Hilf, et al., 1986).

In aerobically grown yeasts, the irradiation activates the fermentation which is shown by the activation of CO_2 formation. As it is known, CO_2 is formed from pyruvic acid by alcohol fermentation (Karu, et al., 1993), so the estimation of ADH activity is of interest.

Alcohol dehydrogenases (alcohol: NAD oxidoreductase EC1.1.1.1) (Maitra, et al., 1971) constitute a large family of enzymes responsible for the reversible oxidation of alcohols to aldehydes with the concomitant reduction of NAD^+ or $NADP^+$. These enzymes have been identified not only in yeasts, but also in several other eukaryotes and even prokaryotes.

The ADHs of S. cerevisiae have been studied intensively for over half a century. Physiologically, the ADH reaction in S. cerevisiae and in related species plays a dual and quite critical role in sugar metabolism. Almost all of the carbohydrate is used fermentatively, regardless of the availability of oxygen, and a specific ADH isozyme serves to regenerate the glycolytic NAD^+, thereby restoring the redox balance, through the reduction of acetaldehyde to ethanol. (Smidt, et al., 2008). Under aerobic conditions, respiration of the accumulated ethanol occurs after depletion of the fermentable sugar, again by the action of specific ADH isozymes. Thus, in S. cerevisiae, the ADH reaction links fermentative and respiratory (oxidative) carbon metabolism (Smidt, et al., 2008).

The aim of this work is to estimate the action of Nd: YAG laser light on some redox activities of cells after irradiation in dose dependences manner.

Materials and Methods

Strain and culture conditions

A commercial baker's yeast (Saccharomyces cerevisiae) was used. The isolate was grown in PDA (potato-dextrose agar) slant after 72 hour of incubation. After growing the cells were transferred to 250ml-conical flask with 50ml of liquid nutritive medium (2% glucose, 1%yeast extract and 0.02% ammonium sulphate, pH 5.5) and incubated in shaker incubator (Pilot shaker) 150rpm for 24h at 28˚C. The flask volume/medium ratio was 5:1 to provide high aeration conditions for growth culture (Gascon and Lampen, 1968).

Cells were separated from the nutritive medium by the centrifugation (2000 rpm for 15 min) by using (Labnet international) centrifuge and washed with twice sterile tap water. The suspension with optical density 0.06 (λ=540nm) in sodium-potassium phosphate buffer (pH=6.0) was prepared for irradiation (Karu, 1996).

Irradiation Procedure

Irradiation experiments were performed at room temperature (20-25℃). Extraneous illumination (sunshine or artificial light, especially that from the fluorescence bulbs) was carefully avoided during preparation of the suspension and the irradiation (Karu,1996).

Flasks with 1.5cm diameter were used for irradiation of yeast cells. AQ-switched Nd: YAG laser (Diamond Beauty) was used for irradiation. The laser was operated with 532nm wavelength, 6 ns pulse duration and 1 Hz frequency. The prepared cells were irradiated using 20, 40 and 60 mJ output energy and for 15, 30 and 60 pulse for each energy. The diameter of the beam was expanded by means of beam expander to 1.5 cm to provide an uniform illumination of whole area .Irradiation from the bottom was preferred to avoid the (meniscus effect) which occurs when irradiating from above. In this case, the meniscus of the liquid acts like a lens and the dose of the light reaching the cells (if reaching the lower layers at all) will be decreased in an uncontrolled way (Fedoseyeva, et al., 1988; Kutomkina, et al., 1991; Tiphlova, et al., 1988). The irradiated cells were incubated for 24 hours, and then further procedures were followed to measure the activity of SOD and ADH.

Cell-Free Extract Preparation

After irradiation, the irradiated yeast cells were grown overnight in 50ml of liquid nutritive medium. The cells were harvested from the medium by centrifugation (2000 rpm for 15 min by using Labnet international centrifuge). The pellet was washed twice with distilled water and resuspended with 0.01M of sodium potassium phosphate buffer pH 6.0 in order to get a cell suspension with an optical density 0.7 at 540nm.

Two ml of Lysozym solution (prepared by dissolving 0.05g of lysozyme powder in 10ml of 1% glucose solution (Kamaya,1970)) was added to 8ml of cell suspension with an optical density 0.7(at λ=540nm) to get a final concentration 1mg/ml of lysozom.

The suspension was incubated in water bath shaker (Jiotechnic) at 30˚C for 4-6 hours. After incubation, the suspension was kept in the refrigerator at -5˚C overnight, therefore; in order to estimate the efficiency of lysozyme treatment; a quick slide was made from the pellet and staining with gram stain.

The suspension was filtered by centrifugation at 12000 rpm for 15 min by using cooling centrifugation (Beckman); the pellet was omitted while the supernatant was used for determination of enzymes activity and total protein concentration in the crude extract.

Measuring the SOD and ADH activities

Superoxide dismutase [EC 1.15.1.1] activity was measured as described by Beyer and Fridovich (1987). One unit was defined as the amount of enzyme causing 50% decrease in the reduction of nitro blue tetrazolium (NBT).

The activity of Alcoholdehydrogenase [EC 1.1.1.1] was determined according to Sakai et al. (1996). One unit of enzyme was defined as the amount of enzyme that catalyzed the reduction of 1.0 pmol of an electron donor per min.

The total amount of protein in enzyme preparations was determined according to Bradford (1976) using bovine serum albumin as a standard protein. This assay was used to determine the specific activities of both enzymes according to:

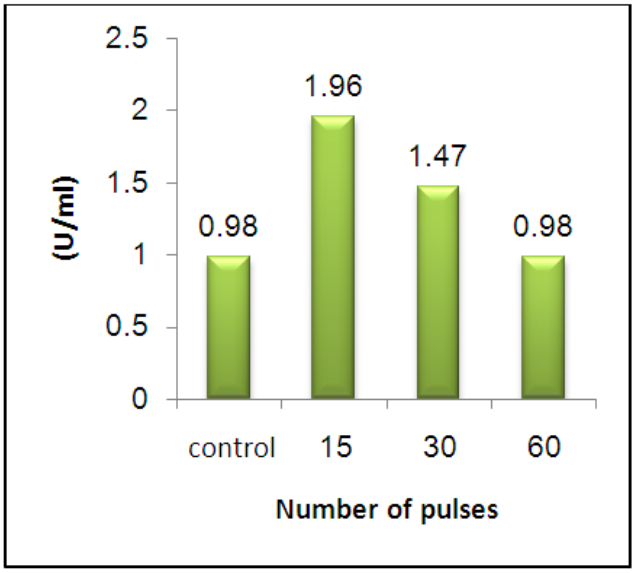
Specific activity of enzymes (U/mg of protein) =

$$\frac{\text{The enzyme activity (U/ml)}}{\text{protein concentration(U/mg)}} \quad \frac{\text{Theenzymeactivity(U/ml)}}{\text{Proteinconcentration(mg/ml)}}$$

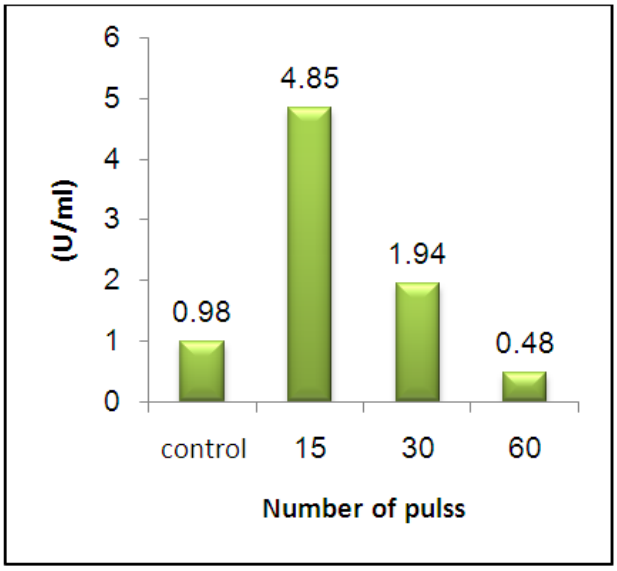
Results and Discussion

The results of the effect of Nd: YAG laser on the activity and specific activity of ADH were illustrated in Figures 1, 2 and 3. An increase in ADH activity and specific activity (Figure 1a, b) was observed using 11.3mJ/cm^2 energy density for different number of pulses.

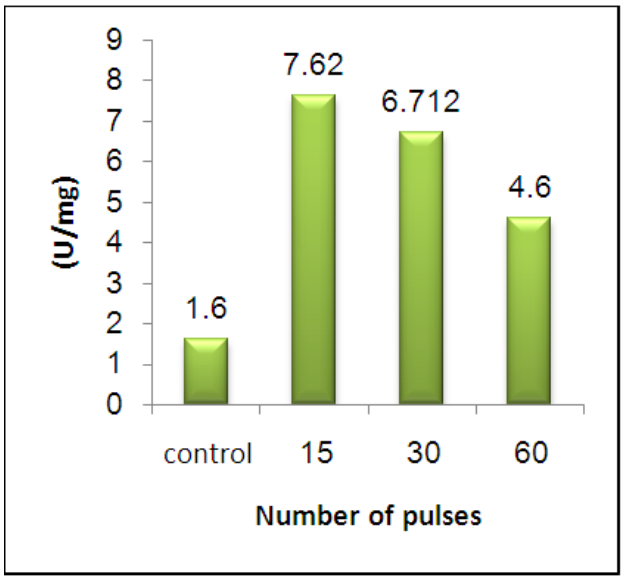
The values of ADH activity reached to 1.96 and 1.47 U/ml at 15 and 30 pulses comparing to 0.98 U/ml in control. While an inhibition was observed in ADH value 0.98 U/ml using 60 pulse. A noticeable increase in ADH specific activity was observed also at 11.3mJ/cm^2 energy density. The values reached to 7.62, 6.71 and 4.6 U/mg using 15, 30 and 60 pulse in their respective order comparing to 1.6 U/mg in control group.



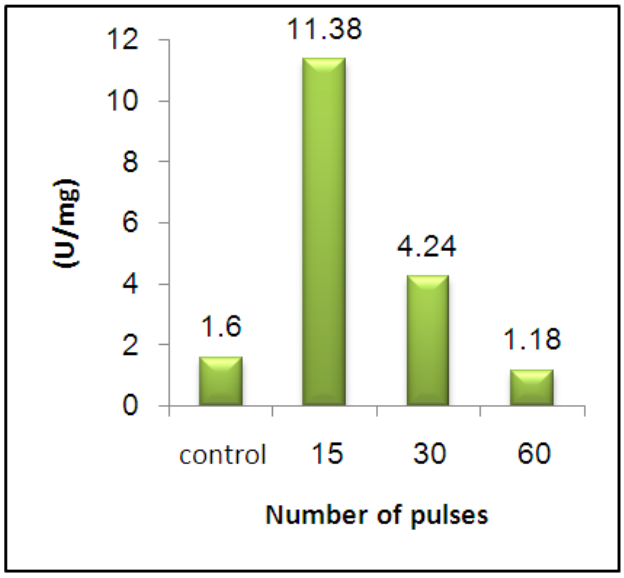
a) Enzyme activity (U/ml).



a) Enzyme activity (U/ml).



b) Specific activity (U/mg protein).



b) Specific activity (U/mg of protein).

Fig. (1): The activity and specific activity values of alcohol dehydrogenase (ADH) after exposure to 11.3mJ/cm^2 energy density at 532nm.

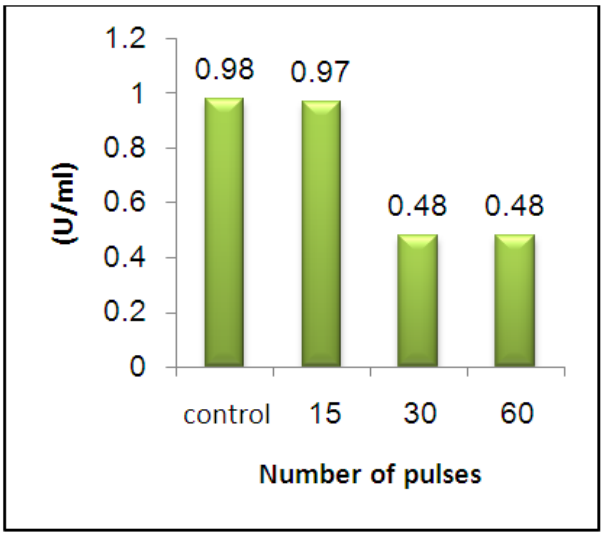
Fig. (2): The activity and specific activity of alcoholdehydrogenase (ADH) after exposure to 22.6mJ/cm^2 energy density at 532nm.

Activation in ADH activity and specific activity was also observed using 22.6 mJ/cm^2 at 15 and 30 pulses with a noticeable inhibition at 60pulse. The values of ADH activity were 4.85, 1.94 and 0.48 U/ml at 15, 30 and 60 pulse in their respective order comparing to 0.98 in control (Figure 2a). While the values of ADH specific activity were 11.38, 4.24 and 1.18 mJ/cm^2 at 15, 30 and 60 pulse in their respective order comparing to 1.6U/mg in control (Figure 2b).

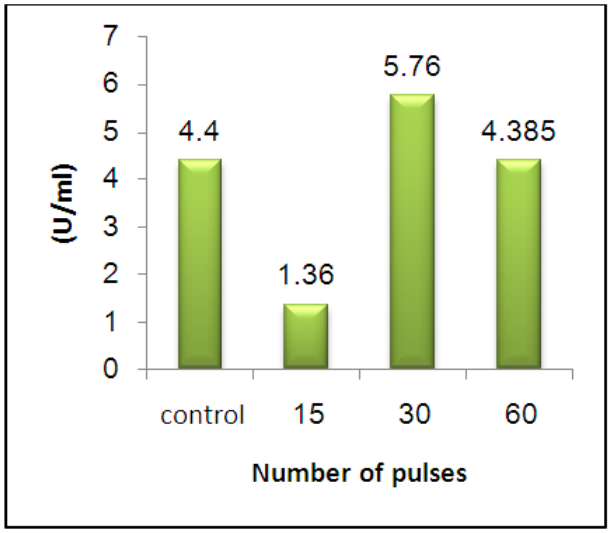
Using the energy density 33.9 mJ/cm^2, an inhibition in ADH activity and specific activity values were noticed at 15, 30 and 60 pulses.

The values at ADH activity were 0.97 at 15 pulse and 0.48 at 30 and 60 pulse comparing to 0.98 in control (Figure 3a).

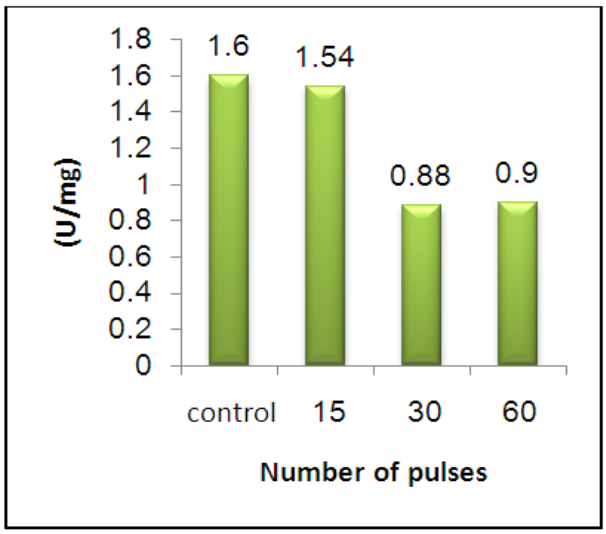
The values of ADH specific activity were 1.54, 0.88 and 0.9 U/mg at 15, 30 and 60 pulses comparing to 1.6 in control (Figure 3b).



a) Enzyme activity (U/ml).

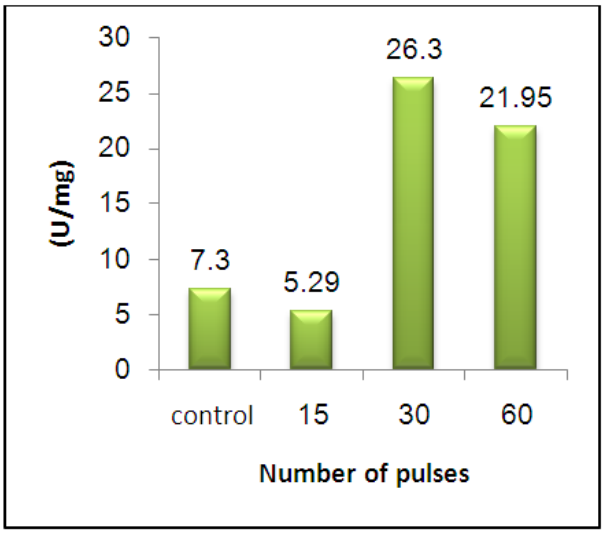


a) Enzyme activity (U/ml).



b) Specific activity (U/mg of protein).

Fig. (3): The activity and specific activity of alcoholdehydrogenase (ADH) after exposure to 33.9mJ/cm^2 energy density at 532nm.



b) Specific activity (U/mg of protein).

Fig. (4): The activity and specific activity values of total Superoxide dismutase (SOD) after exposure to 11.3mJ/cm^2 energy density at 532nm.

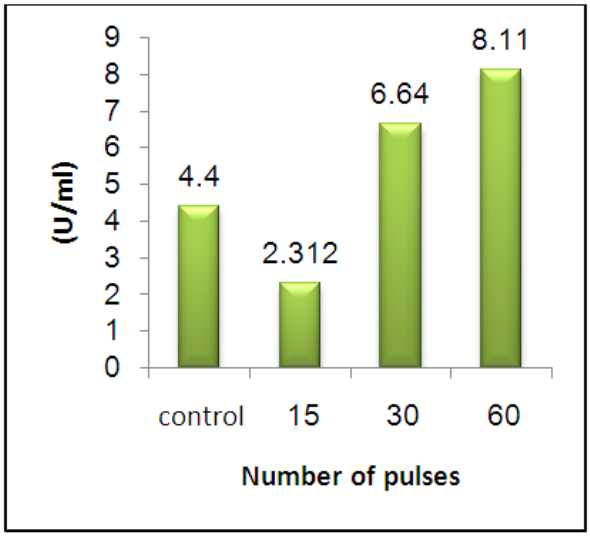
The effects of Nd: YAG laser on activity and specific activity of SOD were illustrated in Figures 4, 5, and 6. Figure 4 showed the effect of laser using 11.3mJ/cm^2 energy density with values of SOD activities was noticed at 30 and 60 pulse reaching to 5.76 and 4.365 U/ml in their respective order. While the value was 1.36 at 15 pulses comparing to 4.4 U/ml in control (Figure 4a).

In Figure 4b, same results were observed regarding the SOD specific activities. High increasing in values were observed at 30 and 60 pulse to 26.3 and 21.95 in their respective orders, while the value was 5.29 U/mg at 15 pulses comparing to 7.3 U/mg in control.

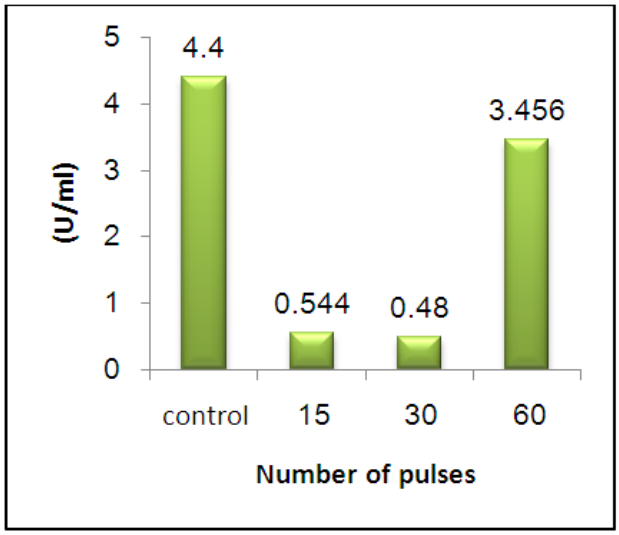
Using 22.6 mJ/cm^2 fluence, a noticeable increasing in activity and specific activity of SOD can be also seen at 30 and 60 pulse. As its clear in Figure 5a, the values of SOD activities increased to 6.64 and 8.11 U/ml at 30 and 60 pulse in their respective order while decreased at 15 pulse to be 2.312 U/ml comparing to 4.4 U/ml in control.

The values of specific activity were 5.43, 14.52 and 20.02 U/mg for 15, 30 and 60 pulse comparing to 7.3 U/mg in control Figure 5b.

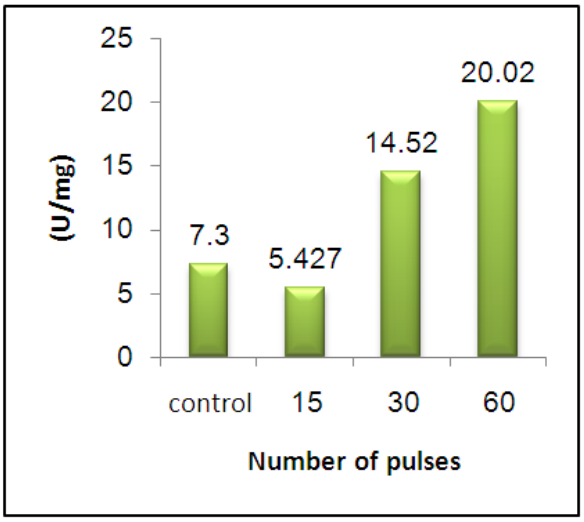
An inhibition in SOD activity and specific activity was observed using 33.9mJ/cm^2 energy for 15, 30 and 60 pulse.



a) Enzyme activity (U/ml).

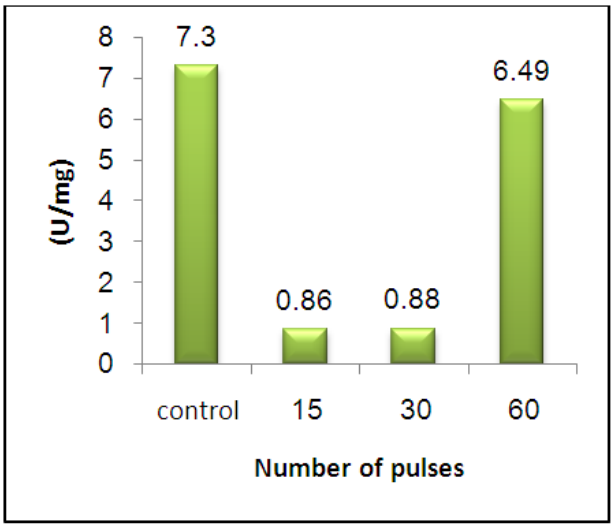


a) Enzyme activity (U/ml).



b) Specific activity (U/mg of protein).

Fig. (5): The activity and specific activity of total Superoxide dismutase (SOD) after exposure to 22.6 mJ/cm^2 energy density at 532nm.



b) Specific activity (U/mg of protein).

Fig. (6): The activity and specific activity values of total Superoxide dismutase (SOD) after exposure to 33.9 mJ/cm^2 energy density and 532nm.

The values of SOD activities were 0.54, 0.48 and 3.49 U/ml at 15, 30 and 60 pulse comparing to 4.4 in control (Figure 6a). While the values of specific activity were 0.86, 0.88 and 6.49 U/mg at 15, 30 and 60 pulse comparing to 7.3 U/mg in control (Figure 6b).

From these results, it could be supposed that the irradiation using energy densities (11.3 and 22.6 mJ/cm^2)at (15 and 30 pulses) encourage the ethanol oxidation reaction that leads to increasing in the NADH production; which appears in increasing in the activity and the specific activity of alcoholdehydrogenase (ADH) as in Figures 1 and 2

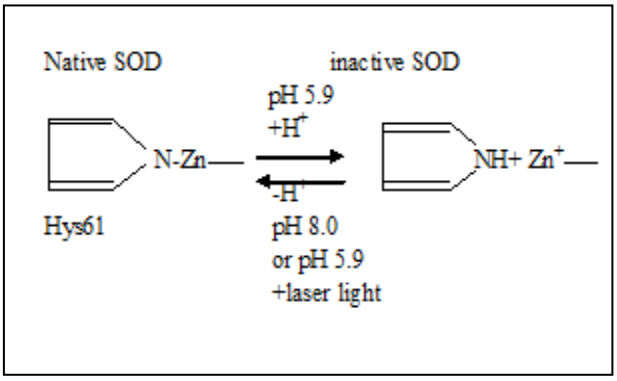
These results could be compared with the conclusion that was reached by Karu (1996), in aerobically grown yeasts; the irradiation activates the fermentation which is shown by the activation of CO_2 formation after irradiation of Saccharomycods ludwigii with He-Ne laser. As known, CO_2 is formed from pyruvic acid by alcohol fermentation (Karu, 1996). It has been shown experimentally that stimulation of the fermentation in S. cerevisiae by irradiation was a result of the weakening of Pasteur Effect, and stimulation occurred to suppression of the respiration (Karu, 1993). In a series of experiment, the activities of some enzymes were

measured in a stimulated culture, for example Fedoseyeva et al. (1986) they irradiated T. sphaerica with He-Ne laser and the irradiated inoculums was incubated for 18h (for at least three division), following which the activity of enzymes was measured.

They found that the irradiation caused considerable activation of respiratory chain components-NADH dehydrogenase and Cytochrome c oxidase, while Tsivunchyk, (2003) found an inhibition in dehydrogenase activity of Saccharomyces cerevisiae after Argon and YAG- laser (355nm and 532nm) irradiation, and the activity of enzyme activity was measured after 40min from irradiation. On the other hand, the decreasing in the activity of SOD at an energy densities 11.3, 22.6 and 33.9 mJ/cm^2 at 15 pulse as in Figures 4, 5, and 6 is could be due to increasing in the rate of oxygen consumption immediately after short time from laser irradiation switching off, this conclusion is agreed with the result that found by Alexandratou (2002), he found that a 15s irradiation evoked a progressive accumulation of H_2O_2 during 8min after the irradiation. Both stimulation and inhibition of the respiratory chain can result in enhanced ROS generation (Brookes, et al., 2004).

Also the acidity of cytoplasm after irradiation may induce inhibition in activity and production of SOD at 11.3mJ/cm^2 and at 15 pulses for example. The acidification of the cytoplasm (rise of intracellular H^+ concentration), caused by the activation of respiratory chain, controls allosterically the activity of the Na^+/ H^+ antiporter situated in the cytoplasmic membrane (Pouyssegur, et. al, 1985). This enzyme plays a key part in the alkalization of the intracellular medium. A short-term increase in the intracellular pH (ΔpH_i) is one of the necessary components involved in the transmission of mitogenic signal in the cell and is a parameter of cellular homeostasis that is closely connected with the cellular redox state (Pouyssegur, et al., 1985; Moolenaar, 1986), so the irradiation at the dose (11.3 mJ/cm^2, 22.6mJ/cm^2 and 33.9m J/cm^2 for 15pulse) acts to shift the intracellular pH (pH_i) and overall redox state of the cells to more oxidized direction.

Recall the irradiation (with 15 pulses and similar cases) cause decreasing in the intracellular pH that leads to decreasing in the activity of Cu-Zn-SOD by deprotonation of histidine and formation of the >N-Zn bond to restore the active center structure and activity of the enzyme (scheme1) (Vladimirov et al., 2004).



Scheme1**:** Native and inactive form of Superoxide dismutase (SOD) as a result of protonation and deprotonation of the SOD active site (Vladimirov, et al, 2004).

Figures 4 and 5 show the increasing in the activity and production of SOD at 30pulse for 11.3 mJ/cm^2 energy density and 30 and 60pulse for 22.6mJ/cm^2. This may result from intensification of irradiation that leads to increase the native form (reduced form) of the enzyme, as well as, increase the ROS concentration (especially $O^{\cdot -}_2$). It was shown in experiments with isolated erythrocyte Cu-Zn-SOD that a decreased pH of the solution and hydrogen peroxide inactivated the enzyme, while its complete reactivation was observed under subsequent irradiation of the inactivated enzyme at pH 5.9 with He-Ne laser light (Vladimirov et al., 2004) .Such a phenomenon can probably be observed in living organisms.

The high energy densities of 33.9 mJ/cm^2 caused inhibition in the activities and the specific activities of both enzymes. On the basis of all of the data, mitochondrial respiration in the absence of light is partially suppressed by nitric oxide synthesized by mitochondrial NO-synthase. Nitric oxide inhibits respiration due to binding with such electron carriers as cytochromes and cytochrome oxidase, and possibly iron sulfur complexes. Irradiation by intense light brings about the photolysis of these complexes and restoration of respiration and ATP synthesis (Vladimirov et al. 2004). Nitric oxide produced in the mitochondria can inhibit respiration by binding to Cox and competitively displacing oxygen, especially in stressed or hypoxic cells (Brown, 2001). Increased nitric oxide (NO) concentrations can sometimes be measured in cell culture or in animals after low

level laser therapy (LLLT) due to its photo release from the mitochondria and Cox (Cytochrome c oxidase). It has been proposed that low level laser treatment LLLT might work by photodissociating NO from Cox, thereby reversing the mitochondrial inhibition of respiration due to excessive NO binding (Lane, 2006). The photodissociation of NO from its binding sites on the heme iron and copper centers where it competitively inhibits oxygen binding and reduces necessary enzymic activity, thus allowing an immediate influx of oxygen and resumption of respiration and generation of reactive oxygen species. (Huang et al. 2009)

Conclusions

Irradiation yeast cells by Nd: YAG laser using (22.6 mJ/cm^2) energy density and (15) pulses leads to increase the activity and the specific activity of ADH. An increase in the activity and specific activity of SOD was obtained using (22.6 mJ/cm^2) energy density (60) pulses. The activity and the specific activity of both enzymes were inhibited using (33.9 mJ/cm^2) energy density (30 and 60) pulses. Irradiation of Nd: YAG laser using different energy densities and pulses may cause fluctuations in cellular redox balance involved in the enhancement or inhibit of oxidoreductase enzymes.

References

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

Anders, J.J.; Borke, R.C. and Woolery, S.K. (1993). Low-power laser irradiation alters the rate of regeneration of rat facial nerve. Lasers Surg.Med. **13:** 72–82.

Beck, Y.; Bartfeld, D.; Yavin, Z.; levanon, A.; Gorecki, M. and Hartman, J.R. (1988). Efficient production of active human manganese superoxide dismutase in Escherichia coli. Biotechnology. **6:** 930- 935.

Beyer, W.F.; JR. and Fridovich, I. (1987). Assaying for superoxide dismutase activity: Some large consequences of minor changes in conditions. Analytical Biochemistry.**161:** 559-566.

Bradford, M.M. (1976). A rapid and sensitive method for quantitation of microorganism quantities of protein utilizing the principle of protein-dye binding.Anal.Biochem.**72:**248-254.

Brookes, P. S.; Yoon, Y.; Robotham, J.; Anders, L. M. and Sheu,S.-S. (2004) .Calcium, ATP, and ROS: A mitochondrial love–hate triangle. Am. J. Physiol. Cell Physiol. **287:** C817–C833.

Brown, G.C. (2001). Regulation of mitochondrial respiration by nitric oxide inhibition of cytochrome c oxidase. Biochim Biophys Acta. **1504:**46-57.

Bull, C.; Niederhoffer, E.C.; Yoshida, T. and Fee, J.A. (1991). Kinetic studies of superoxide dismutase properties of the manganes-containing protein from thermus-thermophilus. J. AM. Chem. Soc. **113:** 4069-4076.

Conlan, M.J.; Rapley, J.W., and Cobb, C.M. (1996). Biostimulation of wound healing by low-energy laser irradiation. A rview. J. Clin. Periodontol. **23:** 492–496.

Fedoseyeva, G. E.; Karu, T.I.; Lyapunova, T. S.; Pomoshnikova, N. A.; Meissel, M. N. and Peskin, A. V. (1986). The effect of low-intensity red light on the activity of yeast enzymes. Microbiologiya. **55:** 944-948.

Fedoseyeva, G.E. Karu, T.I. Lyapunova, T.S. Pomoshnikova, N.A. and Meissel, M.N. (1988). The activation of yeast metabolism with He-Ne laser radiation-I. Protein synthesis in various culture. Laser in the life science. **2(2):** 137-146.

Fedoseyeva, G.E.; Karu, T.I.; Lyapunova, T.S.; Pomoshnikova, N.A. and Meissel, M.N. (1988). The activation of yeast metabolism with He-Ne laser radiation. I. Protein synthesis in various cultures. Lasers Life Sci.**2:**137-146.

Gascon, S. and Lampen, J.O. (1968). Purification of the internal invertase of yeast. The journal of biological chemistry. **243(7):** 1568-1572.

Gordon, S. A. and Surrey, K. (1960). Red and far-red light action on oxidative phosphorylation. Radiat. Res. **12:** 325-339.

Grossman, N.; Schneid, N. and Reuveni, H. (1998). Enhanced proliferation of keratinocyte cultures following 780-nm lowpower diode laser irradiation.

Involvement of reactive oxygen species. Lasers Surg. Med. **22:** 212–218.

Hilf, R.; Murant, R.S.; Narayanan, U. and Gibson, S.L. (1986). Relationship of mitochondrial function and cellular adenosine triphosphate levels to hematoporphyrin derivative-induced photosensitization in R3230AC mammary tumors. Cancer Res. 211-217.

Huang, Y.Y.; Chen, A.C.; Caroll, J. D. and Hamblin, M. R. (2009). Biphasic dose response in low level light therapy. International Dose- Response Society. University of Massachusetts. ISSN: 1559-3248.

Huu, T.P.; MarQuetty, C.A.; Pasquier, C. and Hakim, J. (1984). Luminol assay for microdetermination of superoxide dismutase activity: its application to human fetal blood. Analytical Biochemistry. **142:**467-472.

Jamieson, D. J. (1998). Oxidative stress responces of the yeast Saccharomyces cerevisiae. Yeast. **14:** 1511-1527.

Kamaya, T.(1970). Lytic action of lysozyme on Candida albicans. Mycopathologia et Mycologia applicata. **42:**197-207.

Karu, T.(1996). Activation of metabolism of nonphotosynthesizing microorganisms with monochromatic visible (laser) light: A critical review. Laser in the life sciences. **7(1):** 11-33.

Karu, T.I.; Kutomkina, E. V.; Lyapunova, T.S. and Pomoshnikova, N.A.(1993). The activation of yeast metabolism with He-Ne laser radiation.III. Protein synthesis in Saccharomycodes ludwigii grown n aerobic and anaerobic condition.Laser in the life sciences. **5(4):**259-266.

Kato, M.; Shinizawa, K. and Yoshikawa, S. (1981). Cytochrome oxidase is a possible photoreceptor in mitochondria. Photobiochem. Photobiophys. **2:** 263-269.

Kutomkina, E.V.; Karu, T.I.; Mushengian, M.S. and Duda, V.I. (1991). He-Ne laser induced germination of endospores Anaerobacter Polyendosporus. Laser Life Sci. **4:** 147-151.

Lane, N. (2006). Cell biology: power games. Nature. **443:**901-3.

Longo, V. D.; Gralla, E. B. and Valentien, J. S. (1996). Superoxide dismutase activity is essential for stationary phase survival in Saccharomyces cerevisiae .J. Biol. Chem. **271:** 12275-12280.

Lubart, R.; Eichler, M.; Lavi, R.; Friedman, H. and Shainberg, A. (2005). Low-Energy Laser Irradiation Promotes Cellular Redox Activity. Photomedicine and Laser Surgery. **23 (1):** 3-9.

Ludwig, M.; Metzger, A.; Pattridge, K. and Stallings, W. (1991). Manganes superoxide dismutase from Thermus thermophilus. A structural model and refined at 1.8 A resolution. Journal of Molecular Biology. **219:** 335-358.

Maitra, P.K. and Lobo, Z. (1971). Control of glycolytic enzyme synthesis in yeast by-products of the hexokinase reaction. J. Biol. Chem. **246:** 489.

Moolenaar, W.H. (1986). Regulation of cytochromic pH by Na^+/H^+ exchange. Trends Biochem. Sci. **11:** 141-143.

Passarella, S.; Ostuni, A.; Atlant, A. and Quagliariello, E. (1988). Increase in the ADP/ATP exchange in the rat liver mitochondria irradiated invitro by He-Ne laser. Biochem. Biophys. Res. Commun. **156:** 978-986.

Passarrella, S.; Perlino, E.; Quagliariello, E.; Baldassarre, L.; Catalano, I.M. and Cingolani, A. (1983). Evidence of changes induced by He-Ne laser irradiation in the biochemical properities of rat liver mitochondria. Bioelectrochem. Bioenerg. **10:** 185-198.

Pouyssegur, J.; Franchi, A.; Allimain, G.L. and Paris, S. (1985). Cytoplasmic pH, a key determinant of growth factor-induced DNA synthesis in quiescent fibroblasts. FEBS Lett. **90:** 115-119.

Rochkind, S.; Nissan, M. and Alon, M. (2001). Effects of laser irradiation on the spinal cord for the regeneration of crushed peripheral nerve in rats. Lasers Surg. Med. **28:** 216–219.

Sakai, Y.; Maeng, J. H.; Kubota, S.; Tani, K.; Tani, Y. and Kata, N. (1996). A non-convention at assimilation pathway for long chain n-alkanes in Acinetobacter sp., M1 that starts with a dioxygenase reaction. J. of fermentation and Bioengineering. **81(4):** 286-291.

Sigler, K.; Chaloupka, J.; Brozmanova, J.; Stadler, N. and Hofer, M. (1999). Oxidative stress in microorganisms. I. Microbial versus higher cells-damage and defenses in relation to cell aging and death. Folia Microbiol. **44:** 587-624.

Smidt, O.de; Preez, J.C. and Albertyn, J. (2008). The alcohol dehydrogenases of

Saccharomyces cerevisiae: a comprehensive review. FEMS Yeast Res. **8:** 967–978.

Stadler, I.; Evans, R. and Kolb, B. (2000). In vitro effects of low-level laser irradiation at 660 nm on peripheral blood lymphocytes. Lasers Surg. Med. **27:** 255–261.

Strutz, L.A. and Cullota, V. C. (2002)**.** Superoxide dismutase null mutants of baker' s yeast, Saccharomyces cerevisiae. Meth. Enzymol. **349:** 167-172.

Tiphlova, O.A. and Karu, T.I. (1988). Stimulation of Escherichia coli division by low-intensity monochromatic light. Photochem. Photobiol. **48:** 467-471.

Tsivunchyk, O. (2003). Influence of low intensity laser radiation on different biological systems. Dissertation. Der Philipps, University Marburg. 1-113.

Vekshin, N.L. and Mirinov, G.P. (1982). Flavin-dependent oxygen uptake in mitochondria under illumination. Biophysics (Moscow). **27:**537-539.

Vladimirov, Yu. A.; Osipov, A. N. and Klebanov, G. I. (2004). Photobiological principles of therapeutic applications of laser radiation. Biochemistry (Moscow).**69(1):**103-113.

Yu, W.; Naim, J.O. and Lanzafame, R.J. (1994). The effect of laser irradiation on the release of bFGF from 3T3 fibroblasts. Photochem. Photobiol. **59:**167–170.

Zhu,Q.; Yu Wei, X. and Hicks, G.L. (1997). Photo-irradiation improved functional preservation of isolated rat heart. Lasers Surg. Med**. 20:** 332–339.

تأثير ليزر النيديميوم ياك النبضي 532 nm على نشاط ديسموتاز فوق الأكسيد و Alcoholdehydrogenase من خميرة السيريفيا

نور عبد الجبار جداح [1] **غازي منعم عزيز** [2] **أياد غازي انور**[1]

(1) معهد الليزر للدراسات العليا، جامعة بغداد، بغداد، العراق
(2) قسم التقنيات الأحيائية، كلية العلوم ، جامعة بغداد، بغداد، العراق

الخلاصة دُرِسَ تاثير اشعة ليزر النيديميوم- ياك على فعالية انزيمي superoxide dismutase (SOD) و(ADH) alcoholdehydrogenase. شُعِعَتْ خلايا Saccharomyces cerevisiaeعند الطول الموجي 532نانوميتر باستخدام جهازالليزر Q-Switched النيديميوم- ياك بتردد 1هيرتز; بكثافة طاقات مختلفة 22.6, 11.6 و33.9 ملي جول\سم^2 و عدد مختلف من النبضات 30,15و60 نبضة قد استُعمِلت. حُضِنَتْ الخلايا المشعة في الوسط المغذي السائل مدة24 ساعة ثم أستُخلِصَتْ الخلايا لتحرير الانزيمات الداخل خلوية والتحري عن فعالياتها بالمقارنة مع مجموعة السيطرة غير المشعة, أظهَرتْ الخلايا المشعة زيادة ملحوظة في الفعالية الانزيمية و الفعالية النوعية للانزيم SOD عند الكثافة الضوئية 11.3 و 22.6 ميلي جول\سم^2 عند30 و60 نبضة على التوالي. لوحِظَتْ اقصى قيمة للفعالية والفعالية النوعية لانزيم ADH باستعمال 11.3 و 22.6 ميلي جول\سم^2 عند 15نبضة. بينما ثُبطتْ الفعالية و الفعالية النوعية لكلا الانزيمين عند استخدام كثافة الطاقة 33.9ميلي جول\سم^2 .يُستَنتجْ من ذلك ان الطاقة 40 ميلي جول تُحفزْ الفعالية والفعالية النوعية لكلا الانزيمين.