

# In Vitro Study of He, Ne Laser as an Agent for Melanin Formation to Vitiligo Treatment

Saja I. Dawood<sup>1</sup>, Qahtan A. Abdulqader<sup>2</sup>

Department Physics / college of science / AL Muthanna University / Iraq.

## Abstract

The standard curve for optimum concentration of different lymphocyte samples, were carried by ELISA using the procedure mention in the ELISA kit , using He-Ne laser for irradiated samples for different time, this study found there is a good correlation between the concentration of tyrosinase and optical density  $R^2 = 0.9615$ , by measuring the optical density of lymphocyte samples shows that for healthy person the concentration of tyrosinase more than patients but, the response for producing tyrosinase for patient is more than that for healthy person after using laser for different dose. This result indicates that laser radiation dose to enhance tyrosinase in lymphocyte to produce pigment cells.

**Keywords:** Tyrosinase; Lymphocyte; Melanin; Vitiligo; He-Ne laser.

## Introduction

Melanin is the primary determinant of skin color in human it is also found in hair, the stria vascularis of the inner ear and the pigmented tissue underlying the iris of the eye [1]. Biosynthesis of melanin with tyrosinase transforms the tyrosine into L-DOPA (3, 4-dihydroxyphenyl- L- alanine), which is further converted into dopachrome and auto oxidized to Indol-5, 6-quinone [2]. The Enzyme that plays a crucial role in the formation of Melanin is tyrosinase [3]. Tyrosinases (EC1.14.18.1) see Fig. (1) [4] are involved in the synthesis of melanin pigments in plants, bacteria and mammals [5]. Tyrosinase is nearly ubiquitously distributed in nature. It is found in both prokaryotes and eukaryotic microbes, plants,

invertebrates and mammals [6]. The browning of vegetables and fruits has been interest of research for years [7] the process of browning involves the enzyme-catalyzed synthesis of a dark pigment named melanin [8]. One of a pigmentation disorder is Vitiligo (or leukoderma) that is melanocytes in the skin are destroyed. Vitiligo is a chronic skin condition that causes loss of pigment, causing irregular pale patches in skin. There is some guides suggesting it is result in by a combination of auto-immune, genetic, and environmental factors [9]. The aim of study is to investigate low power laser He-Ne to induce pigment cells as treatment to vitiligo disease.

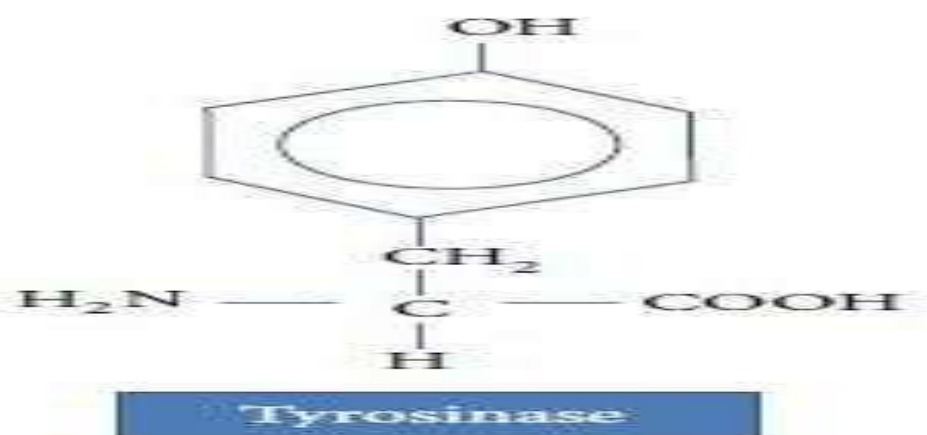


Fig. 1: Basic structure of tyrosinase [4]

## Materials and Methods

### Reagents

Phosphate buffered saline (PBS) (pH =7.4, net Wt. =100 g, HIMEDIA, India), ficoll (sigma, USA), De-ionized water 5L.

### Instruments

PH detector (model 6011A, Taiwan), Balance Acculab (Germany ) (max value 220 gm), He-Ne gas laser (optlectra GmbH) (632.8 nm with power 1 mW), Tubes 10mL (made in H.K.J), thermometer (0-100)°C, Centrifuge (CL008, 150-6000 rpm at 50 Hz, Japan), Incubator (CL011, Japan), EDTA tubes, pipettes 3 mL, P1000 micropipette.

### Preparation of Solutions

PBS stock solution was prepared by dissolving 0.85 gm of PBS powder in 100 mL of de-ionized water in room temperature and leaves it for 30 min before use in order to get a homogenous solution.

### Isolation of Human Lymphocytes

- 3 mL of blood was withdrawn from a healthy person. Leave the blood at room temperature for (~15 min) before proceeding to the second step.
- Prepare tube content 5 mL of PBS and added 3 mL of blood with shake of tube slowly to mitigation the sample.

- Take 5 mL from blood in step above and added it to tube content on 3 mL of ficoll ( take care to put the blood make sure to put the tube that content on ficoll in 45 ° and add the blood carefully so as not to homogenize).
- Centrifuge the tube at 3600 rpm for 30 min at room temperature.
- Following the centrifugation, the lymphocytes have now separated from other components in to the top cell layer. The lymphocytes layer appears as cloudy band (see fig. (2)).
- Carefully remove the yellow-colored upper phase of the blood, above lymphocyte layer, and then used a P1000 micropipette to transfer the lymphocyte layer to a 10 mL to conical tube.
- Centrifuge the lymphocyte tube at 10 min to be sure that content on lymphocyte only.
- Transfer lymphocyte to another tube and add 2 mL of PBS at 37° to tube that content lymphocyte to be useful to use.
- Re-steps above except for the first step, in order to take 3 mL from one patient in vitiligo.

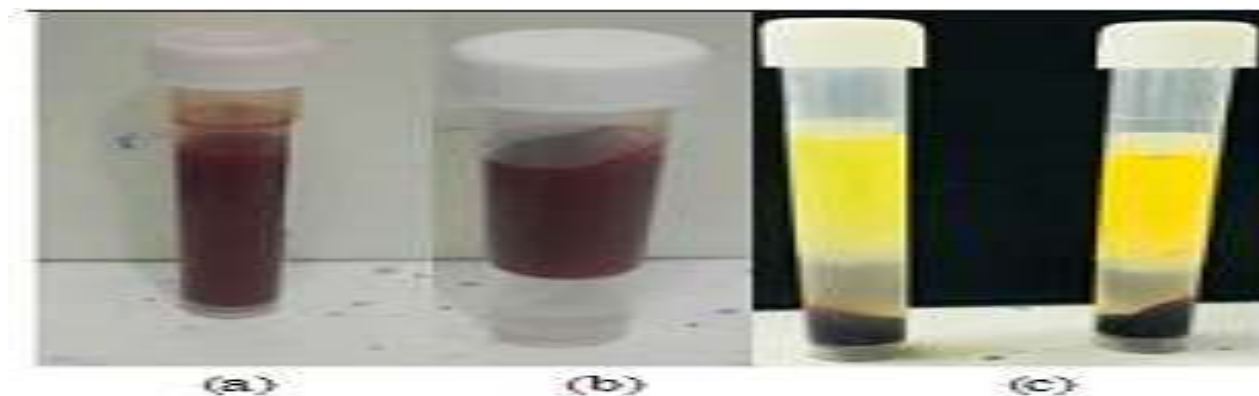


Fig.2: show (a): the blood after added to 5 mL from PBS (b): 3mL of ficoll addition for it 5ml of blood (c): blood sample after centrifuge for 30 min and lymphocyte prepare as cloudy layer

### Irradiation Lymphocyte by He-Ne

#### Laser

A continuous He-Ne laser with an average power output 1mW was used to irradiation lymphocyte in this study. Lymphocyte exposed to He-Ne laser for two period (2 and 6) min, the dose for each exposure was (382.16 J/cm<sup>2</sup> and 1146.49 J/cm<sup>2</sup>) respectively.

### Measurements of Concentrations of Tyrosinase from Lymphocyte

The concentration of Tyrosinase after irradiation samples were obtained by ELISA kit. At room temperature by using the Assay procedure mentioned in the manual (ELISA kit for Human SEA238Hu 96 tests, Cloud-Clone Corp).

## Results

The measurements which carried to get the results, able us to calculate the standard concentration of tyrosine's in lymphocytes, and by which we can get the concentration for any samples in this study. Also by comparing these concentrations with the

absorbed dose of laser, that unable as to investigate the effect of absorbed amount of energy from laser He-Ne by the enzyme. Fig (3) shows the calibration standard curve of the optical densities (O.D (a.u)) at 450 nm and the concentrations (ng/ml).

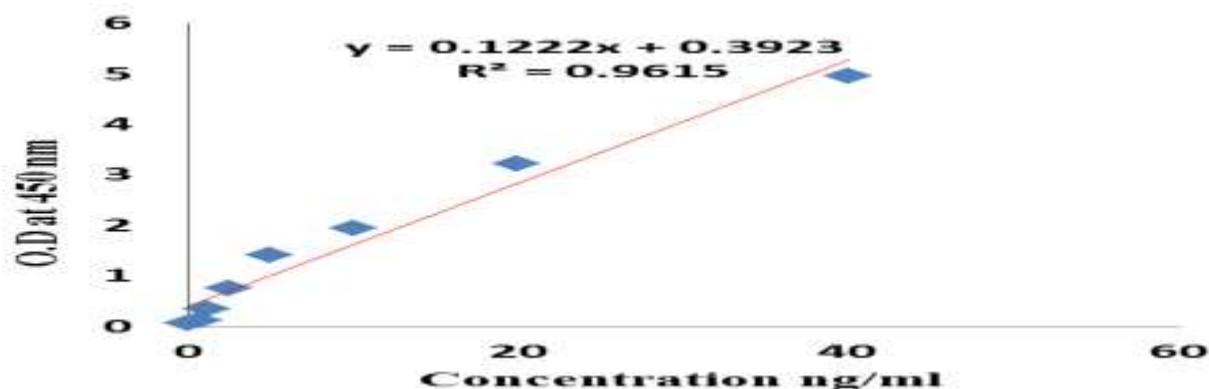


Fig.3: Standard curve for tyrosinase enzyme

This curve able as to determine the tyrosinase in samples for each patients as concentration of show in Fig. (4).



Fig. 4: show the concentration of tyrosinase for patient with vitiligo after exposed to He-Ne laser (1 mW) for (2 and 6) min

The same dependence was shown in Fig. (5) By exposing the samples for healthy person, but the concentration for each identical case

larger for healthy person than patients. This because of the disintegration of melanocytes in patients.

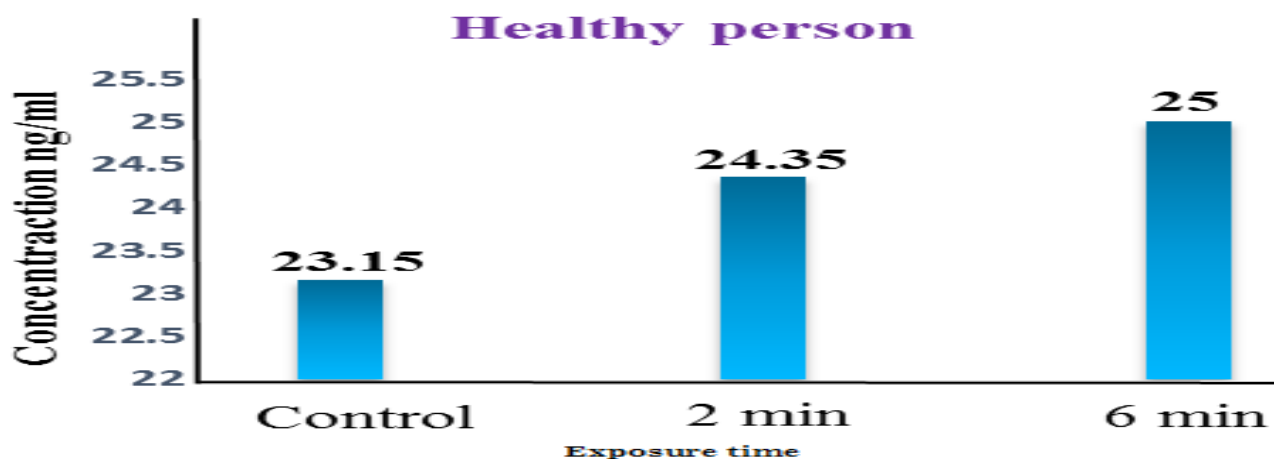


Fig. 5: show the concentration of tyrosinase for healthy person after exposed to He-Ne laser (1 mW) for (2 and 6) min

## Discussion

Vitiligo is an acquired disorder of pigmentation in which depigmentation of skin and hair occurs due to a loss of melanocytes from the epidermis [10]. And it is an autoimmune disorder characterized by the presence of autoantibodies in the patients' sera directed against certain antigens of melanocytes. These autoantibodies lyse melanocytes and lead to the formation of white patches on the skin [11]. Low energy He-Ne laser treatment has been shown to be a safe and effective treatment modality for patients with vitiligo [12].

In this study, He-Ne laser (632.8 nm) used as treatment for skin disorder (vitiligo) to study the physical features of treatment. From Fig. (3) Shown a linear dependence which can be describe first degree equation ( $y=0.122x+0.3923$ ) with a correlation ( $R^2=0.9615$ ). This value of correlation shows there are a good dependence between O.D and concentration, when O.D increased the concentration of tyrosinase increase. Fig. (4) Shows effect of He-Ne laser to induce tyrosinase for a given concentration in patients when exposed to 2 and 6 min. We note change significantly in concentration after irradiation. Attribute this change in concentration to effects physical features of

laser He-Ne (Dose, power, exposure time). In addition Fig.(5) We note change simple in the concentration for healthy person because of healthy person don't have any disease in pigment, but to comparing each identical case with patients. We note that the time required getting the same concentration of tyrosinase for patients to be as healthy person, for our case is (6.119 min). This in vitro study showed the effects of low-power laser He-Ne on vitiligo patients own in this case. This consistent with the other studies have the effectiveness of He-Ne laser treatment type of vitiligo known segmental vitiligo (SV) is comparable to that of conventional therapies for vitiligo [13]. However, we can say that Low- power laser He- Ne can induce pigment cell causing by increasing in tyrosinase concentration. It's easier to used and less expensive compared with other tools and has no harmful thermal effects.

## Conclusion

One can conclude that the low-power laser He-Ne is an efficient tool for enhancing the concentration of tyrosinase in lymphocyte. This result is obtained by using standard method (ELISA) by which the concentrations of tyrosinase in patients with vitiligo compared with healthy person. The present of tyrosinase is doses depended and increase in patient larger than healthy person.

## References

1. Shu ML, Yi SC, Chih CL, Kuan HC (2015) "Hair Dyes Resorcinol and Lawsone Reduce Production of Melanin in Melanoma Cells by Tyrosinase Activity Inhibition and Decreasing Tyrosinase and Microphthalmia-Associated Transcription Factor (MITF) Expression," International Journal of Molecular Sciences, 16: 1422-0067. 1495-1508.
2. Shripad NS, Jyoti P (2011) "A Novel Approach for Biotransformation of L-Tyrosine to Melanin by Microbial System," Thesis, 1-7.
3. Katrin S, Erik V, Armand WJWT, Alexander M, Frieder WS, Gerard WC, Ulla W (2001) "Determination of phenolic compounds using recombinant tyrosinase from *Streptomyces antibioticus*," Analytica Chimica Acta, 427: 201-210.
4. Kamal UZ, Ayesha SA, Sharique AA, Ishrat N (2014) "Microbial Tyrosinases: Promising Enzymes for Pharmaceutical, Food Bio processing, and Environmental Industry," Biochemistry Research International, 1-16.
5. Shun H, Takumi K, Emanuela L, Ellen DW, Noriaki F, Gerard WC (2005) "Oxygen Binding to Tyrosinase from *Streptomyces antibioticus* Studied by Laser Flash Photolysis," J. AM. Chem. Soc., 127: 17966-17967.
6. Sung-Y S, Vinay KS, Niti S (2003) "Mushroom Tyrosinase: Recent Prospects," J. Agric. Food Chem., 2837-2853.
7. Nogurira, Almeida MEMJN (1995) "The control of polyphenol oxidase activity in fruits and vegetables A study of the interactions between the chemical compounds used and heat treatment,"

- Plant Foods for Human Nutrition, 47: 245-256.
8. Friedman Mendel (1997) "Chemistry, Biochemistry, and Dietary Role of Potato Polyphenols. A Review," J. Agric. Food Chem., 45: 1523-1540.
  9. Godbole, Dhanashri (2007) "Role of Proteasomes in the Regulation of Melanin Biosynthesis In Different Phenotypes of Mouse Melanoma Cells," thesis, 2-37.
  10. Chieh SW, Hsin SY, Huog RC, Chu LY, Chia LY, Bin NW (2000) "Cutaneous blood flow and adrenoceptor response increase in segmental-type vitiligo lesions," Journal of Dermatological Science, 23: 53-62.
  11. Pnina F, Ofer M, Ehud B, Yehuda S (1997) "Autoantibodies to Tyrosinase the Bridge between Melanoma and Vitiligo," American Cancer Society, 79: 1461-1464.
  12. His S, Chieh S, Chia LY, Ying HK, Min HC (2003) "Helium-Neon Laser Irradiation Stimulates Migration and Proliferation in Melanocytes and Induces Repigmentation in Segmental-Type Vitiligo," J. Invest Dermatol., 120: 56-64,.
  13. Chieh SW, Stephen CSH, Cheng CGL, Gwo SC, Wen HC, Hsin SY (2008) "Low-Energy Helium-Neon Laser Therapy Induces Regimentation and Improves the Abnormalities of Cutaneous microcirculation in Segmental-Type Vitiligo Lesions," J. Med Sci., 24 (4): 180-189.