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The Role of Nd-YAG Laser with A Wavelength of 1064 Nm for The Treatment of Skin Wounds in Laboratory Mice

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Abstract:

The study showed the potential of prompting and expansion of skin cells in laboratory mice particularly fibroblasts and collagen fibers using Neodymium- yttrium aluminum garnet (Nd-YAG) laser with a wavelength of 1064 nm after compared the laser-exposed groups with control groups This is specified by the results of both statistical analysis and histological examination of samples Which were divided into five groups of each group consisting of six mice, The first group is the control group with four other groups exposed to laser radiation at varying time intervals (one day, three days, five days and ten days) respectively fibroblasts, collagen fibers and blood vessels were more diffused in laser exposed groups near the wound area.

Keywords: Nd-YAG laser, fibroblasts, collagen fibers, skin.

Introduction:

Lasers were used for the first time as a treatment method for healing wounds in the late 1960's and showed good effectiveness in healing the chronic ulcers, incurable tissues. This is the beginning use of the laser as a therapeutic method [1]. Laser exposure accelerates wound healing by forming granular tissue to reduce inflammation, the reintroduction of blood vessels, blood circulation and lymphatic vasculature, as well as promoting the proliferation of fibroblasts and collagen fibers [2]. The ability of the chromophores to transfer absorbed energy to other molecules and thus cause chemical reactions in the surrounding tissues, The optical receptor molecule, in its active form, can also share chemicals for the events of the desired changes, for example in photodynamic therapy (PDT) Cells with specific wavelengths may activate some of their components and thus stimulate cellular metabolism. This type of reaction is thought to form the basis for low-energy laser effects [3]. Radioactive energy is absorbed by separate units or photons and has an inverse relationship with the wavelength. Thus, the longer the wavelength is, the lower the energy. When the photon is reflected or moved, the energy is absorbed into the atoms of the absorbed part by the chromophores. Which in turn changes the temperature level or raises other atoms or molecules (changes in the levels of the energy of electrons), or breaks another chemical (building new chemical compounds) [4]. Evidence suggests that increased wound healing increases the rate of wound healing and reduces bacterial infections, indicating an inhibitory effect on wound infection [5]. The depth of any laser beam penetrates the target tissue along the wavelength, which ranges between 700-1200 nm maximum [6]. Nd-YAG

showed a positive effect in the treatment of glaucoma and no side effects such as redness, swelling, skin scaling or skin discoloration in patients after laser treatment sessions compared to the control group [7]. The Nd-YAG pulse laser is safe even in dark skin types because of its wavelength and deep penetration of the skin with fewer effects on the skin such as pigment changes [8]. A study using the Nd-YAG laser with a wavelength of 1064 nm as a treatment to remove tattoos from different parts of the body (face, chest, hands, and feet) showed high efficacy and no complications, scarring or deformities [9].

The study showed that exposure to laser Nd - YAG wavelength 1064 nm for the treatment of some skin diseases Rosacea in a group of patients, was very effective, including reduced redness of diffuse and remove symptoms such as itching, burning and dry skin in addition to reducing wrinkles by restoring Formation of collagen for patients treated with laser Nd-YAG compared to untreated patients [10]. To study the effects of Nd-YAG at 1064 nm on the skin of laboratory mice after exposure to ultraviolet light, the results indicated that exposure to laser Nd-YAG significantly increased the level of Procollagen and growth factors MMPs, TIMPs followed by an increase in the level of collagen in the skin and wound healing [11].

Materials and Methods:

The Experimental Animals

In this experiment, thirty male and female mice of type *Mus musculus* of age 8-10 weeks were used. The weights were measured by the sensitive balance and the mice weight were (25 ± 5) gm. The mice were putted b in plastic cages and divided into five groups in each group of six mice. The first group was the control group, but the four groups were exposed to Nd - YAG laser with wavelength 1064 nm for different periods of time, group first for one day, group second for three days, group third for five days and group fourth for ten days. The duration of laser exposure is sixty seconds. All animals were left throughout the study time in the animal house of the college of education for pure sciences for the purpose of acclimation under controlled laboratory conditions in period of ventilation, light, and temperature throughout the experiment.

Histological Examination

After exposure the laboratory mice to the laser, all mices were drugged by chloroform for the purpose of earning samples of the skin. The samples were putted in a formalin solution at a concentration of 10% for 24 hours at room temperature for the purpose of fixation. The samples were then washed with tap water for one hour, then samples were transferred to a series of alcohol ethanol with a rising concentration for two hours. The samples were in xylol for 10 minutes and to be clear. The samples were placed in a convection oven containing three groups of pure paraffin wax with a temperature of 60 C° for one hour for each group. Paraffin wax was poured into special templates. The samples were moved to those templates and left to harden to until they became templates and preparation for cutting and then preserved in the refrigerator until cutting. After the trimming of the template, it was fixed on the rotary microtome for the purpose of cutting with a thickness of 5 micrometers, then the tapes, that were resulted from cutting, were put into a water bath with a temperature of 40 C° for spreading them. The sections were putted on clean glass slides then left to dry. The glass slides were placed in the xylol in two stages of 10 minutes. The slides were then transferred to a descending chain of concentration of ethanol alcohol for two minutes per concentration. The slides were then transferred to distilled water for five minutes and then placed in the hematoxylin stain for five minutes and then washed with tap water for five minutes. And then transferred

to the disperse solution (acid alcohol) two seconds for the purpose of removing the excess stain. Washed with tap water for five minutes, then put in Eosin stain for two minutes and were passed through a series of ascending concentration of ethanol alcohol for two minutes for each concentration. The slides were then transferred to the xylol twice for each time 5 minutes D. P.X. substance was used for mounting the sections for the purpose of installing the slide cover and then left to dry at room temperature. Histological slides were examined using an optical microscope type micros. The slides were then photographed using a camera connected to the optical microscope, and these slides were photographed under different magnification forces to detect tissue changes [12]. The statistical analysis was then performed using a program SPSS [13].

Results and Discussion

Histological sections of the mices skin of the control groups showed the essential structures of the skin which consists of the surface layer epidermis which contains several layers of stratified squamous epithelium cells externally covered with keratin and to the inside of the skin layer is the layer of dermis where the spread of collagen fibers can be observed, fibroblasts, blood vessels as well as the structures linked to the skin (sweat glands, sebaceous glands and their ducts and hair). Histological changes of the skin of animals exposed to laser radiation Nd-YAG re-spread epithelium cells in the wound area with the proliferation of collagen fibers and blood vessels near the wound area. This is clearly shown in groups exposed to laser radiation on the fifth and tenth day of experiment

Activity of low-level laser therapy accelerates wound healing. They also agreed with [14] Fibroblasts and collagen fibers showed it was an important factor in the wound healing process. Many collagen fibers were formed in wounds areas, also Collagen makes new structure that gives strength to the tissue that enables to cure. Its normal state, this is consistent with what it pointed out. [15] low-level laser therapy is effective in wound healing and for varying periods of time for a group of laboratory rats in terms of forming collagen fibers and elastic fibers at higher rates than the control group. It is consistent with [16-17] The advancement of wound healing was well in a group of patients treated with low-level laser therapy. It is also consistent with [18] low-level laser therapy improves the spread of fibroblasts in the wound area at the time of laser exposure. They agree with [19] low-level laser therapy promotes the healing of skin lesions in laboratory mice and the results showed significant improvement in wound healing. It is agreed with [20] low-grade laser therapy Showed positive results in accelerating the healing and healing of wounds in laboratory mice. It also agrees with [21] low-level laser irradiation has stimulated the proliferation of collagen, Low-level laser therapy (LLLT) has also shown a positive effect in accelerating the wound healing process due to the spread of fibroblasts, collagen fibers and blood vessels near wound areas. These results are consistent with [22] who noted that the enhancement of wound healing due to low-level laser exposure is due to increased proliferation of Fibroblasts in the wound area. They also agree with [23] low-level laser therapy is used to accelerate wound healing in laboratory mice. The results of the current study are consistent with [24].

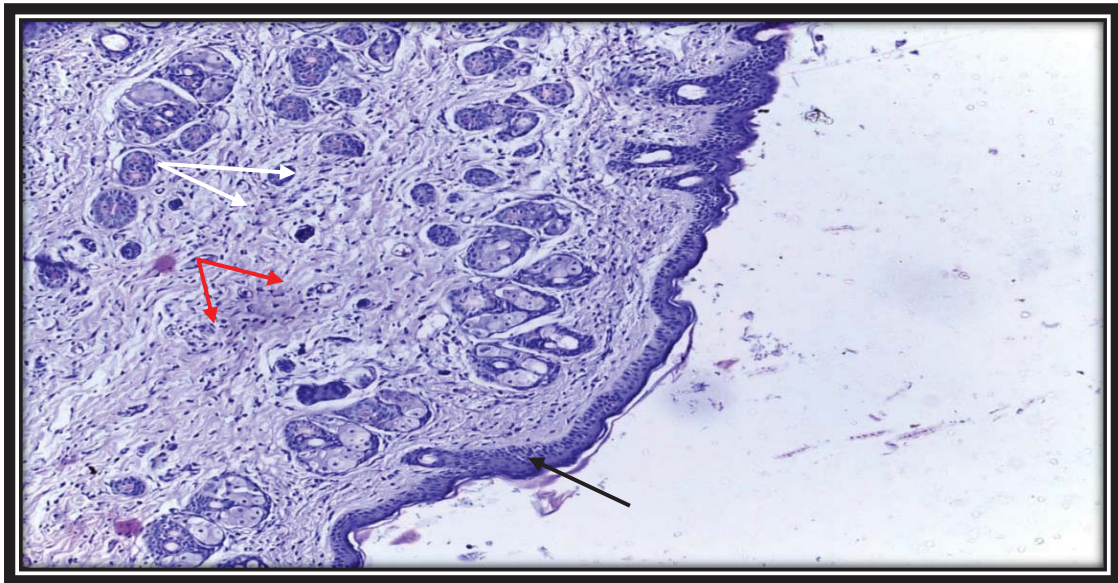
It is consistent with [25] Nd: YAG showed significant improvement in the connective tissue of laboratory rat skin by reconstituting collagen fibers in irradiation zones, the present study does not agree with [26] that the Nd-YAG laser affected the formation of collagen fibers in laser exposure areas. Laser effects on tissues and depends on the determination of the wavelength of the laser, energy, and irradiation time for bioconcentration purposes because the bio-stimulation process is enhanced by the proliferation of

cells. The cause of wound healing in laboratory mice is the effect of the Nd-YAG laser with a wavelength of 1064 nm as shown in Table 1 and these results are appropriate with [27]. Nd-YAG laser therapy showed significant improvement in tissue exposed to Laser Treatment, and agree with [28-29] who pointed out that laser therapy Nd-YAG is widely used for the activity of positive and therefore showed no side effects, It is consistent with [30] that the use of the Nd-YAG laser on the skin of laboratory mice has contributed to the regeneration of skin cells through epithelial cell reconstruction and the distribution of collagen fibers and blood vessels.

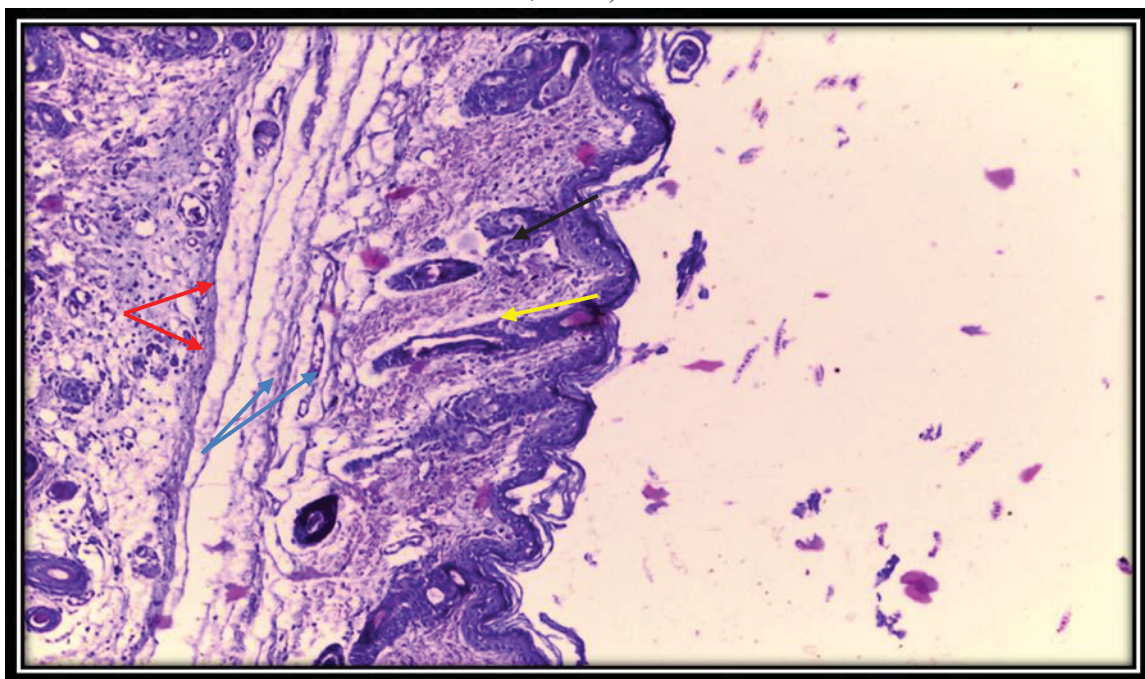
Table (1): shows the density of collagen fibers and epithelial cells of male and female skin lesions for control groups and exposure groups for Nd-YAG wavelength of 1064 nm.

Days	The density of collagen fibers and epithelium cells of male by micrometer.		The density of collagen fibers and epithelium cells of female by micrometer.	
	Control	Exposed	Control	Exposed
First day	100±0.122a	100±0.953aD	95±0.327a	100±0.255aD
Third day	95±0.985a	110±0.264bC	100±0.965a	115±0.461bC
Fifth day	100±0.840a	120±0.372bB	95±0.483a	125±0.685bB
Tenth day	100±0.351a	135±0.566bA	100±0.798a	140±0.342bA
L.S.D	df=3 t= 42.8 (by exposer) Sig. (0.000)**		df=3 t= 2.69 (by sex) Sig. (0.008)**	

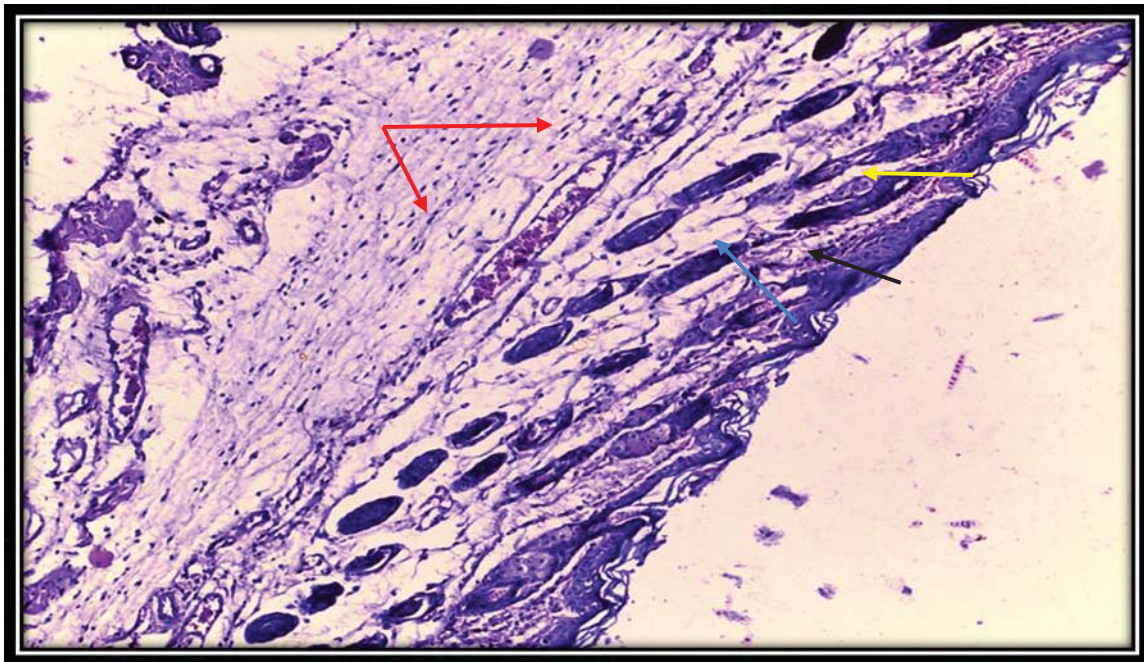
- The numbers in the table Express values Mean (S.D) and L.S.D values.
- The small letters indicate a significant difference under the level probable ($p \leq 0.05$) When comparing horizontally the control and exposure groups to each factor.
- The capital letters indicate a significant difference under the level probable ($p \leq 0.05$) When comparing vertically the control and exposure groups to each factor.



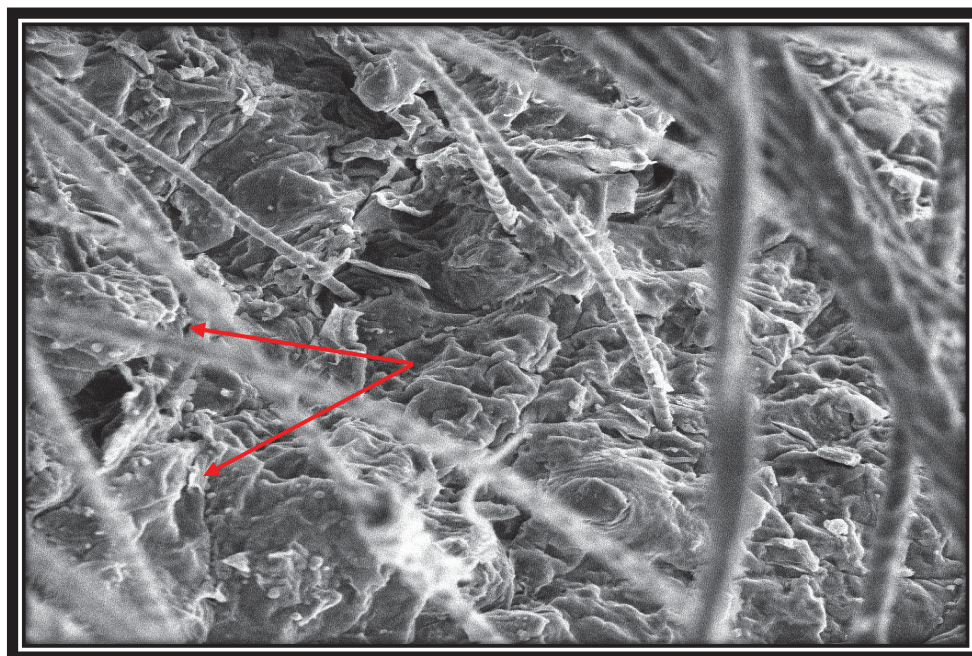
(Figure 1): A longitudinal section of the male skin of the laboratory mice (control group) showing epithelium cells (black arrow), collagen fibers (white arrow) and blood vessels (red arrow) (H & E ,100X).



(Figure 2): A longitudinal section of the male skin the laboratory mice show an increase in epithelial cells in the epidermis (black arrow) and retraction of blood vessels (red arrow) with the presence of inflammatory cells (blue arrows) near the wound area (orange arrow), Nd-YAG irradiation for five days (H & E, 100X).

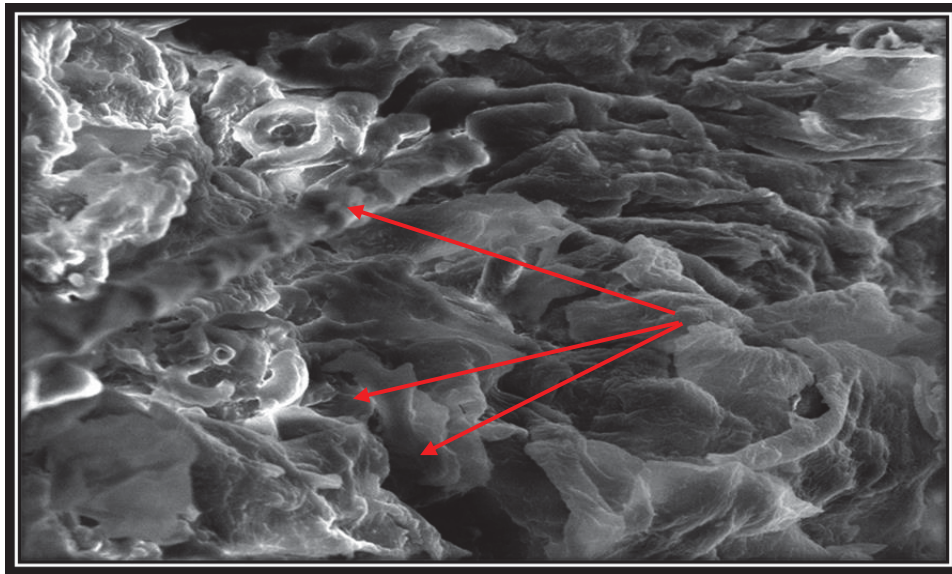


(Figure 3) A longitudinal section of the female skin the laboratory mice show an increase of the epithelial cells in the epidermis (black arrow) and re-spread more in the blood vessels (red arrow) with the presence of inflammatory cells (blue arrow) near the wound area (orange arrow), irradiation Nd-YAG for five days (H & E, 100X).



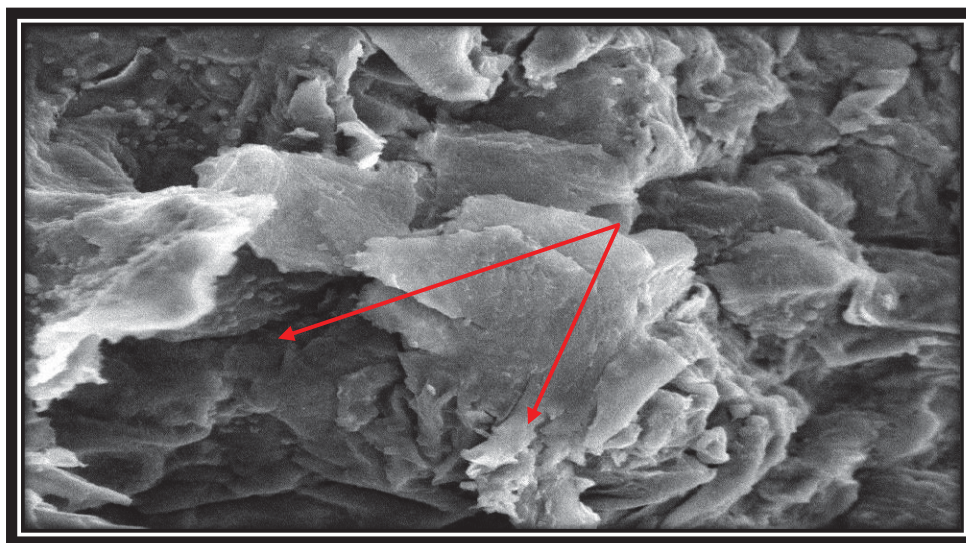
-----100µm-----

(Figure 4): Skin of male laboratory mice from outside, control group showing the Stratified squamous epithelial cells (red arrow), scanning electron microscopy microscope, 100-nanometer magnification power.



-----50 μ m-----

(Figure 5): Female skin the laboratory mice from the outside shows an increase of the Stratified squamous epithelial cells (red arrow), Nd -YAG irradiation for five days, imaging electron microscopy scanner, 50-nanometer magnification power.



-----100 μ m-----

(Figure 6): Male skin the laboratory mice from the outside shows an increase of the Stratified squamous epithelial cells (red arrow), Nd -YAG irradiation for ten days, imaging electron microscopy scanner, 100-nanometer magnification p.

References:

1. Mester, E.; Spiry, T.; Szende, B. and Tota, J.G. (1971). Effect of laser rays on wound healing. Am. J. Surg 122(4);PP:532 – 235.
2. Enwemeka, C.S.;Parker, J.C.;Dowdy, D.S.; Harkness, E.E.; Sanford, L. E. and Woodruff, L. D.(2004).The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study. Photomedicine and laser surgery; VOL: 22 (4); pp: 323-329.

3. **Karn, D.; Timalisina, M. and Gyawali, P.(2014).** Hormonal profile and efficacy of long pulse Nd-YAG laser in treatment of hirsutism .J. Nepal Health Res Counc. Jan.;12(26); PP:59-62.
4. **Pöntinen, P.J. (1992).** Low Level Laser Therapy as a Medical Treatment Modality. Art Urpo Ltd, Tampere. Laser Med. and Surg 20(1);PP:23 –26.
5. **Nelson, J.S. (1993).** Lasers: state of the art in dermatology. Dermatol Clin 11(1);PP:15 – 26.
6. **Health and Safety Manual (2005).** Lasers, Chapter 16 www. lbl. gov/ehs/pub3000/CH16.html Accessed 04 March 2006;pp: 277 – 287.
7. **Neda, Nosrati.; Lindsey, Hunter. and Erica, Kelly.(2012).** Efficacy of Nd:YAG Laser for the Treatment of Hidradenitis Suppurativa. Journal of Cosmetics, Dermatological Sciences and Applications, 2012, 2,pp:83-87.
8. **Alster, T. S.; Bryan, H. and William, C. M. (2001).** “Long Pulsed Nd:YAG Laser-Assisted Hair Removal in Pigmented Skin: Clinical and Histological Evaluation,” Archives of Der-matology, Vol. 13, No. 7, 2001, pp: 885-889.
9. **Fatima, A. Mohammad. And Ali, S. Mahmood.(2009).** Removal of tattoo by 1064 and 532 Q-switched Nd-YAQ laser , Iraqi journal for medical sciences.Volume 3,number 3,pp:66-81.
10. **Joo, Hee. Lee.; Miri, Kim.; Jung, Min. Bae.; Baik, Kee. Cho. and Hyun, Jeong. Park.(2015).** Efficacy of the long-pulsed 1064-nm neodymium:yttrium-aluminum-garnet laser (LPND) (rejuvenation mode) in the treatment of papulopustular rosacea (PPR): A pilot study of clinical outcomes and patient satisfaction in 30 cases. August 2015Volume 73, Issue 2, PP:333–336.
11. **Yousefi, M.; Mansouri, P.; Partovikia, M.; Esmaili, M.; Younespour, S. and Hassani, L. (2017).** The effect of low level laser therapy on pemphigus vulgaris lesions: a pilot study. J. Lasers Med. Sci.;8(4); PP:177-180.
12. **Luna , L.G.(1968).** "Manual of Histological staining Methods of armed Forces Institute of pathology " . 3rd ed. McGraw- hill Book .company .New York .U.S.A. ;34, pp:105- 193.
13. **Denise, Hawkins. Evans. and Heidi, Abrahamse.(2008).** Efficacy of three different laser wavelengths for in vitro wound healing. View issue TOC Volume 24, Issue 4 ,August.PP:199–210.
14. **Schindl, A.; Heinze, G.; Schindl, M.; Pernerstorfer-Schon, H. and Schindl, L. (2002).** Systemic effects of low-intensity laser irradiation on skin microcirculation in patients with diabetic microangiopathy, Microvasc. Res. 64(2); PP:240-246.
15. **Kushibiki,T.; Hirasawa,T.;Okawa,S. and Ishihara,M.(2015).** Low Reactive Level Laser Therapy for Mesenchymal Stromal Cells Therapies. Stem Cells Int.:974864. doi: 10.1155/2015/974864.
16. **Eren,Isman.;Mutan,Hamdi.Aras.;Beyhan,Cengiz.;Recep, Bayraktar.; Umit, Yolcu.;Tolga,Topcuoglu.;Aslihan, Usumez. and Tuncer, Demir.(2015).** Effects of laser irradiation at different wavelengths (660, 810, 980, and 1064 nm) on transient receptor potential melastatin channels in an animal model of wound healing . Lasers in Medical Science, July, Volume 30, Issue 5, pp: 489-495.
17. **Fiorio, F.B.;Albertini,R.; Leal-Junior,E.C. and de Carvalho, P. T.(2014).** Effect of low-level laser therapy on types I and III collagen and inflammatory cells in rats with induced third-degree burns. Lasers Med. Sci. Jan., 29 (1); PP:313-319.
18. **Avci, P.; Nyame, T.T.; Gupta, G.K.; Sadasivam, M. and Hamblin, M.R.(2013).** Low-level laser therapy for fat layer reduction: a comprehensive review. Lasers Surg. Med. Aug.;45(6); PP:349-357.
19. **Posten, W.; Wrone, D.A.; Dover, J.S.; Arndt, K.A.; Silapunt, S. and Alam, M.(2005).** Low-level laser therapy for wound healing: mechanism and efficacy. Dermatol Surg.; 31(3); PP:334-340.

20. Rhee, do. Y.; Cho, H.I.; Park, G.H.; Moon, H.R.; Chang, S.E.; Won, C.H.; Jung, J.M.; Park, K.Y.; Lee, M.W.; Choi, J.H.; Moon, K.C.; Lee, D.C. and Goo, B.(2016). Histological and molecular analysis of the long-pulse 1,064-nm Nd-YAG laser irradiation on the ultraviolet-damaged skin of hairless mice: In association with pulse duration change. *J. Cosmet Laser Ther.*;18(1); pp:16-21.
21. Hopkins, J.T.; McLoda, T.A.; Seegmiller, J.G. and David, Baxter. G. (2004). Low-Level Laser Therapy Facilitates Superficial Wound Healing in Humans: A Triple-Blind, Sham-Controlled Study. *J. Athl Train. Sep.*;39(3); PP:223-229.
22. Kajagar, B.M.; Godhi, A.S.; Pandit, A. and Khatri, S.(2012). Efficacy Of Low Level Laser Therapy On Wound Healing In Patients With Chronic Diabetic Foot Ulcers. A Randomised Control Trial. *Indian J. Surg. Oct.*;74(5); PP:359-363.
23. Mao, Z.; Wu, J.H.; Dong, T. and Wu, M.X.(2016). Additive enhancement of wound healing in diabetic mice by low level light and topical CoQ10. *Sci. Rep. Feb. 2*;6; PP:20-28.
24. Martignago, C.C.; Oliveira, R.F.; Pires-Oliveira, D.A.; Oliveira, P.D.; Pacheco, Soares. C.; Monzani, P.S. and Poli-Frederico, R.C.(2015). Effect of low-level laser therapy on the gene expression of collagen and vascular endothelial growth factor in a culture of fibroblast cells in mice. *Lasers Med. Sci. Jan.*;30(1); pp:203-208.
25. Miri, Kim.; Jongsic, Kim.; Seo-won, Jeong.; Hyunmu, Jo. and Hyun, Jeong. Park.(2018). Long-pulsed 1064-nm Nd: YAG laser ameliorates LL-37-induced rosacea-like skin lesions through promoting collagen remodeling in BALB/c mice. *Lasers in Medical Science*, February 2018, Volume 33, Issue 2; pp:393–397.
26. Montazeri, K.; Mahmoudian, S.; Razaghi, Z. and Farhadi M. (2017). Alterations in Auditory Electrophysiological Responses Associated with Temporary Suppression of Tinnitus Induced by Low-Level Laser Therapy: A Before-After Case Series. *J. Lasers Med. Sci. Summer*; 8(Suppl 1); PP:38-45.
27. Koji, Itai.; Ayumi, Korekawa.; Kayo, Jin.; Takayuki, Aizu.; Akiko, Rokunohe.; Chihiro, Hagiwara.; Katsumi, Hanada. and Daisuke, Sawamura. (2012). Effect of 0.3-Millisecond Multi-Pulsed 1064-nm Nd:YAG Laser in Patients with Postherpetic Neuralgia. *Journal of Cosmetics, Dermatological Sciences and Applications*, 2012, 2; pp: 11-13.
28. Lipper, G. M. and Perez, M. (2006). Nonablative Acne Scar Reduction after a Series of Treatments with a Short-Pulsed 1064-nm Neodymium: YAG Laser. *Dermatologic Surgery*, Vol. 32, No. 8, 2006; pp:998-1006.
29. Farah, A.J. Al-Zahawi. and Ali, S. Mahmood.(2009). Immuno-hematological and biochemical changes in diabetic retinopathy patient's blood after treatment by Nd-YAG laser, *Iraqi journal of laser*, Vol.8, pp: 39-44.
30. Apsari, R.; Nahdliyatun, E. and Winarni, D. (2017). The regeneration of thermal wound on mice skin (*Mus Musculus*) after Q-Switch Nd:YAG laser irradiation for cancer therapy candidate. Published by the American Institute of Physics; Citation: AIP Conference Proceedings 1888, 020015 (2017); doi: 10.1063/1.5004292.