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RESEARCH ARTICLE

Comparative Study of Treatment of Corneal Injury by Using Red Laser and Green Laser in Rabbits

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Abstract

Eighteen mature rabbits were used in the present study to determine the best type of lasers for treatment of wound of the cornea, the results showed that the green diode laser is better than red diode laser in spite of the red laser interacts with the larger tissue, but is causing corneal cataract in rabbits, in conclusion, the use of green laser for treatment of animals cornea be guaranteed and better than others.

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INTRODUCTION

Laser surgery is a modern treatment of the eye, it is founded a suitable for conventional treatment, low level laser therapy (LLLTT) is the user type in the ophthalmic surgery [1]. LLLTT is a light source treatment of a single electromagnetic wavelength that stimulates collagen production, DNA synthesis and improve the function of damaged neurons by multiple mechanism [2]. There are many types of low level laser therapy such as semiconductor diode laser, In-Ga laser, dye laser and He-Ne laser [3,4,5]. Red diode laser is a semiconductor diode laser to have a multiple wavelength from visible red laser 632nm - invisible infrared laser 1290nm is used in soft tissue surgery that characterized by coherent, monochromatic and climate are used in tattoo removal kidney stone treatment, hair removal and eye surgery [6,7]. Red laser also used in the treatment of hard tissue such as tendons and cartilage was investigating the effect of low level red lasers (904nm-60mw) are administered 30 minutes after injury of tendon [8]. Whereas red laser therapy was used in (890nm-80mw) reveal to influence of laser therapy to control the 3rd degree of burn [9]. A Green semiconductor diode laser is a one laser used in many purposes, recently was used in medical surgery, there wavelength between (450nm -632nm) with different energy (1-1000 watt) [10]. Green laser irradiated on platelet activation and glycoprotein [11], other researchers were used double beam green laser in renal surgery in some cases [12], while oncologists were used double beam green laser (532nm) to treat skin cancer and cosmetic medicine [13].

Corneal surgery is delicate surgery that deals with a sensitive tissue and sensitive function, corneal tissue composed of collagen fiberlis of stroma 81% water, is about 0.6-0.8 nm thick and consist of four layers; epithelium outer layer, the conjunctival epithelium (basal cell layer), mucous layer and cuboidal epithelium layer, its avascular organ, its function as a window and as a part of a strong refraction medium [14]. Cornea injury and ulcer are the most common diseases in animals specially pet animals [15]. There are two types of corneal treatment; conventional treatment and physical therapy such as laser therapy, there are many types of laser whose used are neodymin yttrium aluminum garnet Ne-Ya laser, excimer laser and erbium laser [16]. Red laser is most common used in corneal surgery and have many types depending on wavelength (620nm-950nm) [17], whereas the green laser (532nm)

either argon laser or diode laser that used in ophthalmic surgery, which were used in corneal bleeding, corneal wound and in conjunction by indirect ophthalmoscope for photocoagulation [16]. All researchers were summarized the mechanism of action of laser that is depending on three factors, animal corneas, laser wavelength and laser intensity the mechanism includes the thermal effect on biological interactions between cornea and laser beam, the energy of laser converted to heat source in the area and has an effect on corneal blood vessels and corneal stroma cause increase corneal circulation and the corneal bubble [18]. Mechanical effect can result from either the creation of plasma is the intensity of luminous flux over a small area ionized atoms, vaporization is a thermal effect on the area without diffusion cause explosive vaporization, cavitation phenomenon is mean bubble formation or due to shock wave that mean propagation of ions cause pressure destruction effect [19].

Materials and Methods

1-Rabbits were used in the present study, their age more than 6 months, live in similar condition at $25\text{C}\pm 2$, their food was green grass and dried bread, light period was 10-12hr.

2-Tools and anesthesia were used surgical blade and scalpel, ophthalmoscope, red diode laser, green diode laser, balanced anesthesia of ketamine and xylazine as well as gentamicine ointment and injection
RL, red diode laser. GL, green diode laser

4-Surgical procedure and laser therapy

All animals were incised 1cm (longitudinally, 2-3 mm deep) under iris in cornea without bleeding [20], under general anesthesia by ketamine 12mg/kg. bw + xylazine 3mg/kg.bw, see in figure (3) [21]. According to experimental design the 1st group was treated by gentamicine ointment and methadon-N drop see figure (4), the 2nd group animals was treated by red diode laser (635nm, 10mw), daily for 10 days, by range 5 minute exposure directly on the wound, the 3rd group animals were treated by green diode laser (532nm, 10mw), daily for 10 days, by range 5 minute exposure directly on the wound see figure (5).

5- Gross images and histopathology images

Daily images were taken after the treatment period, histopathology were achieved after eye extirpation [20], to prepare histopathological slides [22].

Results

Figure (1) Red diode laser and green diode laser



Figure (2a) Ophthalmoscope



Figure(2b) ophthalmoscope



3-Experimental design

Animals (18 rabbits) were divided randomly into three groups, 1st group was a control group, 2nd group red laser group and 3rd group were a green laser group

Therapy Period	Total animals		
	Control Group	Red laser Group	Green laser Group
0 days	Conventional therapy by medicine	Exposure to RL for 5 min/day/ daily	Exposure to GL for 5 min/day/ daily
5 days			
10 days			

Figure (3) surgical procedure of corneal incision



Figure (4) conventional treatment

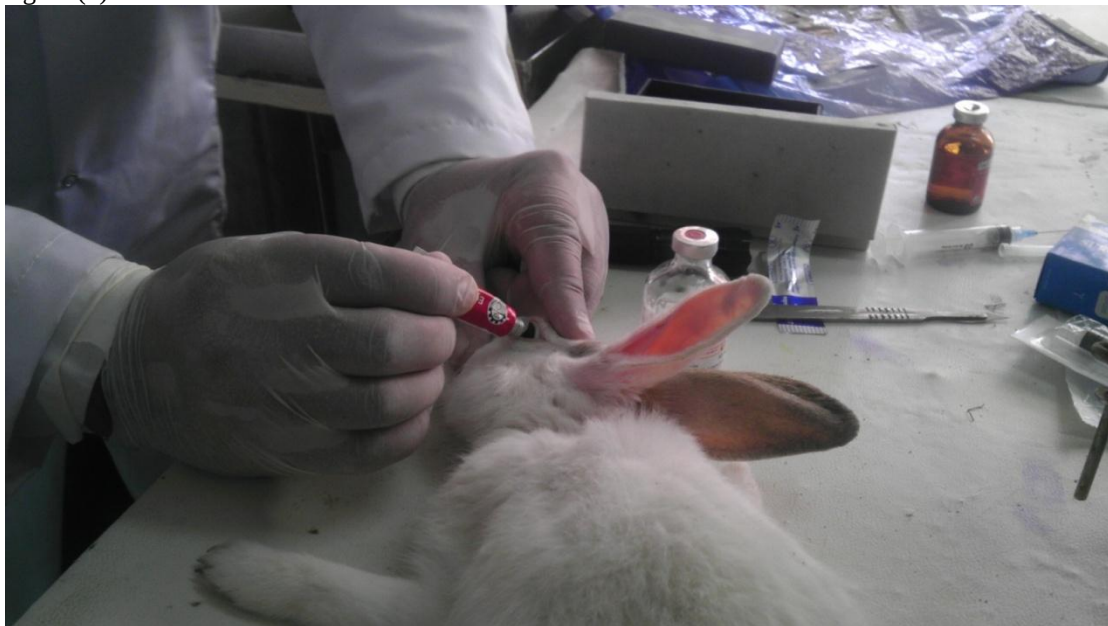


Figure (5) low level laser treatment



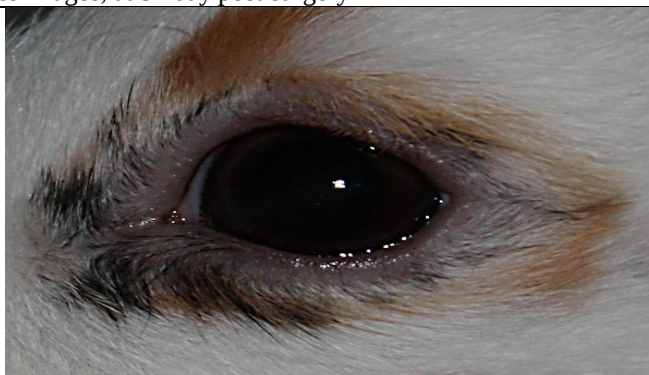
1-Follow the case clinically: there were no version deviation or corneal cataract due to laser therapy because the wound is far away from the iris and low density of laser power

2-Gross images were taken on the 1st day, 5th day and 10th days

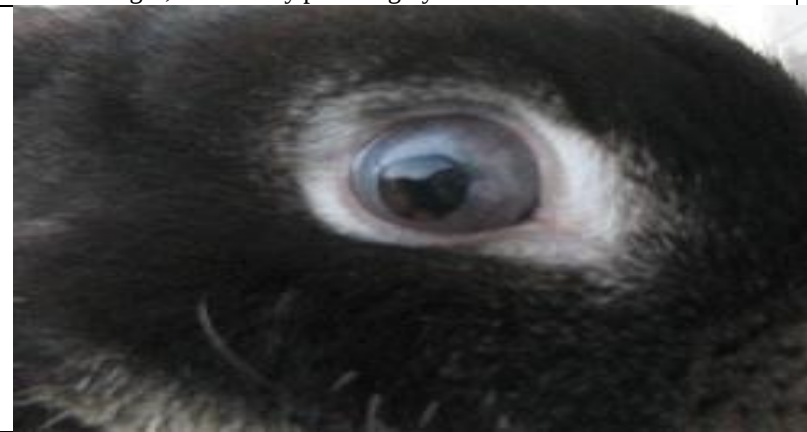
Gross images, at 5th day post surgery

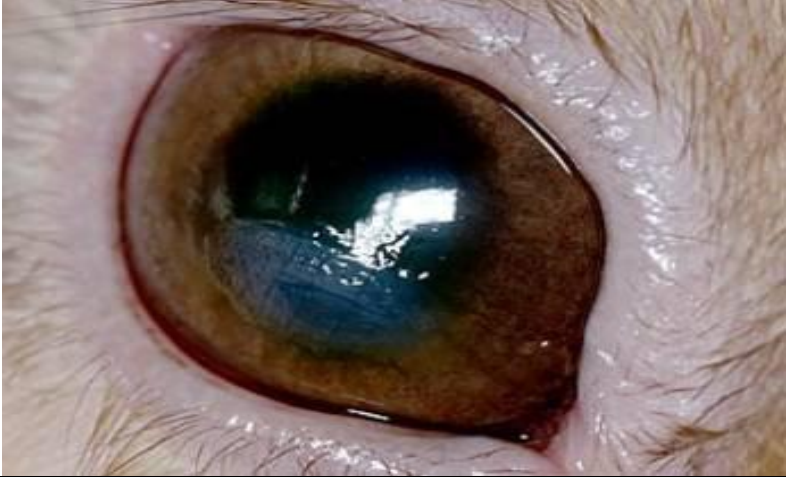
2a-Control group

The cornea is clear with lacrimation, wound line sees clearly beside iris, semi- healing.



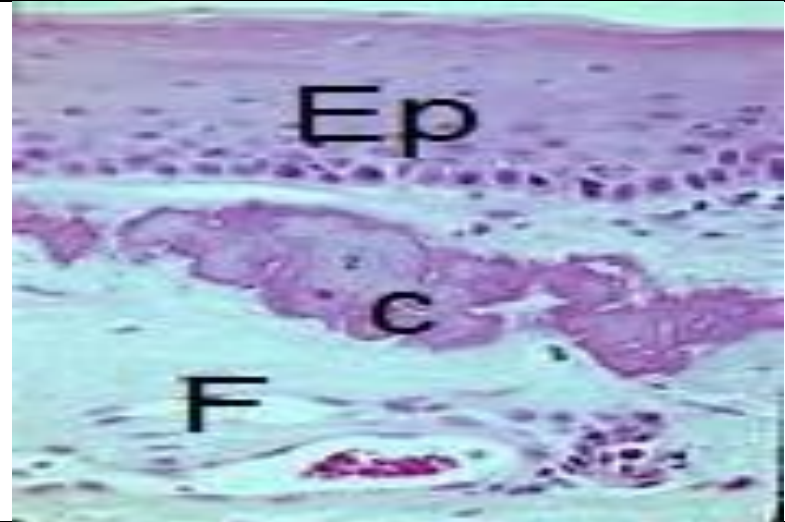
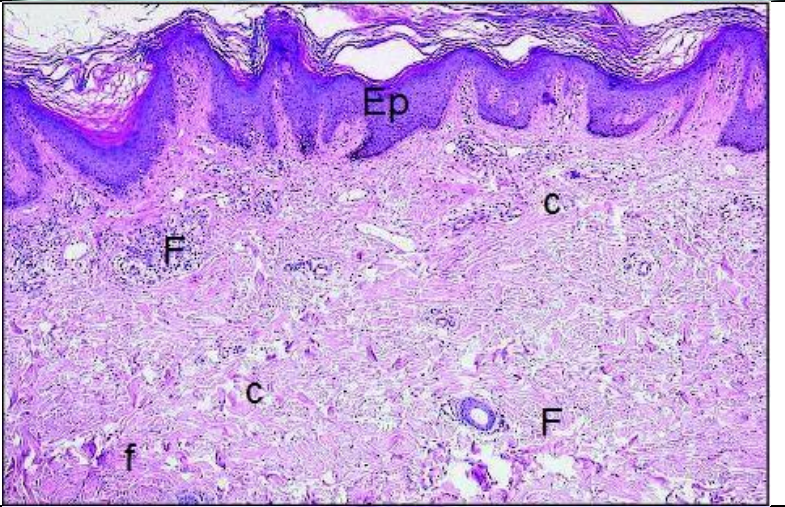
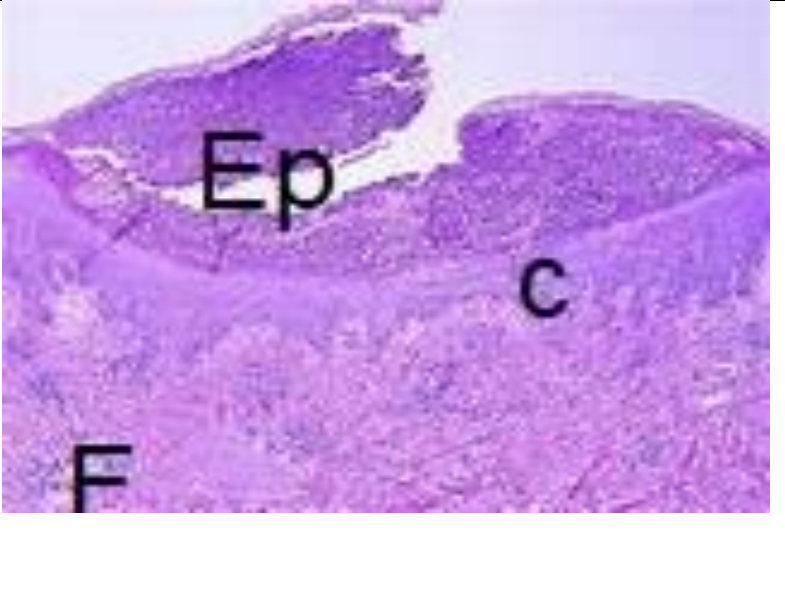
2b-Red laser group The cornea is inflamed and dried with disappearing wound line	
2c-Green laser group, normal cornea with semi healthy, wound line no complete healing an inside the iris	

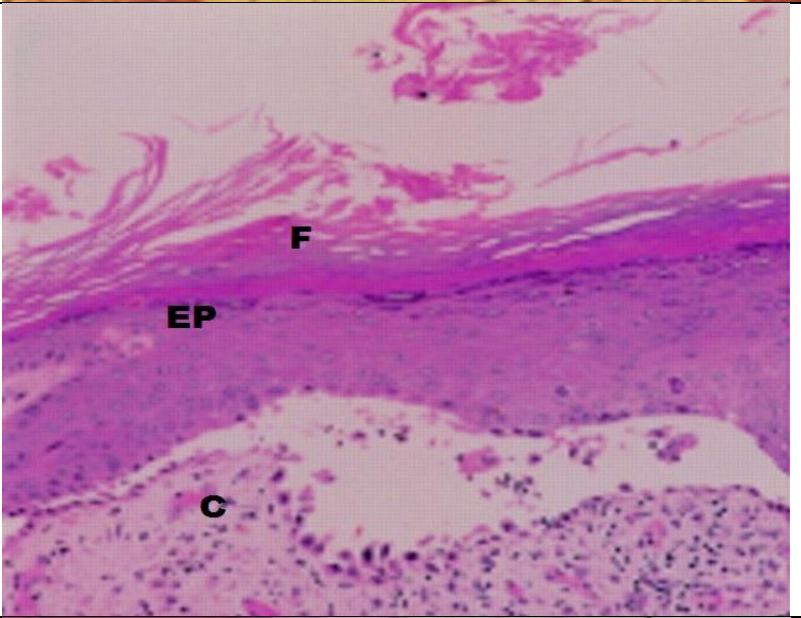
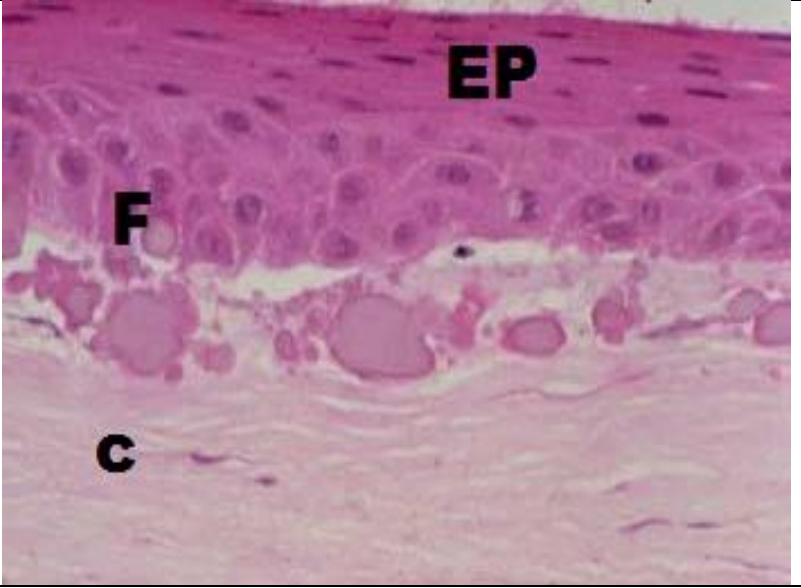
Gross images, at 10 th day post surgery	
2d-Control group Good healing with edema and wound line is appearing an inside the iris	

2e-Red laser group Cataract clearly appear and inflamed of the cornea, clearly lacramation	
2f-Green laser group, cornea is a good healing, healthy eye, wound line heal completely.	

3-Histopathological images were taken at 5th days, 10th days after surgery and treatment

Histopathological images, at 5th day post surgery

3a-Control group, histopathological section, was showed increase epithelial cells (Ep) with normal collagen (c), and fibrils is founded (f). X100 by E&H stain	
3b-Red laser group, histopathological section, was shown decrease epithelial cells (Ep) with copious collagen (c), and fibrils is founded (f). X10 by E&H stain	
3c-Green laser group, histopathological section, was showed normal epithelial cells (Ep) with normal collagen (c), and fibrils is founded (f). X100 by E&H stain	
Histopathological images, at 10 st day post surgery	

Control group, histopathological section, was showed increase epithelial cells (Ep) with normal collagen (c), and fibrils is founded (f). X100 by E&H stain	 A histopathological section of the control group stained with E&H. The image shows a thick layer of epithelial cells (labeled EP) at the top. Below the epithelium, there are collagen fibers (labeled c) and fibrils (labeled f). The overall structure appears normal for the control group.
Red laser group, histopathological section, was shown decrease epithelial cells (Ep) with copious collagen (c), and fibrils (f) is founded as well as rabush extra-structure of tissue . X10 by E&H stain	 A histopathological section of the red laser group stained with E&H. The image shows a thinner layer of epithelial cells (labeled EP) compared to the control. Below the epithelium, there is a large amount of collagen (labeled c) and fibrils (labeled f). A distinct rabush extra-structure of tissue is visible in the lower part of the section.
Green laser group, histopathological section, was showed normal epithelial cells (Ep) with normal collagen (c), and fibrils is founded (f). X100 by E&H stain	 A histopathological section of the green laser group stained with E&H. The image shows a layer of epithelial cells (labeled EP) that appears normal. Below the epithelium, there are collagen fibers (labeled c) and fibrils (labeled f). The overall structure is similar to the control group.

Discussion

The clinical signs of eyes after corneal surgery were healthy and don't showed complication, whereas the inflammation gradually develop in the red laser group, therefore the red laser had bio-activity and bio-interaction more than green laser and medicinal treatment, these bio-activity was reaching into to corneal cataract at 10 days after red laser treatment, which account for the main reason of red laser bio-activity was regarded to wavelength not to intensity of red laser energy, similar to Wang *et.al*, Brinkmann *et. al* and Envmka *et.al* [23,24,25] because of equal intensity between red laser and green laser or may be an agonisity between wavelength and intensity of laser, this opinion approaching to Nagy and Talmo *et.al* [26,27]. To knowlge the processing of formation of cornea cataract in red laser group therapy, should be know the histopathological changes among groups and changes in same group with defferint time, in laser group at 5 days post treatment of red laser reveal to highly precess of collagen formation due to the effect wavelength of red laser (632nm), means the wavelength of red laser more penetration than green laser to cornea tissue and more bio-interaction as well as thermo-effect of red laser (10mw) to cornea, this evident to cornea cataract formation in red laser group through 10 dayes of therapy compared with green laser and control groups, the effect of long wavelength more than short wavelength agreed with Invarsen *et.al* and Oliveira *et.al* [28,29]. Green laser (532 nm) also causes increase collagen moderately more than normal tissue in the control group except epithelial tissue showed increase more than control group histopathologically [3,30]. The wavelength enhances of the corneal blood vessel and thermal effect had played role to increase ocular circulation to create the morphological and histopathological changes in red laser and green laser groups [31].

Conclusion

Laser has therapeutical effect to cornea according to laser type and in the present study showed green laser is better than red laser.

References

- 1- Bjordal J M, Basfor J R, and Anderson T E, 2003. A systemic review of low level laser therapy with location-specific doses for pain from chronic joint disorders. *Australian Journal of Physiotherapy*, 49:107-109.
- 2- Fagerholm P, Hamberg-Mystrim H, Tengroth B, 1994. Wound healing and myopic regression following photorefrastine Keratectomy. *Journal of Refract Surgery*, 72 :229-234
- 3- Reddy G K, Bittel-Lisa S, Enwemeka C S, 1998. Laser photostimulation of collagen production in healing rabbit Achilles tendon. *Laser in Surgery and Medicine*, 22(5):281-287.
- 4- Salette A C, Barbosa G, Gaspar P, Koek P U, Parizoh N A, Benze B G, and Foschani D, 2005. Effect of In-Ga-Al-P diode laser irradiation on the angiogenesis in partial ruptures of Achilles tendon in rat. *Photomedical laser surgery*, 23(5): 470-475
- 5- Delori F C, Webb R H, and Sliney D, 2007. Maximum permissible exposure for ocular safety with emphasis on ophthalmic device. *Journal of optic society*, 24(5): 1250-1265.
- 6- Eriksen H A, Pajala A, and Leppilahti J, 2014. Histomorphological evaluation of level laser therapy in dog Achilles tendon injuries. *European journal of experimental biology*, 4 (2): 143-147.
- 7- Olivier C. A, Holden B.A., Epstein D. , Tengroth and Forrer GM, 2007. Factors affecting epithelial hyperplasia after photorefrene .*Journal of cataract ophthalmology surgery*, 23: 1042-1050.
- 8- Joensen J, Gjerdet N R, Hummeiund S, Iversen V, Martins R A, and Bjordal M, 2012. An experimental of low-level laser therapy in rat Achilles tendon injury. *Lasers in medical science*, 27(1): 103-110.
- 9- Bayat M, Ezzati A, Tahri S, and Mohesenifar Z, 2009. Low level laser therapy with pulsed infrared laser accelerates third degree burn healing process in rats. *Journal of rehabilitation research, development*, 46 (4): 543-547.
- 10- Huang Y M, Zhou F, and Zhai G, 2003. Effects of red laser and green laser irradiation on optical properties of an azo-containing bent-core liquid crystal. *Engineering materials*, 5: 428-429.
- 11- Gresner P, Watala C, and Sikuroval L, 2005. The effect green laser light irradiation on whole blood platelets. *Journal of photochemistry and photobiology B:biology*, 77(1): 43-50.
- 12- Genina E A, Bashkator A N, Simoneko G V, Odoevskay O D, Tuchin V V, and Altshuler G B, 2004. Low intensity indocyanine-green laser phototherapy of acne vulgaris: pilot study. *Journal of biomedical optic*, 9(4): 828-834.
- 13- Brinkmann R, Sliney H D, Liue W, and Vincelette R L, 2012. Real-time temperature determination during retinal photocoagulation on patients. *Journal of biomedical optics*, 17(6): 121-132.
- 14- Stact F C, Wyman M, and Boeve M, 2006. *Ophthalmology for veterinary practitioner*. 2nd edu., Willy-Neumnn pub., 129-140.

- 15- **Abergel RP, Lyons RF, Castel J C, Dwyer R M, and Hiltto J, 1987.** Biostimulation of wound healing by lasers, experimental approach in animal models and fibroblast cultures. *Journal of dermatological and surgical oncology*, 13:127-133.
- 16- **Matthews J, Walsh J, Fischer W, and Junhasz A, 1999.** Surgical application of ultra-short pulsed laser technology. *Applied optics*, 64:181-189.
- 17- **Romo-Cardens G, Perez F G, Mina A, Lopez E and Aguilar G, 2001.** Study of NS and f's pulse-induce effects in biological tissue models and the corneal tissue. *Applied physics*, 64: 902-910.
- 18- **Narasimhan A, John K, and Gopal L, 2010.** Transient of heat transfer in human eye undergoing laser surgery. *International journal of heat mass transfer*, 53 (3): 482-490.
- 19- **Kathy B, and Ffrangcon E T, 2002.** Veterinary ocular emergencies, 1st adult, Gillingham, Kent, UK, Elsevier Science Ltd, 47-56.
- 20- **Gelatt K N, and Janica P G, 2001.** Small animal ophthalmic surgery, Oxford & Philadelphia, Butterworth and Heinemann, 60-77.
- 21- **Fish RE, Biown MJ, Danneman PJ and Karas AZ., . 2008.** Anesthesia and analgesia in laboratory animal ^{2nd}, American collage of laboratory series, 420-421.
- 22- **Luna L G, 1968.** Manual of Histological Staining Methods Blackstone Division, McGraw-Hill, 44-57.
- 23- **Wang J, Sramek C, Paulus Y M Lavinsky F, Schuele G, Anderson D, Dewey D, and Palanker D, 2012.** Retinal safety of near-infrared in cataract surgery. *Journal of biomedical optics*, 17(9): 095001.1-095001.12.
- 24- **Brinkmann R, Palanker D V, Kadam R S, and Gown G, 2000.** Origin of retinal pigment epithelium cell damage by pulsed laser irradiance in the nanosecond to microsecond time region. *Laser in surgery and medicine*, 27(5): 451-464.
- 25- **Envemka C S, 2001.** Attenuation and penetration of visible 632nm and invisible infrared 904nm light in soft tissue. *Official journal or world association for laser therapy*, 13: 95-101.
- 26- **Nagy Z Z, 2014.** New technology update: femtosecond laser in cataract surgery. *Open access to scientific and medical research*, 8: 1157-1167.
- 27- **Talamo J H, Gooding P, Angeley D, and Kotcyn J, 2013.** Optical patient interface in femtosecond laser assists cataract corneal applanation versus liquid immersion. *Journal cataract refraction surgery*, 39: 501-510.
- 28- **Ivarsen A, Laurberg T, and Pedersen-Moller T, 2003.** Characterization of corneal fibrotic wound repair at LASIK flap margin. *British journal of ophthalmology*, 78: 1272-1278.
- 29- **Oliveira F S, Pinfidi CE, Parizoto N A, Liebano R E, Bossini P S, 2009.** Effect of level laser therapy (830 nm) with different therapy regimes on the process of tissue repair in partial lesion calcaneous tendon. *Wiley Inter Science. Laser in surgery and medicine*, 41: 271-276.
- 30- **Nicolau R A, Martinez M S, Rigau J, and Tomas J, 2004.** Effect of low power 655nm diode laser irradiation on neuromuscular junction of the mouse diaphragm. *Laser in surgery and medicine*, 34:277-284.
- 31- **Nakamura K, Kurosaka D, Yoshino M, Oshima T, and Kurosaka H, 2002.** Injured corneal epithelial cells promote myo-differentiation of corneal fibroblasts. *Association for research in vision and ophthalmology*, 43(8) : 2603-2608.