

Efficiency of Therapeutic Laser for Treatment of Chronic Non Healing Wounds in Cats

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The study on wound healing has focused on similarities rather than on differences between species. Recent studies by authors have revealed significant differences between the cat and the dog with respect to cutaneous healing. These differences made us to reconsider wound care dogma of the feline species. Research has shown significant quantitative and qualitative differences in wound healing between cats and dogs and wound heals in the former more slowly. Since, Laser Therapy enhances the phases of wound healing, the present study was undertaken to access the efficiency of laser for chronic non healing in cats. Clinical cases of cats that were presented to the small animal surgical outpatient unit of madras veterinary college for conservative treatment. The wounds that underwent therapy were subjectively evaluated, histopathology, pain score assessment and color flow doppler ultrasonography were performed. Based on the findings from Subjective Evaluation, Wound Planimetry, Histopathology and Pain Score Assessment it was inferred that wounds of Group I animals treated with Therapeutic Laser healed faster with earlier epithelialisation, granulation, wound healing, increased collagen density and significant reduction in pain when compared to Conservative Treatment group.

Keywords: Feline - Chronic Wounds - Therapeutic Laser - Wound healing.

Introduction

The study on wound healing has focused on similarities rather than on differences between species and there is a general assumption that wound healing is more or less homogenous across species lines, at least among mammals. The aim of wound healing was to promote rapid wound closure and to prevent excess scar formation. Laser Therapy modulates wound healing and is stimulatory in origin. In addition, Laser Therapy has a positive effect through acceleration of inflammation, increased collagen synthesis and tensile strength. Laser Therapy also appears to be inhibitory for pain receptors and sensory nerves thereby increasing the threshold for chronic wound pain. The beneficial effects of Laser Therapy is more focused to the proliferative phase of wound healing since it increases fibroblast activity and is rather inhibitory in the early inflammatory phases of the wounds. Research has shown significant quantitative and qualitative differences in wound healing between cats and dogs and wound heals in the former more slowly. Since the Laser Therapy enhances the phases of wound healing, the present study was undertaken to access the efficiency of laser for chronic non healing wounds through Subjective valuation, Color Flow Doppler, Wound planimetry and histopathology.

Materials and Methods

The study was conducted on twelve cats brought to the Small Animal Outpatient Unit at Madras Veterinary College with a history of chronic non-healing wounds. These cases were divided into 2 groups with 6 animals in each group. The cases were selected based on the following inclusive and exclusive criteria viz., Traumatic wounds, Lacerated wounds, Radiation wounds, Photosensitisation injuries and Burn injury and exclusive criteria viz., Parasitic wounds, Systemic septic wounds and Self mutilated wounds. Group I Cases were treated with therapeutic laser, Group II were treated with conservative treatment. The cats were sedated with Xylazine (Xylaxin, Indian Immunologicals) @1mg/kg body weight and Butorphanol (Butodol-2, Neon) @ 0.2mg/kg body weight. Highly uncooperative and aggressive cats were additionally administered Ketamine Hydrochloride @10 mg/kg (Aneket, Neon) and then intubated. Brachycephalic breeds such as Persian cats were sedated with Butorphanol @ 0.2 mg/kg, followed by Midazolam (Mezolam/ Neon) @ 0.2mg/kg and Ketamine @10 mg/kg body weight. The wound was lavaged and debrided, for the Conservative or Laser Therapy was performed.

All animals of Group I with chronic non healing wounds were subjected to Laser Therapy on Days 0, 3, 7 and 14 or until granulation was evident. The device was placed at 6ft distance from the electrical outlet

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(100- 240V). The hand piece was held perpendicular to the wound surface of the patient and the laser emission commences once the foot/finger switch is activated. The hand piece is moved slowly over the entire wound area in a zig zag manner for the duration of the treatment. Therapeutic Laser treatment at 4.8 Watts for 1.5 minutes was given, at a distance of 1-2 inches from the patient (Plate no.1 &2)

The animals of Group II were subjected to Conservative Wound Management on the day of presentation and on alternate days (48 hours) or till the wound healing was evident. The Conservative Therapy was done by lavaging, debridement and appropriate wound dressings.

In Group I and II, the wound healing was evaluated based on the physical observations such as colour, odour and presence of exudates on day 0, 3, 7 and 14 days of treatment. In all the three groups, the colour coding namely red, yellow and black as reported by Wilson (2012) were followed and observations. Chronic non-healing open wounds were observed for the presence of any mal, offensive, repulsive, acrid or putrid odour. Open wounds were observed for the type of exudates, serous as clear without blood, pus or debris, serosanguineous as bloody and bright red fluid and purulent discharge as thick, cloudy and yellow (Jones *et al.*, 2003).

Wound planimetry was used to determine the area of epithelization by subtracting the area of contraction and area of granulation of the wound. The planimetry values have also been used to calculate the cumulative percentage of wound contraction, wound epithelization and wound healing. Color flow Doppler ultrasonography was used to perform a subjective assessment of the wound bed's vascularity on the 3rd, 7th and 14th day (Plate No.3). A 3.0 mm punch biopsy instrument was used to take tissue sample from the wound center and wound bed margin on 3rd, 7th and 14th day with or without sedation.

Punch biopsy is considered as a module for histological analysis to evaluate collagen density using mason's trichrome stain (Plate No.4). Using Abramov's histological scoring system, the histological parameters were graded on Days 3, 7 and 14 for both groups of animals. The Glasgow CMPS-SF pain scoring system developed by Reid *et al.* (2007), was used to assess the pain scores according to six behavioural

signs, viz. vocalisation, attention to wound, mobility, response to touch, demeanour and posture/activity. The scores for each response varied from 0 to ≥ 3 (i.e. least pain to most pain). The pain scores of each animal were calculated as a sum of the individual scores of each behavioural category (e.g., pain score of animal A = i+ii+iii+iv+v+vi). This assessment was performed on Days 3, 7 and 14 for all animals of both Groups I and II. Table No. 9.

Results and Discussion

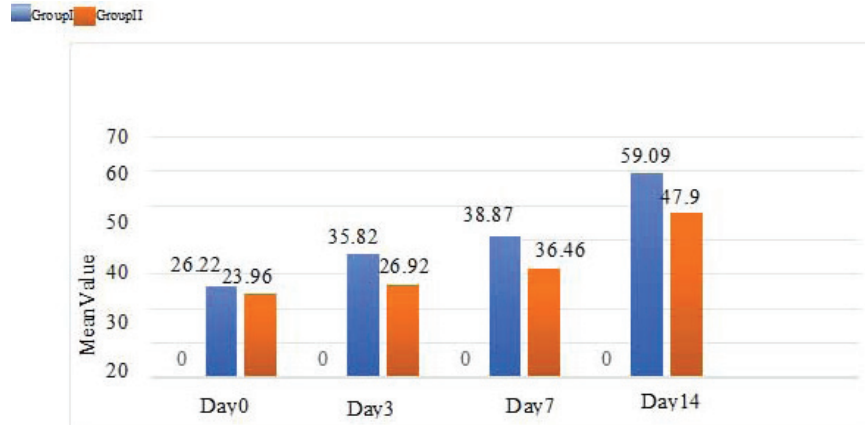
The wounds of the groups were subjectively evaluated for the colour, odour and exudate on days 0, 3rd, 7th and 14th after initial presentation. The animals of Group I and II were evaluated subjectively on the basis of the wound colour, odour and exudate on Days 0, 3, 7 and 14 or until the day of complete granulation. On the day of initial presentation, most of the wounds were yellow or black, with offensive odour and copious exudate on the wound bed. These observations concurred with Amalsadvala and Swaim (2006), who stated that chronic wounds in felines were usually pale, unhealthy and weak with friable granulation tissue.

On Day 14, all animals of Group I had red-coloured wound beds and only five animals had red coloured wound bed in Group II, which indicated all the animals of Group I had healthy granulation tissue by Day 14. This is in accordance with Pryor and Millis (2015) who mentioned Laser Therapy showed effective healing of chronic wounds in two to three weeks duration (Plate No.5,6,7,8). The observations also concurred with the study of Weller and Sussman (2006) who observed that red-coloured wounds were in the terminal stages of healing process. On Days 3, 7 and 14 of wound healing, there was an increased percentage of epithelialisation in Group I than Group II (Plate No.9,10,11,12). This could be due to the property of Therapeutic Laser to accelerate epithelialisation as reported by Hochman (2018) and Perego *et al.* (2016).

Significant difference was noticed in the percentage of epithelialisation within the Groups I and II. These observations concurred with the study of Pope (1993) who stated that the number of inflammatory cells dropped during the transition to proliferative phase and the epithelialisation commenced, as the granulation tissue served as a smooth surface for the epithelial cells to migrate from the wound borders (Table No.1 and Figure No.1).

Table No. 1: Percentage of Wound Epithelialisation of Group I and II (MEAN±S.E)

Parameters	Day 0	Day 3	Day 7	Day 14	F-Value
Group1	26.22±0.63 ^a	35.82±1.14 ^b	38.87±2.51 ^b	59.09±1.78 ^c	67.87**
Group-11	23.96±1.27 ^a	26.92±3.68 ^{ab}	36.46±1.21 ^b	47.91±2.79 ^b	34.68**
t-Value	1.58 ^{NS}	4.70**	3.46**	3.37**	

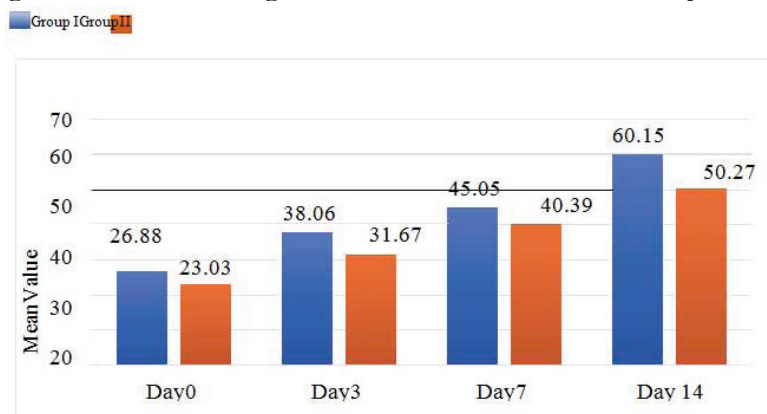
Figure No. 1: Percentage of Wound Epithelialisation in Group I and II

On Days 3, 7 and 14 of wound healing, there was increased percentage of wound contraction in Group I than Group II (Table No.2 and Figure No.2). This could be due to the property of Therapeutic Laser to increase fibroblast production, proliferation and collagen deposition as suggested by Suárez Redondo(2015) and

Perego *et al.*(2016). Significant difference was also noticed in the percentage of contraction within the Groups I and II due to the formation of fibroblasts, which peaked around the 7th day after injury and is responsible for commencement to angiogenesis, epithelialization and wound contraction, as stated by Neagos *et al.* (2006) in their study.

Table No. 2: Percentage of Wound Contraction of Group I and II (MEAN±S.E)

Parameters	Day 0	Day 3	Day 7	Day 14	F-Value
Group1	26.88±0.84 ^a	38.06±0.92 ^b	45.05±2.65 ^c	60.15±1.03 ^d	80.22**
Group-11	23.03±1.32 ^a	31.67±2.00 ^b	40.39±0.99 ^c	50.27±2.42 ^c	35.57**
t-Value	2.43*	2.92**	3.23**	3.74**	

Figure No. 2: Percentage of Wound Contraction in Group I and II

On Days 3, 7 and 14, there was increased percentage of wound healing in Group I compared to Group II (Table No.3 and Figure No.3). This could be due to property of Therapeutic Laser to enhance wound healing by increasing neovascularisation, fibroblast proliferation, keratinocyte proliferation, early epithelialisation, growth factors and greater tensile

strength of the wound as stated by Pryor and Millis (2015) in their study. Significant difference was also noticed in the percentage of wound healing within the Groups I and II. This concurred with the study of Sharpe and Martin (2013) who stated that the combination of epithelial migration and contraction of the wound bed achieved wound healing.

Table No.3: Percentage of Wound Healing of Group I and II (MEAN±S.E)

Parameters	Day 0	Day 3	Day 7	Day 14	F-Value
Group I	26.27±0.77 ^a	37.93±1.09 ^b	43.81±1.98 ^c	55.49±2.20 ^d	55.80 ^{**}
Group-II	22.72±1.11 ^a	31.60±1.51 ^b	40.38±1.06 ^c	48.02±2.46 ^d	44.55 ^{**}
t-Value	2.62 [*]	3.38 ^{**}	3.51 [*]	2.25 [*]	

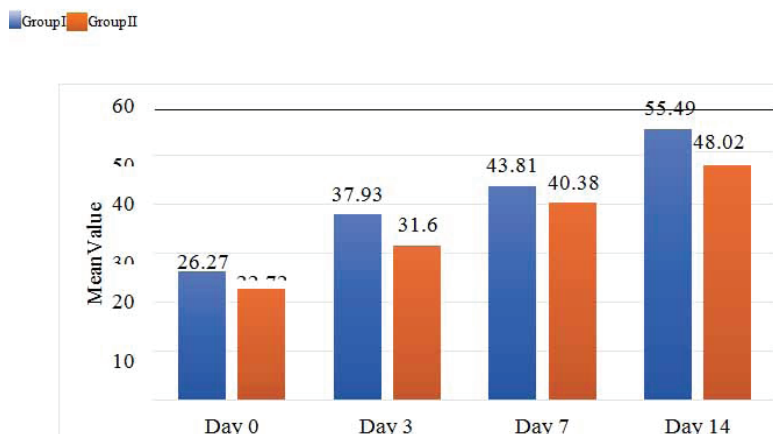
Mean bearing similar superscript do not refer significantly

NS = Nonsignificant ($P > 0.05$)

* = Significant ($P < 0.05$)

** = Highly significant ($P < 0.01$)

Figure No. 3: Percentage of Wound Healing in Group I and II



The statistical inference for acute inflammation, chronic inflammation, granulation tissue density, granulation tissue maturation, re-epithelialisation and neovascularization were found to be non-significant between the groups. However, there was significant increase in collagen density of Group I compared to Group II (Plate No.13,14,15,16). The increased collagen density in Group I can be attributed to the property of Therapeutic Laser which increases collagen deposition and organization as stated by Lopez and Brundage (2019), Pryor and Millis (2015), Hochman (2018), Dycus (2014), Fesseha (2020).

Although there was no statistically significant difference in the histological parameters between the groups, microscopic evaluation revealed increased granulation tissue density, re-epithelialisation and

neovascularisation in Group I compared to Group II which may be due to the reason that photobio modulation enhances angiogenesis, collagen deposition and re-epithelialisation.

Colour Flow Doppler Ultrasonography was done on Days 3, 7 and 14. The non-parametric study was undertaken to determine the presence of healthy vascular supply to the wound bed which would reflect on the status of the wound. This was in accordance to the work of Gokulakrishnan *et al.* (2018), who used colour flow Doppler ultrasound to subjectively assess the vascularity of skin flap in their study. Non-parametric *Chi* square test was used to statistically analyse the ultrasound grading between Group I and II. The result from the statistical analysis revealed no significant difference between the two groups on Days 3, 7 and 14 of evaluation. Even

though there was no statistical significance between Group I and II, on Day 7, five animals of Group I showed high degree of confidence for detection of the blood vessels while Group II had only two animals with high degree of confidence for detection. This might be attributed to the property of Therapeutic Laser to increase the production of nitric oxide which caused vasodilation and which in turn triggered angiogenesis as explained by Hochman (2018) in his study.

Interpretation from the statistical analysis revealed significant difference in the animals of Group I in comparison with Group II animals (Table No.4). Thus, animals which underwent Laser Therapy had significant pain relief and this might be due to the property of Therapeutic Laser to enhance serotonin and beta-endorphin secretion which in turn had a positive effect on pain relief as stated by Wardlaw *et al.* (2019) and Cotler *et al.* (2015) in their study.

TableNo. 4: Non parametric statistical analysis of pain assessment of Group I and II (Kruskal-Wallis Test and Mann-Whitney Test)

Groups Testvalue (Between Groups)	Days			Test Value (Withingroups)	
	3	7	14	K-W Value	P-Value
I	1.50±0.50	0.67±0.49	0.00±0.00	8.57*	0.012
II	4.33±1.38	2.50±0.80	0.17±0.16	8.39*	0.015
M-W Value	22.0^{NS}	28.5*	21.0^{NS}		
P-Value	0.240	0.043	0.699		

NS =NonSignificant(P>0.05)

* =Significant(P<0.05)

** =Highly significant(P<0.01)

The profound pain relief in animals that underwent Laser Therapy may be due to the ability of Therapeutic Laser to relieve both acute and chronic pain by modulation of peripheral nerve conduction velocities as explained in the study by Fesseha (2020).

It also concurred with the work of Lopez and Brundage (2019) who suggested that biostimulation by Laser Therapy promoted regeneration of tissues as it reduced inflammation which in turn reduced the subsequent pain.



Plate no. 1
Therapeutic laser instrument



Plate no. 2
Laser therapy application at 4.8 watts for 1.5 minutes



Plate no. 3
Colour Flow Doppler Ultrasonography procedure



Plate no. 4
Punch Biopsy Procedure



Plate no. 5
Group I Contaminated wound with blackish necrotic tissue on the wound bed – Day 0



Plate no. 6
Group I Granulation tissue and partial wound contraction – Day 14



Plate no. 7
Group I Blackish necrotic tissue and purulent discharge on the wound bed –Day 0



Plate no. 8
Group I Evidence of wound contraction– Day 14

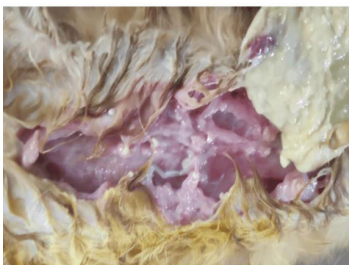


Plate no. 9
Group II Severely contaminated yellow colored wound bed with copious purulent discharge – Day 0



Plate no. 10
Group II Wound contraction and granulation tissue – Day 14



Plate no. 11
Group II Chronic wound after debridement– Day 0



Plate no. 12
Group II Evidence of Wound contraction– Day 14

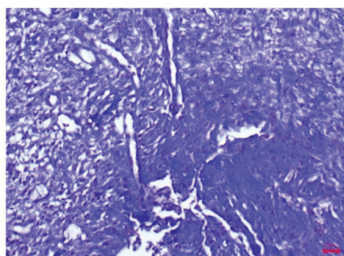


Plate no. 13
Group I- Fibroblasts arranged loosely, low collagen tissue density- masons trichrome staining day 0

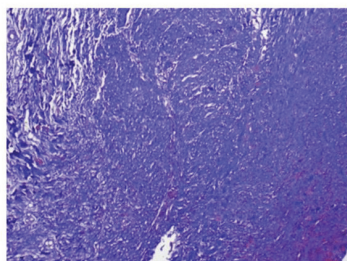


Plate no. 15
Group II –Moderate collagen densitywith mild angiogenesis masons trichrome staining day 0

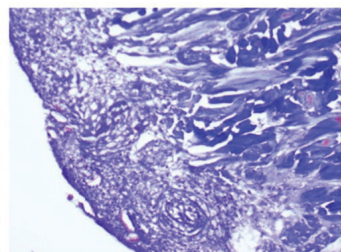


Plate no. 14
Group I - Fibroblasts thin and well arranged,increased collagen density masons trichrome staining day 14

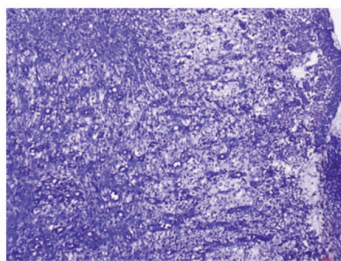


Plate no. 16
Group II - partial epithelialisation with moderate collagen activity masons trichrome staining day 14

Conclusion

Based on the findings from Subjective Evaluation, Wound Planimetry, Histopathology and Pain Score Assessment it was inferred that wounds of Group I animals treated with Therapeutic Laser healed faster with earlier epithelialisation, granulation, wound healing, increased collagen density and significant reduction in pain when compared to conservative treatment group.

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