



*Ministry of Higher Education and
Scientific Research
University of Al-Qadisiyah
College of Veterinary Medicine*



Comparison of PRP and LLL in the Treatment of Second Degree Burn in Sheep

A Research Project

*Submitted to the council of Department of the Surgery
and Obstetrics College of Veterinary Medicine/
University of Al-Qadisiyah in Partial Fulfillment of the
Requirements for the Degree of Bachelor in Veterinary
Medicine & Surgery*

By

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Dedication

- ♠ *To whom I had the honour to bear his name... my father.*
- ♠ *To the bright face of my life... my Mother.*
- ♠ *To the spirit of my uncle the martyr Aras Abdullah.*
- ♠ *To the candles whom illuminate my road... my brother and sisters.*
- ♠ *To the twin of my soul ... Roqaya Tariq Alhabeb.*

ZAHRAA

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ZAHRAA

Certificate of Instructor

We certify that *Zahraa Ali Gameel* has completed the fulfillment of her graduation project entitled *Comparison of PRP and LLL in the Treatment of Second Degree Burn in Sheep* for the year 2016/2017 under our construction.

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Dr. Muthanna Hadi Hussain

March 2017

Certificate of supervisor

I certify that *Zahraa Ali Gameel* has completed the fulfillment of her graduation project entitled *Comparison of PRP and LLL in the Treatment of Second Degree Burn in Sheep*

for the year 2016/2017 under my construction.

Lecturer

AHMED KADHIM MUNAHI

March 2017

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

وَقُلْ يَا رَبِّ زِدْنِي عِلْمًا

صَبْرًا وَتَوْفِيقًا

سورة طه، من الآية / ١١٤

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Summary

This experiment was conducted in order to compare the effect of PRP and LLL therapy on healing of second degree burn wound in sheep. Twelve adult rams were divided into three groups of equal numbers. A second degree burn was generated on the back of all animals the burned area in the first and second group were exposed and applied for continuous five days with autologous PRP and LLL ($6\text{J}/\text{cm}^2$) respectively. Fourteen days later, samples were collected from all animals for histopathological examinations. Histopathological evaluations on the second week showed the burn healing to be better in the PRP than LLL and both of them were best with respect to the control group.

In conclusion, application of PRP for continuous five days is effective than LLL in healing burn related skin wounds in sheep model.

1.Introduction

In veterinary medicine; burns can be resulted from a various household items including electrical, chemical as well as thermal equipment (1). Burn was still considered as one of the emergency cases which affecting both sexes and all ages leading to serious physical disabilities with a trend to morbidity & mortality (2). As well as regarding most painful to be treated and managed with high health care cost (3). Furthermore, burns were among the most devastated injuries (4).

Deep second degree burn involved the whole epidermis and most dermis and distinguished by the existence of non-elastic red or white layer on the upper part of the burn which did not blanch under pressure (5). A commune intermediation in the healing of burn was still on important challenge to prevent infection and reduce the scar tissue besides availability.

Discovering more effective agent with fewer complications for treatment of burn had evermore been a concern of scholars, different techniques were used to treat burn injury one of them is low level laser therapy which had been employed clinically since 1971 during the first successful cases reported by professor Ander Mester and colleague (6). The frequency of the laser light and the type of irradiating tissue were delimiting the depth which be penetrated by the light as well as the 99% of the low level laser was absorbing in the superficial layer of skin (7).

The effect of diode laser on burn headling had few studies demonstrating inconsistent results (8). Irradiation of the burn with

990 nm laser was effective for enhancing the healing of 3rd degree burn in diabetic rats (9). Several studies appeared the effects of pulsed diode laser on skin wound healing (10) .

Plateletrich plasma had a high concentration of thrombocytes and the alpha granules of platelets include platelet released growth factors than contain molecules as platelet derived growth factors, vascular endothelial growth factor, these stimulate proliferation of calls and differentiation leading to tissue formation (11). This preparation was an autologous product that concentrated a huge number of platelets in a little volume of plasma, platelet rich plasma act as a fibrin tissue adhesive with hemostatic and tissue sealing activities , and supplied a unique ability to enhance wound healing and promote osteogenesis (12).

For our knowledge, no study existed in the literature had compared the effects and distinguished the better effect of low level laser and PRP on histological healing rates of second degree skin burn in sheep.

2. Materials and Methods

Material	Origin
12 Awasi local sheep	teaching station of the college
laser diode System	Omega Laser System Ltd. the United Kingdom
Spiral Balance	China
Silk suture	Demophorius healthcare China
Alcohol solution 70%	L'Origine sal Lebanon
Bovidone Iodine	Ve imalyeri Turkey
Lidocaine Hcl	Ibn Hayyan Pharma Syria
Surgical gloves	SMG Canada
Examination gloves	SMG Canada
Sterile syringe	JIANGSU LTD. China
Medical Cotton	Gaziantep Turkey
Normal saline	Elnaser Pharmaceutical Co. Egypt
Formalin 10%	Al-Jubail Saudi Arabia
Urine cup	Himedia India
Surgical blade	Beijing China
Medical gauze	Gaziantep Turkey



❖ Animals

Permission was obtained from deanery of veterinary medicine college in University of AL-Qadisiyah as well as from the administration of the animal teaching station to achieve this experiment .

In a randomized clinical trial, twelve rams weighing (30 ± 5) Kg and aging (8 ± 2) months were kept in identified stalls. All animals were preserved under similar conditions and received same drugs and vaccines against endemic diseases, water was providing *ad libitum* and food presented with regular times, the experiment expended from January to February in 2017.

❖ Laser and PRP

Low level laser therapy with the following criteria:

660 nm, 50 mw, 10Sec, 6 J/cm^2 , 146 pulsing rate for second (Fig. A). Autologous platelet rich plasmas twenty milliliters of whole blood were aspirated from jugular vein of certain animals, centrifuged two times at (4000 rpm) for (10 minutes), the layer had the yellow color containing plasma, platelets and growth factors is collected gently , adding ten percent CaCl_2 after that preserving solution in an incubator at 37°C for a whole day to activate platelets, so that they release high concentration of growth factors and keeping under $(- 8^\circ \text{C})$ (12) .

❖ Burn formation

Local analgesia was achieved by using a single dose of injection field block of lidocaine HCL 5cc (Ibn Hayyan Pharma Syria) in the

back region of the animals after shaving and antiseptic applying with 10% with povidone iodine solution.

A controllable and adjustable temperature machine with probe (Ya Xun 702, China) was used with 155°C by applying the probe over the skin of the local anesthetized animal for ten seconds with no pressure (Fig. B and C), using this procedure a second degree partial thickness burn was formed.

After formation of burns, all animals were divided equally and randomly into three groups of four animals each.

Group one (P); covering affected area with PRP one time per a day for five continuous days.

Group two (L); applying of LLL on the burned area with a single dose per a day for five days.

Group three (C); the control in which the animals were left without any treatment.

❖ Histopathological examination

Samples were taken from the burned skin tissue at the second week from all groups for histopathological examination assessments.

Fig A: Application of low level laser over the burned area



Fig B: A controllable and adjustable

temperature machine

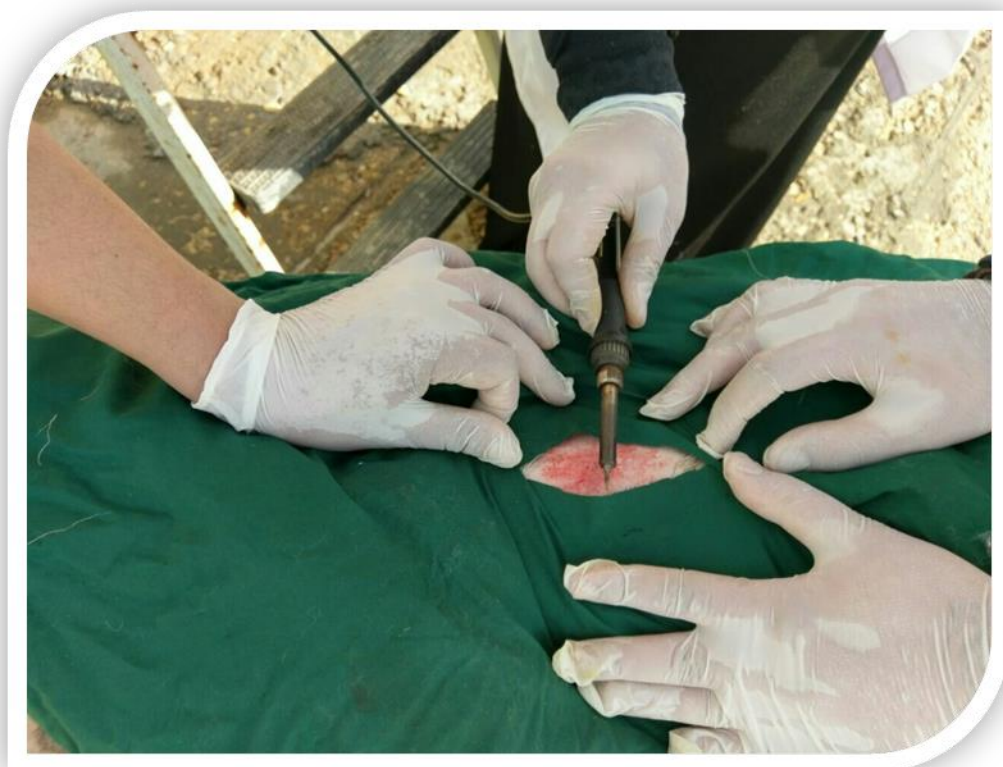


Fig C: Applying the probe over the skin of the local anesthetized animal for ten seconds with no pressure.

Results

Burn wound model at 155C for 10 seconds produced uniform second degree burn on the skin of sheep, on day 14 post burn, they are initiated to promote healthy tissue in both group one and two with a degree more than that of the control group.

Wound contraction was higher in the group one than both group two and control, clinically inflammatory reaction and exudation were less in group one than group two and control group. Also there were no adverse outcomes (such as infection) noted in any of the groups.

Group one shows that there a thickening of keratinized layer and marked downward hyperplasia of epidermis as well as profuse collagen with infiltration of inflammatory cells in the dermis and formation of new blood vessels with fibrosis (Fig. 1 and 2), all these developing changes revealed that the group one has the superiority in the healthy rates when compared with the other two groups.

Accordingly, the second group noted that the healing rate is better than the control group but with less improvements than the first group, group two recorded complete sloughing of keratinized layer and destruction of hair follicle as well as there was a few collagen in the dermis (Fig. 3 and 4).

Healing processes in the group three were in there beginning in comparison with the group one and two, control group exhibited there were a destruction of the keratinized layer also there is sloughing and destruction of epidermis as well as absence of hair follicle with few collagen in dermis (Fig. 5 and 6).

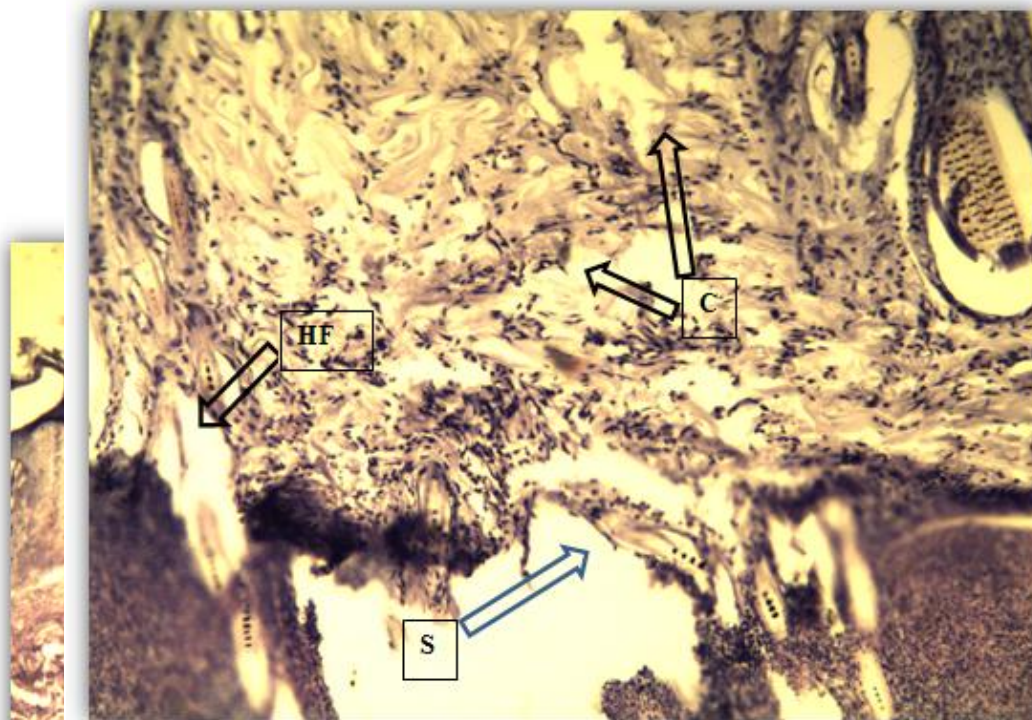


Fig. 3: Complete sloughing of keratinized layer (S), destruction of hair follicle (HF) with few collagen in the dermis (C). (H&E, 10X)]

Fig. 5: Destruction of keratinized layer (K), sloughing and destruction of epidermis (EP), absence of hair follicles with few collagen (C) in dermis . (H&E, 4X).

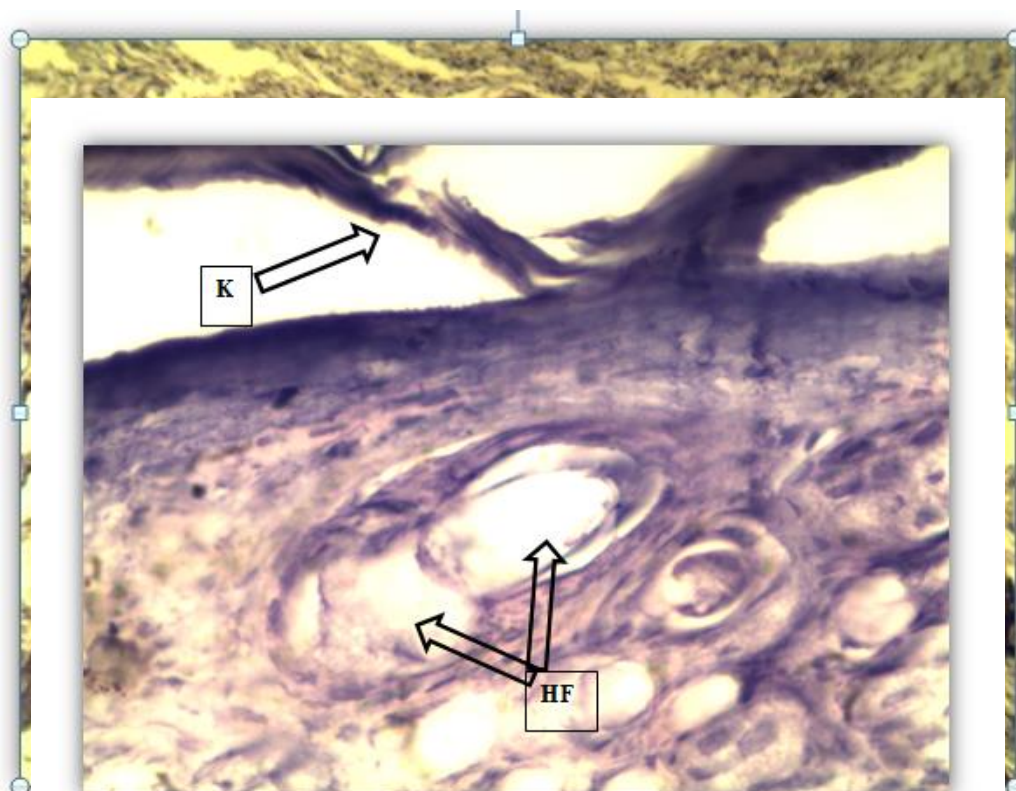


Fig. 6: Destruction of keratinized layer (K),with destruction of hair (D), follicles (HF), in dermis. (H&E, 40X).

Discussion

Burn are one of most widespread health issues, when the treatment become topically; it considers a very important matter in burns. Multiple materials and drugs have been used for this purpose, numerous which applied for a long time, some have only lately approved by the researchers, the ideal topical treatment must heal the burn in short time with best results as well as decrease mortality and morbidity rates by prevent sepsis and they should be available and not expensive and restore the damaged tissue as normal as possible, the researchers are ongoing to discover the ideal agent. Burns can be classified by the depth into four degrees, the second one (the subject of the current study) involves the whole epidermis and may be superficial or deep (13).

The final aim at all contemporary burn treatments is to accelerate healing of skin and avert infection of burn (14)(15). The succession of the occurrence that renovate the injury is listed into three sequent phases; inflammation, proliferation and tissue remodeling, the normal healing process could be hindered at any step along its course by a different factors (15).

In the light of such researches, it may be proposed other agent have the more beneficial effective action with less side effects.

Although; disenchantment of some researchers to clarify the advantageous effects of low level laser on burn healing on healthy animals (16), the current study showed that low level laser therapy can noticeably fasten the wound healing rate of second degree burn in healthy sheep.

The biostimulatory effects of low level laser which applied in the present study have marked increase of the healing rate of the burned are with better changes of the group two and have better healing process than group three.

It seems that the group two displayed beneficial effects due to presence of laser light when it used appropriately can stimulate the healing rate of burned tissue and this agreed with (17) who improved that the dermis can be regenerated with better results

when irradiated with low level laser and explicate that treatment with low level laser both *in vitro* and *in vivo* ; the mast cells and macrophages can be activated to produce growth factors which give the desired effects of healing.

The suggested mechanism of increment the burn healing process by low level laser therapy is by absorption the mitochondria of light energy and this lead to sustain the cell energy and stimulate the production of chemical mediators (18).

Our result of study controverts the results collected by (16)(19) who clarified that the low level laser therapy has no significant improvement in the burned area when compared with control group and this may because of the irradiated dose of laser was $2.3\text{J}/\text{cm}^2$ and this insufficient low energy may unable to give the beneficial effects by stimulation the microphages and release growth factors.

The current results propose that the low level laser irradiation with a dose of $6\text{J}/\text{cm}^2$ produces a noticeable acceleration in the burn healing rate in comparison with control group and this confirms the consequences of (6) (20) whom verified that low level laser significantly increased the burn closure rate.

We conclude that low level laser therapy with a $6\text{ J}/\text{cm}^2$ for continuous five days in second degree burn in sheep increase the healing process rate when compared with control group and these results resemble the findings which harvested by group two.

Platelet rich plasma is an autologous agent yielded from whole blood through the process of gradient density centrifugation, these concentrates have a huge numbers of platelets in a little volume of plasma (21)(22). Platelet rich plasma has been demonstrated to applied exogenously to soft tissues to enhance wound healing and tissue sealing (23). There are few researches about using and affection of platelet rich plasma on the burned tissue the available ones are related to human, however, the platelet rich plasma considered as a safe treatment supposed its autologous source and long persistence application without any life threatening complications.

The application of autologous platelet rich plasma for continuous five days shows a noticeable improvement in the healing rate of burned area when compared with group three and two, these results are accompanied with (24). The variable mechanisms of action of platelet rich plasma are extensive due to the yielding of a numerous bioactive materials. The advantageous application of the platelet rich plasma for enhancement the injured tissue may be because of the presence of concentrated platelets in the site; whenever the stimulation is occurred, the consequences are a huge amount of growth factors (25).

Our results of the current study in the group one show an important healing process rate with better of what had been demonstrated in the group three and this accord with (26) who found that application of platelet rich plasma in the intractable skin ulcer with no complication occurred and the wounds achieved complete epithelialization.

Platelets participate toward hemostasis at locations of vascular damage and they include a numerous growth factors and cytokines that play an important role in the inflammation and repairing of tissue (27). These peculiarities of platelet have conducted to the notion of application of platelets as a therapeutic device for bolstering wound healing, especially in animals with impaired or delayed tissue repair.

Platelet rich plasma stimulates angiogenesis and proliferation of fibroblast and application of these agents improve wound healing alike soft and hard tissue; these findings are evinced by (28) and accord with our results of the current study. A deep dermal burn can treated by platelet rich plasma because of its hemostatic and antimicrobial abilities also the affirmatory impact can be seen in wound healing (29), who mentioned these results and these agreed with what we have in the results of group one.

Platelet rich plasma seems to partially meliorates healing of burn and apparently this type of treatments can be used beneficially (30).

Conclusions and Recommendations

Conclusions

1. PRP and LLL can improve second degree burn healing in sheep.
2. PRP can improve the healing with a rate better than application of LLL therapy and both of them are best than control group.

Recommendations

1. Application of PRP in the different degrees of burn.
2. Application of this technique in other species of animals in case of other injuries.

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