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EFFECTS OF LOW LEVEL LASER THERAPY ON WOUND HEALING IN GOAT

أثر الليزر العلاجي منخفض القدره في التئام الجروح في الماعز

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Dedication

We dedicate our Dissertation to:

Our Mothers “Our angels in life”

Our father's “Whom we hold their names with pride”

Our Sisters and Brothers “With whom we shared the most beautiful

moments of our lives

&

Whom were always supported us whole heartedly

Preface

Our work in this thesis has been carried out in the Department of Surgery, Collage of Veterinary Medicine, Sudan University of Science and Technology. Under supervision of Doctor Maria Al Rayah Osman.

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Abstract

The aim from our research to assess the clinical effects of the low level of laser therapy(LLLT) on healing process of full thickness skin wounds.

Full thickness skin wounds were surgically created on the lateral and medial aspect of the thigh of 5 cross breed from Nubian and red sea goats. According to the time of scanning with continuous waves of Dioedlaser .scanning with 4j/cm^2 for 240 second respectively for five days. LLLT treated wounds showed faster finishing of contraction and regression in wound size. Also use of this type of laser can be helpful in elimination of the pain.

خلاصة الاطروحة

هدفت هذه الدراسة الي تقييم الاثار السريريه لاشعاع الليزر العلاجي منخفض المستوى على عملية التأم الجروح الجلدية .

عُملت جروح الجلد الكثيف على جانبي الفخذ لخمسه من الاغنام الهجين الناتجة من سلالتي الاغنام النوبية واغنام البحر الاحمر .

وقد تم تطبيق اشعة دايود بكثافة قدرها 4 جول/ سم2 لفترة 240 ثانية في اليوم ولمدة 21 يوم غير متتالية على الاغنام.

وقد اوضح مسح الانسجة انتهاء سريعا في عملية تقلص الجرح .

كذلك توضح النتائج ان استعمال هذا النوع من الليزر قد تساعد بشكل ايجابي على ازالة الالم.

Contents

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INTRODUCTION

Wounds are one of the main problems in animal health, whether surgical or non surgical. The clinicians seek to obtain wound care for healing promotion and to decrease hazards for a variety of skin wounds, which include abrasions, burns, surgical incisions and ulcerations.

Livestock plays an important role in the economy of the Sudan (Anon, 2003). Most of goats in some towns in the Sudan are not kept indoors but straying in the streets and hence are more exposed to the risk of injury. In this animal species wounds are usually manifested in the skin of limbs and udder as a result of penetration or laceration by sharp objects and fences. They can lead to complications, such as infections, contractures, or limb amputation.

Many studies investigated the enhancement of the healing process in animals using different methods (Gillette, *et. al*, 2001; Scardino, *et. al*, 1998). One of these methods used is the LLLT (Mester and Nagi, 1973).It is a form of phototherapy that involves the application of low power monochromatic and coherent light to injuries and lesions. It has been used successfully to induce wound healing in non healingdefects(Hawkins and Abrahamse, 2005) and to relief acute and chronic pain (Ladalardo, *et al*, 2005).

The objectives of this research work are:

-To identify the effect of LLLT on healing of experimental full-thickness skin wounds model in the cross breed goats.

-To compare the findings of this study with other previous works.

-To encourage veterinary surgeons to use laser therapy in veterinary fields.

Results of this study will provide information that may encourage the use of laser therapy in veterinary surgery in the Sudan to minimize the complications of wound on animal health and production.

CHAPTER ONE

LITRATURE REVIEW

LITERATURE REVIEW

1.1 Goat Breeds in Sudan

The goat population in the Sudan is estimated to be 38.0 million head (internet I). It is increasing at an annual rate of 3%.Four local types of goats are distinguished in the Sudan: Nubian goat, Southern goat, Sudan desert and Hill goat (Mason and Maule., 1960; Muffarah, 1995).

1.2. Wound healing

A wound whether surgical or accidental is described by Swaim (1980) as a traumatic separation of skin, mucous membrane, or organ surface modality.It is either simple, if no deeper tissues are involved or compound, when muscle,nerves, tendons and bones are involved.

Wound healing is the process whereby the body destroys and removes the irritant and returns the part to as near as normal functional state (Runnels,*etal.*, 1967).

These events are instituted in proper sequence and the final outcome is generally connective tissue scar. The processes involved in wound repair should be precisely controlled (Jones *et al*., 1997).

The essential feature of wound healing are similar and had been subdivided into the inflammatory,debridement, repair and maturation stages (Swaim, 1980, Jones, *et al*., 1997). Those stages function to fill the wound gap, replace lost cells within the wound, and finally to reestablish tissue function.

1.2.1. The inflammatory phase

Is recorded to be the initial phase in wound healing, and it can be subdivided into vascular and cellular components that have been shown to occur in a sequence of events (Peacock, et al., 1984).

1.2.2. The debridement or destructive phase

The debridement or destructive phase is described by Oehme (1988), to begin approximately six hours after injury and to last for variable amount of time, depending on the amount of tissue debris and contamination in the wound area.

1.2.3. The proliferative phase

The proliferative phase is characterized by fibroplasia epithelialization, granulation tissue formation, and wound contraction, (Silver, 1984; Clark and Henson, 1988; Jones, et al., 1997).

1.2.4. The maturation phase

It is accompanied by a reduction in the numbers of fibroblasts within the wound area and a leveling-off of collagen production. By that time epithelialization is shown to be completed and the wound has regained the majority of its strength (Dunphy, and Winkle, 1969).

1.2.5. Demolition following acute inflammation

The clot in the centre of the wound is recorded to be invaded and replaced by granulation tissue. But the clot which grows from the subcutaneous tissues at the wound edge is important in causing wound contraction (Walter, and Israel, 1987).

1.3.Laser therapy

Low level therapy (LLLT) is described as a form of phototherapy which involved the application of low power monochromatic and coherent light to injuries and lesions (Hawkins, et al., 2005, .Hawkins and Abrahamse, 2005).

The principles upon which all laser devices are based are developed at the turn of the century. Einstein published a paper in 1917 which outlined the key principles for stimulated emission of photons (Baxter, *et al*., 1991).

The device termed as MASER (Microwave Amplification by stimulation Emission of Radiation) works at microwave wavelengths which is part of electromagnetic spectrum or radiation (EMR). (Gordon,*etal*., 1955). EMR is fundamental form of energy that exhibits waveproperties, starting on the long-wave length; low-photon energy end of spectrum .EMR includes radio waves microwaves infrared radiation visible light ultraviolet radiation and X-ray (Anderson, 1994).

The use of laser and photodiodes at relative low intensities has been promoted as effective for several conditions includingarthritis, soft tissue injuries and pain, as a result of which that therapeutic modality is gaining widespread acceptance within the professions (Payter, *et al.*, 1991).

1.3.1. Element of laser

According to Baxter, *et al*., (1994) four functional elements in laser systems are necessary to produce coherent light by stimulated emission of radiation.

1.3.1.1.Active medium

Is collection of atoms or molecules that can be excited to a state of inverted population, where more atoms or molecules are in an excited state than in some lower energy state .The active media may be gas, liquid, solidmaterial, or junction between tow slabs of semiconductor materials (Baxter,etal., 1994).

1.3.1.2.Excitation mechanism

The excitation mechanism is source of energy that excites atoms in the active medium from alower to a higher energystate.

1.3.1.3.Feedback mechanism

The feedback mechanism returns a portion of coherent light originally produced in the active medium, back to it for further amplification by stimulated emission.

1.3.1.4.Output copular

The output copular allows a portion of laser light between the two mirrors to leave the laser in the form of a beam (Noel and peter, 2006)

1.3.2. Types of laser

Laser classified according to the active medium or excitation mechanism or duration of laser output.

There are many different types of lasers depending of the active medium, the laser medium can be a solid, gas, liquid or semiconductor.

1.3.2.1.Solid state laser

Have lasing material distributed in a solid matrix (such as ruby or neodymium: yttrium-aluminum garnet (Nd-YAG laser) TheNd-YAG laser emits infrared light at 1064nanometer (nm) (internet ii).

1.3.2.2.Gas laser

The most popular type of gas laser which contain a mixture of helium and laser (internet iii)

Helium-Neon lasers (He-Ne)

The helium-Neon (He-Ne) laser is the most common laser in photobiostimulation on tissue repair (Abergel, *etal*., 1987b; Kokino, 1985; Rochkind, 1989; Enwemeka, 1990).

He-Ne is first laser used in clinical and research application such as cytometry and for diagnostic, relief of pain and wound healing purposes (Lam, *et al* m., 1984; Abergel, etal., 1984).

1.3.2.3.Semiconductor lasers

Sometimes called diode lasers,these electronic devices are generally very small and use low power.

They may be built into largerarrays, such as the writing source in some laser printers or CDplayers (Internet iii).

Diode lasers use microscopic chips of Gallium-Arsenide or other exotic semiconductors to generate coherent light in a very small package.

1.3.3. Irradiant power

According to (Baxter, *et al.*, 1994) the power is generally defined as the rate at which work had been developed, used or transferred.

Baxter, *et al.*, (1994) stated that "The power of a laser beam is the rate at which optical energy is developed by the beam". In radiometric terms the output or the radiant power of laser is measured and specified in watts (w) which is equal to one joule per second (J/sec). The power of low-intensity light is measured in mill watts (mw = 10^{-3}w) or micro watts, as in the He-Ne laser. The mill watt is more commonly used to specify radiant power for such systems.

The optical power density (irradiance) is the power per unit area of a light beam striking a surface, and will decrease with increasing distance between the treatment unit and the target tissue. Irradiance or power density is defined by this equation:

Irradiance (W/m^2) =incident power (W) ÷ Area of irradiance (m^2) (Baxter, *et al*., 1994).

1.3.4. Laser-tissue interaction

Medical laser produce deference wavelengths of light depending on their medium, the target tissue composition and the laser wavelength.

When absorbed within the target tissue, laser light converted to other forms of energy (Chemical, thermal or mechanical-acoustic energy). These laser-tissue interactions classified as a photochemical, photothermal or photoplasmolytic (Anderson,1994).

Photothermolysis occurred when the energy of laser light was absorbed by the skin and converted to heat. Once the heat was absorbed, causing rising rapidly of the tissue temperature, tissue absorbs varying wavelengths of lasers light, hyperthermia may contract, constrict or destroy blood vessels resulting in tissue hypoxia and cell death (Noel and Peter, 2006). The interaction may be considered at three levels: atomic, molecular and macromolecular levels. Laser-tissue interaction at the wavelength commonly used in LLLT applications was predominantly at the molecular and macromolecular levels (Baxter, *et al.*, 1994). The zone of direct interaction because of changes induced by direct action of laser on the tissue causing vaporization of intracellular water and tissue ablation, the zone of irreversible thermal damage and denaturation with necrosis, and the zone of reversible thermal damages with collagen shrinkage and minimal necrosis.

1.3.5. Laser and wound healing

He-Ne laser and light-emitting diode irradiation on skin wounds in rats was found to stimulate the transition of the inflammatory phase of the wound healing into the reparative (proliferative) and scarring phases sequentially (Klebanov, *et al*., 2005). Effect of LLLT on the stimulation of the healing process of post operatively aseptic wounds in humans foots was found to be an effective process (Herascu, *et al.*, 2005).

According to Hopkins, *et al.*, (2004) the LLLT resulted in enhanced wound healing in humans wounded arms as measured by wound contraction.

Berman and villa, (2004) recorded that for atrophic scars, different types of lasers represented modern treatment modalities with satisfactory results in human.

In a meta-analysis of the literature, Woodruff, *et al.,* (2004) concluded that laser therapy is an effective tool for promoting wound repair in animal experiments as well as in human clinical studies.

Laser therapy now forms a part of most if not all physiotherapy training programs within the UK, and it is rare to find hospital, physiotherapy departments or private clinic without access to this modality. Yet there remains a considerable amount of ignorance about its efficacy (Patel, 1965). A survey among physiotherapist showed that 94% of respondents were dissatisfied with the amount and quality of information on LLLT upon which to base their clinical practice (Baxter, *et al.,* 1991).

Dyson and Young, (1986) reported local wound healing effects in mice with helium- neon laser which may be explained by the action of low intensity light on cell proliferation in the area of injury, same results in human obtained by Hopkins, *et al.,* (2004)

Accordingto(Abergel, *et al.*, 1987). Improve in tensile strength in laser treated wounds in mice and pigs by infrared and He –Ne laser was found. They concluded that laser irradiated may cause the release of tissue factors into the systemic circulation which increased tensile strength of wounds. Same result obtained in rabbits by Braverman, *et al*., (1989). .

This would seem an acceptable mechanism of laser- stimulated healing in view of the role of these cells in the healing process using human embryonic foreskin fibroblasts and adult human skin.

Chapter two

Materials and methods

MATERIALS AND METHODS

2.1. Experimental animals

Five apparently healthy males and females cross breed between Nubian and Red sea goats, 2-8 years of age and 5-15 Kg body weightClinically examined to insure their clinical fitness.

2.2. Housing and feeding

The animals were housed in closed site (5x5 miters) in kuku Zoo, in Eastern Nile State.These goats were given water and green-AlfaAlfa(Medicago sativa).

2.3. Materials for surgical procedure

Soap (Dettol antibacterial soap, Reckitt and Colman {overseas} Ltd. Hull, UK), scalpels, razor, gauze, disposable syringes (5ml), tissue forceps, artery forceps, lidocaine 2% (lignocaine hydrochloride 50mg/ ml. P.P.L, Malta), tincture of iodine (povidone-iodine 10%USP Yamani Medical Products, Sudan- Khartoum), 70% alcohol (Balsam Company for Chemicals).

2.4. Surgical methods

2.4.1. Wounding

2.4.1.1. Site

The lateral aspect of the left and right thigh over the biceps muscles of all the experimental animals,left wound for laser and right one for control.

2.4.1.2. Positioning

All surgical procedure was comes out with the animals in the standing position.Laser it dosing in recumbence.

2.4.1.3. Preparations

An area of 20x20 cm was clipped against the pattern of hair growth to obtain a closer clip with sterile scissor (Theresa, *et al.,* 2002) then shaved with sterile razor. It was cleaned and scrubbed with mild detergent containing tincture of iodine. Scrubbing was started at the incision site using sterilized gauze. The prepared area was then allowed to dry. After that70% Alcohol was applied to produce a fast kill of bacteria and acts as a de-fating.

2.4.1.4. Anesthesia

Local infiltration anesthesia was achieved by subcutaneous injection 2 ml lidocaine at the surgical site (Thurmon, *et al.,* 1996).

2.4.1.5. Sugary

An area of 2x2 cm^2 was measured using a flexible measuring tape. Using a sterile scalpel and tissue forceps an incision was made to perform an open wound involving all the layers of the skin (dermis and epidermis).

The bleeding from the superficial capillaries was controlled by direct pressure with sterile gauze.

The wound was kept without dressing, cleaning or antibiotics to insure the maximum benefit from laser treatment.

2.5. Dosing

After wounding, and after the bleeding has stopped the right woundswas used as control and was not subjected to any laser radiation while the left wounds were exposed to LLLT at the rate dose of 0.640J/cm^2 for 200 second. Dosing were done during the same period of time in all animals and repeated for 5days.

2.5.1. Laser scanning

Five minutes post wounding, manual scanning with continuous laser waves (cw) was applied to the wound from a diode laser probe (wavelength 675nm, max. output 1mW, united kingdom). The dose was calculated as: joule = "power (mW) Time (s)/ centimeter (cm) of laser irradiation(Baxter, *et al.*, 1994). The wound surface area was 4cm^2.

2.6. Parameters:

2.6.1. Clinical manifestations

Visual qualitative observation was used for testing bleeding, swelling, scab thickness and size of scar. Thus could have been converted into simple quantitative scale such as: + for slight, ++ for moderate, +++ for sever and ++++ for very sever.

2.6.1.1. Pain

Pain was detected by limb withdrawal reflex, according to the method designed by Hellebrekers and Sap, (1997).It was considered positive when the animal withdraws its limb as a response to the application of digital pressure on the skin around the wound.

2.6.1.2. Bleeding

Bleeding was tested by visual qualitative observation (Rochkind, *et al.*, 1989) immediately after the first irradiation of wounds with LLLT.

2.6.1.3. Swelling

The wounded area was examined for swelling after 24 hours from wounding. Swelling was tested grossly in all experimental animals by visual qualitative observation of the elevation on the area around the wound; it's extending in the adjacent area (Rochkind, *et al*., 1989) which was measured by a flexible meter.

2.6.1.4. Scab thickness

Scab thickness was examined by visual qualitative observation and all wounded areas were assessed photographically at periodic intervals (Braverman, *et al.*, 1989).

2.6.1.5. Contraction and wound size

Size of wound was measured to analyze wound contraction as a function of healing by using a measuring tape (Hopkins, *et al.,* 2004). Photographic assessment of wound size was also used as described by Braverman, *et al*., (1989).

2.6.1.6. . Scar size

Scar size was tested by visual qualitative observation, (Rochkind, *et al*.,1989) and all wounded areas were assessed photographically at periodic intervals (Braverman, *et al.*, 1989).

2.6.1.7. Infection

Wounds were examined grossly for infection by detection of pus in the whole or part of the wounded area (Rochkind, *et al.*, 1989).

2.7. Data analysis

2.7.1. Imaging

All gross appearance recorded by standard digital photos using a digital camera (AIPTEK, China). Digital images were then recorded under the same conditions (distance, lighting, settings) as the original images.

CHAPTER THREE

RESULTS

RESULTS

3.1. Clinical manifestation

3.1.1. Pain

Compared with control wound, pain relief' was improved in all laser treated wounds. Desensitization was detected after laser irradiation by the third hour after wounding.

3.1.2. Bleeding

The cutaneous bleeding started again and continued for few second after the first exposure to LLLT treated wounds, bleeding was found to be more in laser wound no. one and very little in wound no. five.

3.1.3. Swelling

The effect of LLLT on the swelling is found in both , level and extend of swelling with the time taken for the swelling to be stable ,or to start to decrease. LLLT decreased the swelling in all treated wounds and it extent was decreased within the adjacent tissue and confined to the boundaries of the wound. It was extended to about 1.5 cm in the control group and about 0.33 cm maximum around the wound boundaries in all treated groups. The swelling continued to increase up to 24 hours after wounding in control wounds no. Two, three and fourandit are found to be stable in wound no. one and five.

3.1.4. Scab thickness

In the control wounds the scab was thick and covered only part of the wound in one animal. It was sloughed due to re-infection in this wound by day twenty post wounding. Scab formation was affected positively by the level of LLLT used. In laser wounds no one very thin or no scab formation was noticed. Thick scab is formed at the edges of the wound in laser wound no two, three and four, and in middle of the wound in laser wound no five. Whenever there is a scab formed in all treated groups, it is sloughed by day fifteen post wounding.

3.1.5. Contraction and wound size

Compared with control wounds, decrease in wound size is obvious after three days in the LLLT treated groups. All of them show well wound contraction after 21 days.

3.1.6. Scar

No complete healing, or scar formation was seen in control wounds during the fifteen days. Small scar is observed grossly in four treated wounds. And complete healing and very small size scar by day 21post wounding. Very small scar was formed by day 21 in control wounds. The skin resembled the normal skin with hair growth in all control and LLLT treated wounds.

3.1.7. Infection

In the control wounds infection with purulent exudates was detected in two wounds at day three post wounding. The infection was detected again after ten days post wounding leading to the sloughing of scab formed. Apparently non infected wounds (early healing) with no obvious pus were seen in the treated wounds.

Figure.1. Bleeding in full-thickness skin wound in a cross-breeding goat no. four after wounding (not receiving LLLT).

Figure.2. Bleeding in low level laser therapy treated full-thickness skin wound in a cross-breeding goat no. five after wounding

Figure.3. Swelling in anon treated full-thickness skin wound in a cross-breeding goat no. three days post wounding.

Figure.4. swelling in low level laser therapy treated full-thicknessskinwound in a cross-breeding goat no. one after

Figure.5. Formation of thick scab in a non treated full-thickness skin wound in a cross-breeding goat no. twoand it sloughed by daytwenty post wounding.

Figure.6. Absence of scab in a low level laser therapy treated full-thickness skin wound in a cross-breeding goat no.one and it sloughed by dayfifteen post wounding.

Figure.7. wound contraction in a full-thickness skin wound in a cross-breeding goat no.five , twenty one days post wounding (not receiving LLLT)..

Figure.8. wound contraction in a low level laser therapy treated full-thickness skin wound in a cross-breeding goat no. one , twenty one days post wounding.

Discussion

One of the main indications of the LLLT is the relief of pain (Mckibben, 1983; Bromiley, 1991). It was described by Baxter ,et al.(1991) to be rated over all as effective method of relief for post-operative pain .He considered it as an important treatment objective in physio-therapists use of low power laser.

In the present study relief of pain has been detected in all laser treated wound. Starting since the first hour of wounding and this correlates with the results of Li (1990).He demonstrated relief of pain of tennis elbow by He-Ne laser irradiation in human. Pain relief in LLLT treated wounds may be due to the altered (Walker and akhanjee, 1985) or decreased (Zarkovic, et al., 1989) metabolism of serotonin induced by the laser irradiation in human to treat chronic pain (Walker and akhanjee, 1985) and on feet of experimental mice (Zarkovic, et al., 1989).

Laser may have signification effects on the synthesis, release and metabolism of a range of neuro-chmicals, such as acetylcholine (Zarkovic, et al., 1989 lombard, et al., 1990).

Our primary finding was that the bleeding which stopped after wounding in all experimental animals occurred again and continued for a short period of time after application of laser therapy in the treated wounds. Previous clinical studies and research reports have shown apparent increase in cutaneous and deep blood flow as a result of laser irradiation (Martin,et al.,1991;singer,et al.,2005).

Vascular reactivity to injury is rapid and results in a neither immediate, short-lived vasoconstriction mediated by nor adrenalin. This partially controls tendency to hemorrhage as partial occlusion of small vessels occur due contraction of endothelial lining which become sticky (peacock, et al., 1984; kloth, et al., 1990).

The swelling was greater in the control wounds which suggest the effectiveness of LLLT in reducing edema. These results agree with Christensen (1989); Zhou (1988) and Zhukov, et al., (1979). Edema is produced due to leakage of fluid from dilated vessels, due to change in vascular permeability brought about by histamine release from damage cells and degranulating mast cells which also release prostaglandin, serotonin and heparin. Histamines cause swelling in the endothelial cells lining blood vessels; its lasts not longer than 30 minutes. Then other factors alter permeability (Peacock, 1984). Decrease of histamine levels and mast cell numbers after a course of irradiation with He-Ne unit in human bone fracture, has been detected by Trelles, *et al.*, (1989).

In the present study the scab size has been found to decrease with the increase of the laser dose. The thickest scab was seen in control wounds it sloughed a way in LLLT wounds before those of the control group. The same result have been described by Han and Kim, (1997).complete wound healing, closure and contraction were more rapid in LLLT wounds receiving the least LLLT dose while the slowest wound healing and closure and minimum wound contraction was seen in control wound. It was obvious that the LLLT enhanced wound healing and wound contraction that was showed by the time taken by the wound to heal and the size of wounds. This result agrees with that observed by Han and Kim, (1986).

Several theories may help explain the enhanced wound contraction observed here. In vitro studies have shown an increase in fibroblast proliferation after irradiation, also Dyson and young (1986) found and increase in wound contraction in irradiated wounds in mice ones. These data indicate that LLLT is an effective modality to facilitate wound contraction of full thickness skin wounds. Our data support this suggestion

CONCLUSIONS AND RECOMMENDATIONS

The results of the present study indicate that LLLT improves full skin wound healing. It is concluded that the LLLT is an effective treatment for enhancing wound healing, contraction and pain relief of full thickness skin wounds. Thus LLLT may be useful especially in difficult or chronic wounds.

Better understanding of the utility of LLLT in cutaneous wound healing and well controlled clinical processes are needed to formulate meaningful conclusions regarding LLLT. Also further researches is required in recommended of LLLT for the management of infected wounds studies in the physiological effects of LLLT upon blood flow and neural function and hypo analgesic effects in laboratory. So we recommended using the LLLT:

- To induce the healing in none or delaying healing defects such as to accelerate the healing in diabetics ulcers or wounds.

-To accelerate the healing of postoperative incisions.

- To relief of acute and chronic pain such as in tendonitis and strains in equine.

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