

## **Histopathological Study Of The Effect Of Low Level Laser And Biatain Ag Non-Adhesive Dressing On The Avulsion Wound Healing In Rabbits**

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### **Abstract**

The aims of this study was to compare between the effect of Low level Laser and Biatain Ag non-adhesive dressing on healing of avulsion wound in rabbits. Eighteen adult healthy rabbits were used. It is divided in to two equal groups , Laser group , and Biatain Ag group, also each group divide into three equal sub group for histopathological study at 1 week , 2 week and 3 week post operation. The avulsion wound was done on the back(dorsal) after general anesthesia by blade 1 cm . post-operation , in laser group, the rabbits were exposed to low level laser for 5 minute/daily to period 1week, 2week ,3week continuously , but in Biatain Ag group, the avulsion wound was dressing by Biatain Ag non-adhesive dressing and it fixed on wound by blaster and continuous to 1week, 2week, 3week.

The histopathological examination appear in first week, both groups showed ulceration but in biotin group showed more ulceration than laser group.

In two week, laser group some animals showed absence of ulceration with dermal fibrosis. In two week, in biatain group showed reduce the size ulcer with dermal fibrosis.

In three week, laser group showed complete healing with moderate thickening of epidermis and hyperkeratosis with dermal fibrosis. While biatain group showed complete healing with marked dermal fibrosis but presence of foci of hair folliculitis. Conclusion In two week, laser group all showed healing of ulcer except one animal while biatain group there was only reduce size of ulcer but not complete healing..

**Key words:** Laser, Biatain, wound, Radiation

دراسة نسيجية لتأثير الليزر وضمادة البياتين الغير ملصقة على شفاء الجروح القلعية في  
الارانب

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الهدف من هذه الدراسة , المقارنة بين تأثير الليزر وضمادة الببائين الغير ملصقه على شفاء الجروح القلعية في الارانب. استخدمت ثمانية عشر ارنبا سليم وبالغ وقسمت الى مجموعتين متساويتين (مجموعة الليزر ومجموعة الببائين) عملت الجروح القلاعية على الظهر بواسطة المشروط الجراحي بعد التخدير العام بعد اجراء العملية , مجموعة الليزر عرضت الارانب الى الليزر لمدة خمس دقائق يوميا لفترة اسبوع , اسبوعين , ثلاث اسابيع. اما مجموعة الببائين ضمدت الجروح بواسطة ضمادة الببائين وثبتت على الجروح بلاصق (بلاستر) واستمرت لمدة اسبوع , اسبوعين , ثلاث اسابيع. الفحص النسيجي يبين في الاسبوع الاول ان في كلا المجموعتين وجود تقرح لكن في مجموعة الببائين التقرح اكثر شدة. في الاسبوع الثاني يرى عدم وجود التقرح في ارنبين في مجموعة الليزر مع تليف الادمة. اما في مجموعة الببائين يرى صغر حجم التقرح مع تليف الادمة. في الاسبوع الثالث وجد في مجموعة الليزر شفاء تام للتقرح مع وجود بؤر التهاب الجريبات . الاستنتاجات في الاسبوع الثاني في مجموعة الليزر يكون شفاء تام للتقرح في ارنبين ما عدا ارنب . اما مجموعة الببائين لوحظ قلة حجم التقرح لكن لا تشفى تماما..

### Introduction:

Wounds are the most common injuries encountered in both humans and animals. Depending on the depth and the possibility of contamination, wounds may produce local or secondary phenomena, local or general, affecting the health status and patient behavior. In the case of simple and uncomplicated wounds, the healing process is conducted in the form of sequence of morphological changes which is named "sanatio per primam intentionem" and usually lasts between 8 and 12 days (1).

Complete healing may occur after 3 weeks This healing period can be shortened by using the low power laser radiation Wound healing and tissue repair are complex process that involve a series of biochemical and cellular reaction (2,3) . Immediately following an injury the healing process begins by getting rid of damaged tissue, then rebuilding

healthy connective tissue in a step – by – step manner(4).

The phases of normal wound healing include homeostasis, inflammation, proliferation and remodeling. Each phases of wound healing is distinct, although the wound healing process is continuous with each phase overlapping the next(5). The redness, swelling, heat and pain of inflammation are natural signs of the healing process (3). Wound healing must occur in a physiologic environment conducive to tissue repair and regeneration (5)

LLLT; (also known as biostimulation and photobiostimulation) is a form of phototherapy that involves the application of low-power monochromatic and coherent light to injuries and lesions in order to stimulate wound healing. LLLT has been shown to increase the speed,

quality and tensile strength of tissue repair, resolve inflammation and provide pain relief (supporting literature is reviewed later in the article). Lasers are already used in a variety of medical and surgical fields, including dentistry, chiropractic, osteopathy, physiotherapy, cosmetic, pain attenuation, wound healing and acupuncture to name but a few (Walsh, 1997). Therapeutic mechanisms of laser, laser promotes healing and reduces pain through several therapeutic mechanisms: Stimulation of collagen production, Alteration of DNA synthesis, Improves the function of damaged neurological tissue, Increased ATP production by the mitochondria and increased oxygen consumption on the cellular level, which may result in muscle relaxation, Increased serotonin and increased endorphins, Increased anti-inflammatory effects through reduced prostaglandin synthesis, Improved blood circulation to the skin in cases like neuralgia and diabetes mellitus, Decreases permeability of the membrane of the nerve cells for Na/K causing hyperpolarisation, Increased lymphatic flow and decreased edema (6). Biatain foam was introduced as early as the 1970s. Biatain foam is a soft, pliable dressing which is comprised of an outer layer that helps guard against bacteria and infection, and an inner foam layer that assists with absorbing fluids (exudates) during the healing process. Biatain dressing "locks" in the secretions while still allowing for a moist atmosphere, which aids in the healing process(7).

Biatain foam dressing is used in wounds with low-to-high exudates levels. Currently, Biatain foam may be used for a wide variety of wounds such as [pressure ulcers](#), [venous leg ulcers](#) and non-infected [diabetic foot ulcers](#). It may also be used for second-degree [burns](#), wounds due to [surgery](#) (post-operative wounds) and even minor abrasions(8).

Wounds normally produce a fluid discharge composed of tissue fluids, proteins, and cellular materials such as white blood cells and other substances. This discharge has a tendency to leak and cause damage to surrounding normal tissues. Furthermore, this discharge may provide an environment in which harmful bacteria may grow. Biatain is able to reduce the potential harmful effects of this exudates by effectively absorbing it while still providing a moist wound healing environment. This results in enhanced wound healing and reduced wound leakage(7).

Biatain foam also provides higher patient comfort due to its soft, flexible nature, and offers more convenience since the dressings don't have to be changed as frequently(9,10)

#### **. Materials and Methods:**

##### ***Experimental Animals:***

In this study were used 18 from adult health rabbits were randomly selected, 5-month old, weighting 1.5-2 kg, were housed in clear wood cages and fed with vegetables and tap water under the same of laboratory environment. These animals divided into two groups: irradiated group include He-Ne laser group and biatain group each group contain 9 rabbits, first group was exposed to first wavelength 632.8 nm

and the second group was contain 9 rabbits.

**Laser Devices:**

• He-Ne (632.8nm) laser unit emitting red light with 1 mw maximum power was radiated with a manual k1 probe in direct and continuous wave (Cw) from (Griffin &George-London) figure (1)..



Fig (1): He-Ne Laser

**. Method:**

**. Animals Preparation and Anesthesia:**

All the rabbits were anesthetized with intramuscular administration (I.M.) of 10mg/kg body weight xylazine hydrochloride and 0.5 mg /kg body weight ketamine hydrochloride(22). Make avulsion wound in the back (dorsal) of the rabbit by scalpel after that give the rabbits systemic antibiotic (penicillin and streptomycin) for four days.

**Experimental Design:**

All the adult animals divided randomly in to two groups:

1-Biatain group: contain 9 rabbits, and divided in three sub groups, post make the avulsion wound , put the

COMFEEL PURILON™ GEL (Sterile) High viscosity hydrogel maintains moist wound environment, while assisting in autolytic debridement fig(4), and then dressing the wound by biatain foam dressing for three period 7 days , 14 days , 21 days fig(5). At the end of all period , the specimens were taken from target tissue and fixed immediately in 10%formalin.

2-Irradiated group: contain 9 rabbits and divided in three subgroups:

♦**He-Ne laser group** (9 rabbits), animals of this group were fixed on coach and prevent them from the motion during exposure period . The animals were exposed to 632.8 nm wavelength of this laser after put them on distance 10 cm between the light

source of laser and the target tissues of the wound fig(3). Laser rays were given once daily at 5minutes for7, 14 and21days.

At the end of exposure time of each group with the laser group, the

specimens were taken from the target tissue include the skin , all the specimens of whole target were fixed immediately in 10% formalin and Bouin's solution for at least 48 hrs for histological examination



Figure(2):Make Avulsion wound



Figure(3):Rabbit exposure to Laser

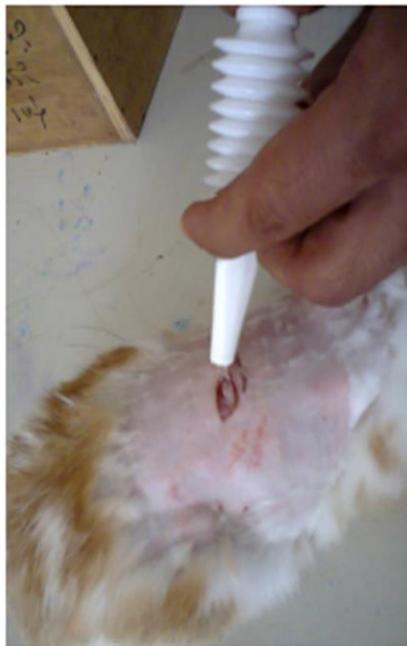


Figure (4):Put gel(purilon)on wound



Figure(5):Dressing the wound by Biatain

**. Photographic and Microscopic examination:**

The histological sections were studied under the light microscope on 10x, 40x and the photographic pictures were taken for the sections of tissues by using microscope with camera (Leica DM 500 from Germany).

**Results**

**Histopathological Findings:**

**1-Biotin group:**

**A-One week period:**

Very large and sever ulceration and with moderate dermal fibrosis with absence of hair follicles and sebaceous glands, in adjacent area moderate epidermal thickening and hyperkeratosis.

**B-Two week period:**

Reduce size of ulceration and dermal fibrosis and reduce hair follicles and sebaceous glands.

**C-Three week period :** no ulceration but mark dermal fibrosis extending into subcutaneous all so some areas in the dermis of mononuclear cell infiltration as evidence of hair folliculitis of hair follicle.

**2-Laser group:**

**A-One week period:**

Large area of ulceration in adjacent moderate thickening of epidermis with hyperkeratosis, Permanente dermal fibrosis associated with reduce number hair follicles and sebaceous glands , there were version in the side of ulcer and thickening of epidermis and hyperkeratosis but all showed marked dermal fibrosis with reduce hair follicles and sebaceous glands.

**B. two week period:**

Some of animals were large area of ulceration, prominent dermal fibrosis associated with reduce of hair follicles and sebaceous glands, in adjacent moderate thickening in 4. folliculitis in the dermis.

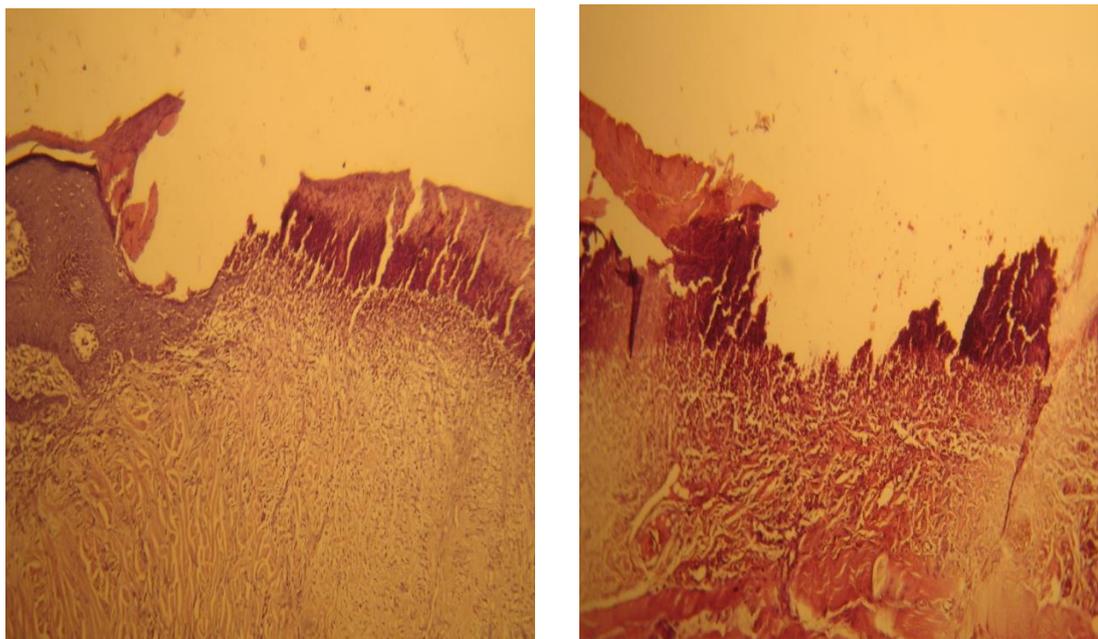
hyperkeratosis. And in other animals no ulceration but moderate thickening of epidermis and hyperkeratosis and prominent dermal fibrosis with reduce hair follicles and sebaceous glands.

**C-Three week period:**

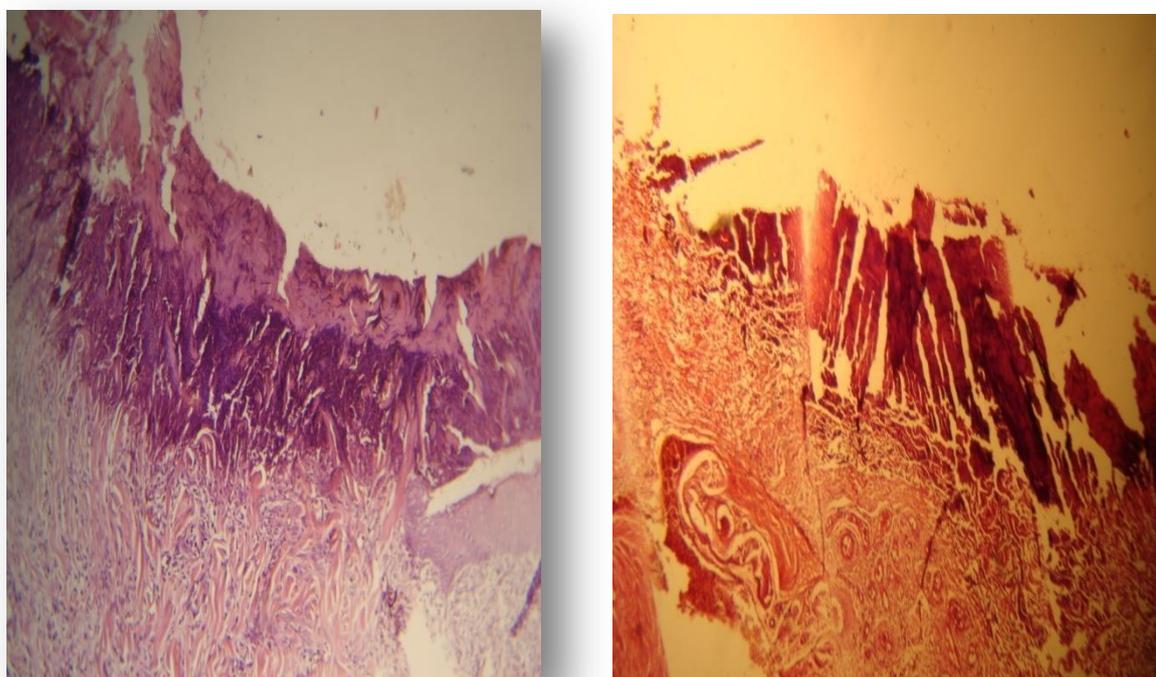
1. No ulceration and moderate thickening of epidermis and hyperkeratosis , with mark dermal fibrosis associate with reduce hair follicle and sebaceous glands . Also sever dermal fibrosis.

The difference between laser group and biotin group as follow:

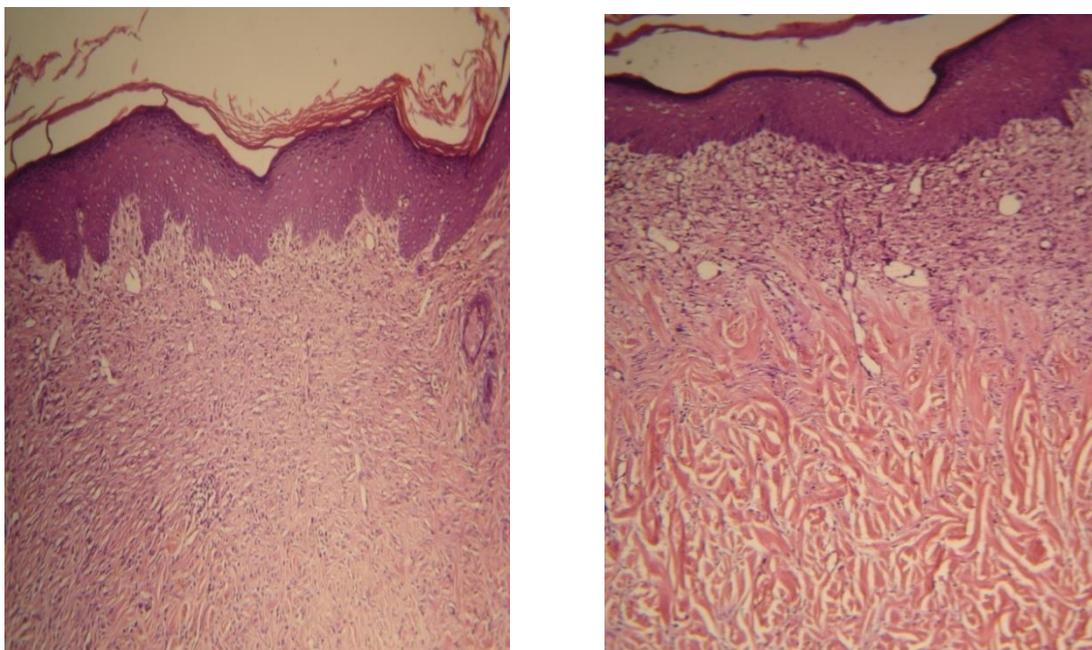
1. in first week, both groups showed ulceration but in biotin group should more ulceration than laser group.
  2. In two week, laser group some animals showed absence of ulceration with dermal fibrosis. In two week, in biotin group should reduce the size ulcer with dermal fibrosis.
  3. In three week, laser group showed complete healing with moderate thickening of epidermis and hyperkeratosis with dermal fibrosis. While biotin group showed complete healing with marked dermal fibrosis but presence of foci of hair folliculate.
- Conclusion In two week, laser group all showed healing of ulcer except one animal while biotin group there was only reduce size of ulcer but not complete healing. In the end of three week, laser group showed complete healing with moderate dermal fibrosis and epidermal thickening but biotin group showed also complete healing but with more dermal fibrosis and presence of foci of hair folliculitis in the dermis.



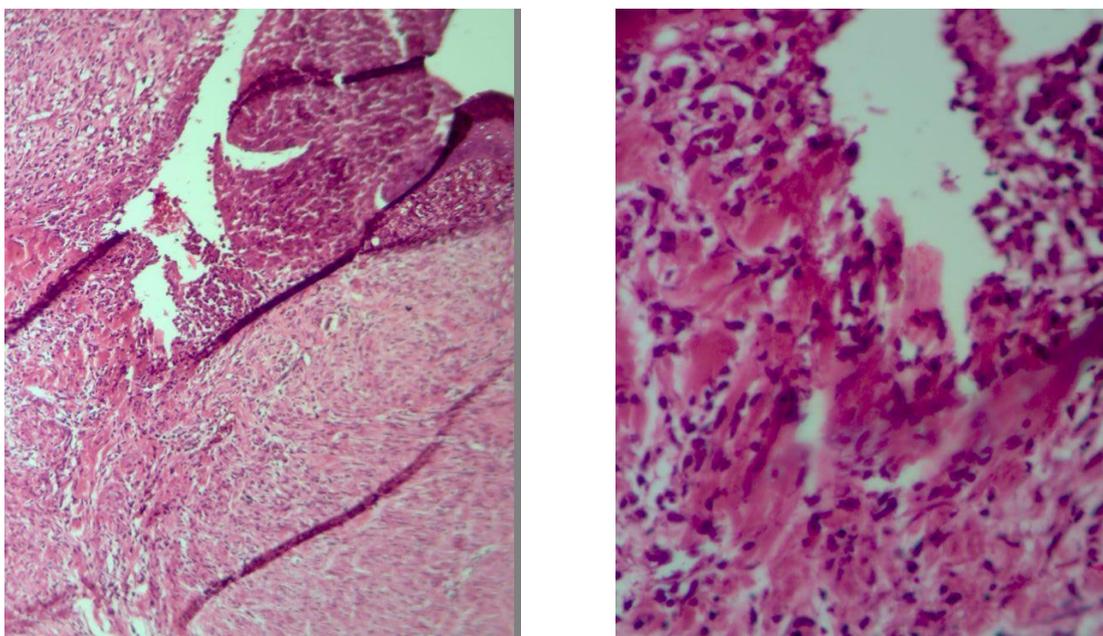
**Figure (3): Laser group one week( sever ulceration with scar formation and inflammatory cells)X10.**



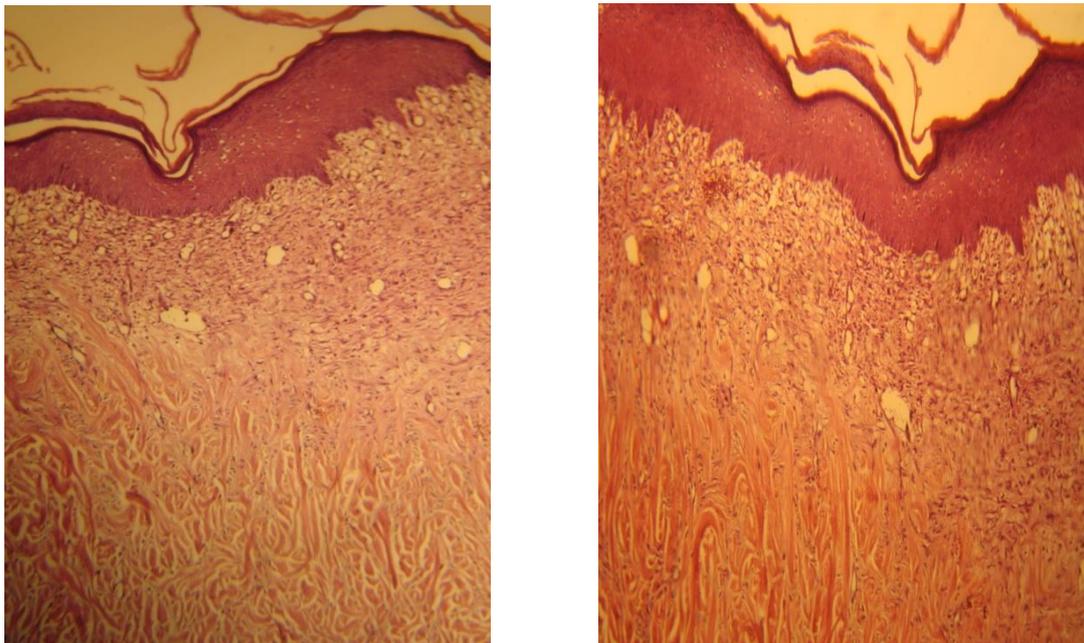
**Figure(4): Biatain group one week(sever ulceration with scar formation and inflammatory cells)X40 .**



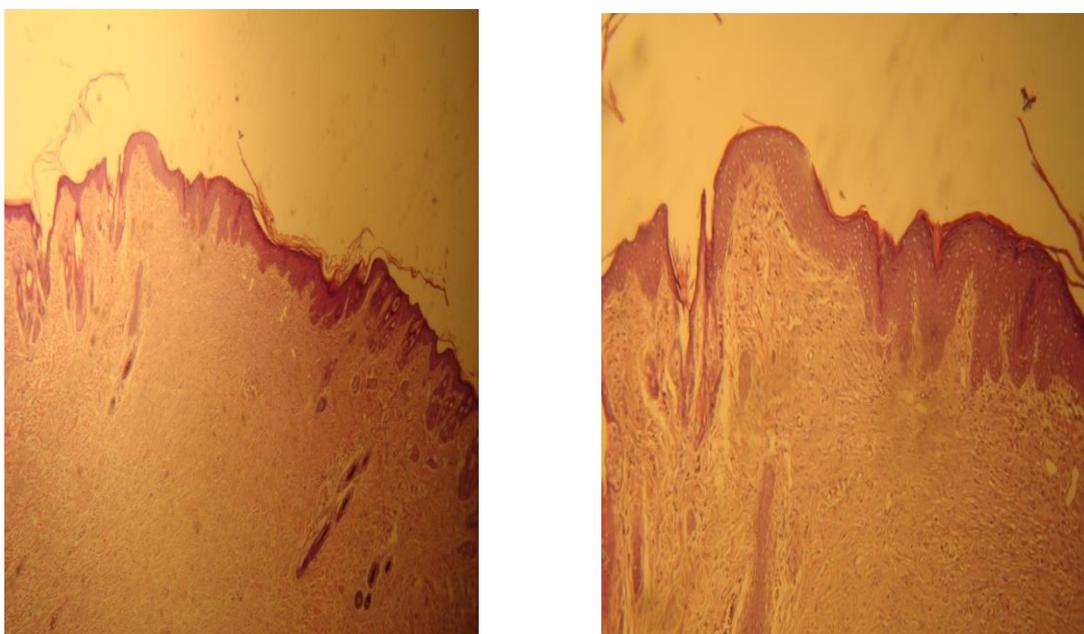
Figure(5) : Laser group tow week(Healed ulcer with epidermal thickening and marked dermal fibrosis).X10



Figure(7) : Biatain group tow week(Area of ulceration associated with loss of epidermal lining and heavy infiltration of inflammatory cells)X10, X40.



Figure( 8): Laser group three weeks (complete healing with marked epidermal thickening and dermal fibrosis , absence of hair follicles and sebaceous glands)X10.



Figure(9): Biatain group three week( Moderate epidermal thickening and marked dermal fibrosis , absence of hair follicles and sebaceous glands)X10.

**Discussion:**

Low power laser radiations have been used in medicine for over three decades in many medical centers of the world to treat a wide variety of diseases (acute and chronic musculoskeletal aches and pains , edema , indolent ulcers , chronic inflammation and autoimmune diseases .In all these medical applications, clinical observation, imaging techniques, biochemical and histological examination were used for the evaluation of the effects of laser radiation on the biological tissue(1).

In this paper we have proposed the use of laser and Biatain Ag non adhesive as a useful, rapid and way to evaluate the effect of low power laser radiation and biatain dressing on wound healing based on the changes in the histopathological of the injured area.

Tissue healing is a complex process that involves local and systemic responses. The use of Low Level Laser Therapy (LLLT) for wound healing has been shown to be effective in modulating both local and systemic response . Additionally, LLLT causes the release of healing enzymes, which were required for optimal functioning, as well as the production of

endorphins, which are natural pain killers[Adel et al,2011].

Acceleration wound healing is an important goal in general surgical practice , more importantly wound healing with minimal scar formation is highly advocated in plastic surgery.

In all groups of laser and Biatain group, there were ulceration formation in 1and 2week of treatment, then absent in 3week , and this result agreed with (Vogensen et al,2008),in that which after one week of treatment the wound(ulcer) was reduce by 30%, and after two week , the ulcer area was reduce by 69%. The reduction continued to a relation reduction of 96% after three weeks. The Biatain Ag non-adhesive demonstrated fast improvement of the wound bed composition and complete healing was obtained within the four &a half weeks treatment period odour was reduce and exudates level decrease continuously (7).

Dermal fibrosis and epidermal thickening can result from increase in inflammatory cells infiltration or epidermal odema associated with enhanced blood flow .

In general, fibroblasts are known to be essential in the healing of tissue injuries including surgical wounds, the epithelialization and granulation tissue formation was created in the repair stage; fibroblasts begin to synthesize collagen and ground substances(11,12). The laser-treated group in comparison with the Biatain Ag group showed higher numbers of fibroblast proliferation in the early stage. These observations confirm the results achieved by a previous study(13) that indicated a possibility of laser-induced fibroblast proliferation during healing mechanism. The effect of laser stimulation of fibroblasts on wound regeneration is by the maintenance of a high mitotic activity of the fibroblast in the later healing period(14), in which it was demonstrated that LLLT preferentially stimulates resting cells rather than proliferating ones. The macroscopic and microscopic results in group II could be due to the increased collagen production by fibroblasts. This in turn affects LLLT in enhancing the job of mononuclear cells through production of leukotrien-B4, which is derived from arachnoid acid and production of interleukin-8, which promote

fibroblasts(15). These results are similar to those in this study. The results showed clear promotion of healing in the treated group in comparison with the control group . LLLT can facilitate wound healing, which may be due to acute inflammation is resolved more rapidly and the proliferation phase of healing begins earlier(16) therefore, the LLLT decreased the inflammatory reaction of wound healing. During the healing stage, some studies revealed the presence of neutrophils at the site of injury at 48-96 hours and reached a peak in 72 hours(17,18).

Biatain Ag releases silver ions according to the amount of exudates present in the wound. The more exudates the more silver is released. The release persists for up to 7 days (19,20). The silver is evenly distributed in the foam. When the dressing is applied, silver is released continuously, depending on the amount of exudates from the wound. Biatain Ag demonstrates wide spectrum antimicrobial efficacy against bacteria that are commonly present in non-healing wounds3, 8(20,21).

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