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Use of Povidone Iodine plus Diode Laser for Treatment of Infected Wound in Mice

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Abstract

Objective: The study was aimed to evaluate the 660 nm (6J/cm^2) laser treatment alone, povidone iodine alone, and the combination of both on healing of induced infected open wounds in mice.

Methods: Forty adult male mice were utilized. Two open full-thickness (0.5cm in diameter) skin incision on the back of the animal were made. Infection was creating in the cranial incision with *Pseudomonas aeruginosa* (inoculation of 0.2 mL bacterial suspension containing 2x10^8 CFU/mL of *P. aeruginosa* after 24 hrs. from wounding). The caudal incision not inoculated, and left without treatment as control in the same animal. Animals were randomly divided into four groups (n 10). G1; Control group; not treated with povidone iodine or diode laser. G2; treated with single dose 6J/cm^2 diode laser / day for 7 successive days post infection (PI). G3; treated only with topical application of 10% povidone iodine for 5 min/day for 7 successive days PI. G4; treated with a topical application of povidone iodine plus diode laser for 7 successive days PI. Direct measurement of wound dimensions at 7th and 14th days PI was done and specimens of healed skin for histopathological examination was taken.

Results: Cranial wounds in G1 exhibit more pus at the 7th day PI, and at 14th day the size of wound become more wider, large quantity of pus under scab was seen, and the healing did not occur, also the infection was seen involving the distal wound in some animals. The cranial wounds of G2 at 7th day PI display inexistent of infection, the size of wound decreased more than 50% and become small, dry, with no scab. Histologically complete normal skin layers dermis and epidermis was seen. At 14th day PI, the wound become barely visible. In this time also, histologically complete epithelialization of both cranial and caudal incisions was developed. Cranial wounds of G3 at the 7th day PI display vanish infection, while wound still covered with scab, and little reduction in size in compare with control in same animal, and control group at 7th day. On the 14th day PI, complete epithelialization, and more reduction in size were seen. Histologically, regenerated epidermal layers with severe inflammatory infiltration were seen at 7th day, and normal skin structure seen at 14th day. Cranial wounds of G4 at the 7th day PI show inexistent of infection, presence of scab with more reduction in size in compare with day 0, and little reduction in size in compare with wounds of control in same animal. On 14th day PI the treated wounds show more reduction in size, no scab cover the incisions and complete epithelialization of both incisions reach hard to distinguished from the normal skin. Histologically demonstrate formation of an epithelized epidermis bridging between the wound edges, and newly formed hair follicle were seen at 7th day, and completely normal skin layers and cells were seen at 14th day.

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Conclusions: The 660 nm, 6J/cm^2 LLLT used in this experiment effectively inexistent of the wound bacterial infection caused by *Pseudomonas aeruginosa* in mice, and enhancement of open wound healing. The 10% povidone iodine eradicates the infection, with negligible delay in wound healing in compare with laser group. The povidone iodine and laser interact additively or synergistically in enhancement of wound healing, and against wound infection in this experiment.

Key Words: LLLT, PDT, aPDT, *P aeruginosa*, Povidone iodine, Wound healing.

Introduction

Skin is the largest external organ of the body, serves as a protective barrier. It performs a variety of activities to maintain body homeostasis, which aims to preserve life, including protection, thermoregulation, sensory, and immunological and endocrine processes (1). The wound is a break in the skin that can cause infection and sepsis if left untreated. The bacteria that ordinarily live on the skin, such as coagulase-negative *Staphylococci*, *Streptococci*, *Bacillus* species, and *Corynebacterium* species, quickly contaminate open wounds. *Pseudomonas aeruginosa* (PA) was invariably related with chronic intractable wounds, and it was found in 50% of these instances. The difficulties in treating PA wound infection were mostly due to drug-resistant strains and the production of bacterial biofilms. As a result, novel treatment techniques for eradicating PA and associated biofilms on a local level are required (2).Antibiotics are the most common treatment for infected wounds. Antibiotics are becoming less effective for bacterial wound infections as a result of widespread antibiotic abuse and an increase in the number of multi-resistant bacterial species such as *Pseudomonas aeruginosa* and methicillin-resistant *S. aureus* (3). Dressings, topical medications like iodine, and, more recently, negative pressure therapy is all traditional local remedies for infected wounds (4). Bacterial resistance to antibiotics and certain antiseptics is a problem that must be addressed (5). The increasing and widespread resistance to antibiotics necessitated the development of new medications and/or alternative therapies capable of eradicating resistant germs and preventing the emergence of new forms of resistance (6). Photodynamic therapy (PDT) and phtobiomodulation (PBM) are two promising approaches. PDT is a technique that is regularly approved for the treatment of certain cancers. Antimicrobial photodynamic therapy (aPDT) refers to the use of photodynamic therapy as a therapeutic modality for treating localized microbial infections. aPDT kills microbial cells by combining light with a photosensitizer (PS) and oxygen. In vitro, photosensitizers like methyl blue, toluidine blue, and aluminum disulphonated phthalocyanine have been demonstrated to increase the lethal impact of lasers on bacteria (7).Low level laser therapy (LLLT), or (PBM), is thought to have three main effects: pain reduction, inflammatory management, and healing acceleration. The use of LLLT as a biostimulatory modality for the treatment of chronic wounds is being investigated. When used correctly, laser irradiation appears to be beneficial to wounds. The presence or absence of bacterial infection could be one criterion for choosing appropriate cases for LLLT. Laser therapies designed to induce protein synthesis in wounds may also encourage bacterial development, interfering with wound healing and thus explaining the lack of efficacy

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observed in experimental trials (8). Laser irradiation has been shown to have both a stimulatory and suppressive effect on microorganisms in vitro. The growth stimulation and inhibition were largely dependent on bacterial species and wavelength of irradiation and that effects were produced by low rather than high radiant exposures (8, 9).Povidone Iodine (Polyvinylpyrrolidone-iodine (PVP-I) (PVI) is a foam solution composed of iodine, used for scrubbing, antisepsis of healthy skin, preoperative shower; for which, it should be used in its purest form. For the cleansing of contaminated wounds, it is recommended that one-third be diluted with water (10, 11). The PVI preparations are well known to be effective against multiple bacteria, fungi and protozoa. They are also effective against enveloped and non- enveloped viruses. PVI is thought to have a multimodal activity against pathogens, which includes oxidizing vital structures such as amino acids, nucleotides and membrane components (12, 13, 14). The described beneficial sterilization properties of PVI include broad antimicrobial spectrum, lack of emergence of resistance, ability to penetrate biofilms, low cytotoxicity to host cells, tolerability, cost- effectiveness and overall favorable risk/benefit profile (12, 13). The study was aimed to evaluate the effect of 660 nm laser irradiation alone, the povidone iodine alone, or the combination of both, on healing of *Pseudomonas aeruginosa* infected open wounds in mice.

Materials and Methods

Forty adult male mice were utilized. Two open circular full-thickness (0.5cm in diameter) skin wounds on the back of animal were made. Wound infection was induced by inoculation with 0.2 mL bacterial suspension of *Pseudomonas aeruginosa* (approximately $2x10^8$ CFU/mL) in the cranial wounds after 24 hrs. from wounding, the infection was occur after 72hrs. Then the infected wound was treated with povidone iodine or laser or both. While the caudal wound was not inoculated with bacteria, and left without treatment as control in the same animal. Animals were randomly divided into four groups (n 10). Control group (G1); in which the infected wound (cranial) not received any treatment with povidone iodine or diode laser and served as control. Laser group (G2); in which cranial wounds treated only with diode laser (GaALAs) (Omega Laser Systems Limited, UK) (660 nm wave length, $6J/cm^2$ (energy density) single dose/day for 7 successive days post infection (PI) (15). Povidone iodine group (G3); in which cranial wounds treated only with topical application of 10% povidone iodine for 5 min/day for 7 successive days PI. Povidone iodine + Laser group (G4); in which cranial wounds treated with a topical application of povidone iodine plus diode laser for 7 successive days PI. Morphometric assessment of the wound healing organized, by direct measurement of wound dimensions at 7^{th} and 14^{th} days PI. Specimens of healed skin (1 cm^3) were taken after (7, and 14) days PI, for histopathological examination after sectioned in 6µm and staining with H&E stain to evaluate the progress of healing process (16).

Results

All animals (control and treated) throughout the time of experiment (14 days) post infection (PI) clinically were seen healthy, and active, with the exception of three animals dead from the control group 5 days after inoculation of bacteria were recorded.

Cranial wounds of G1 at 7^{th} day PI show increase the size of wound, more signs of

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infection, wounds was covered with thick scab, and a large quantity of pus (creamy pale yellow secretions) beneath the scab was found in compare with day 0 of infection. On the 14th day no signs of healing was seen, the size of wound become wider, also the scab was still found, and hug quantity of pus under the scab were seen. the infection was seen extending and involving the distal wound in some animals. Cranial wounds of G2 at 7th day PI show inexistent of infection, the wound become small, dry (no exudate), no scab, and early epithelialization was developed. At 14th day post infection, it exhibit marked reduction in size of wound, the wound become small and fad with tiny scar. The size of the wound after the 7th day decreased more than 50%, and at the 14th day were become barely visible and their sizes approached zero. Complete epithelialization of both incisions were occur, the cranial incision exhibit fleshy raw epithelialization, while the control incision show more advanced epithelialization reach to difficult to distinguished from the normal skin. Cranial wounds of G3 at the 7th day PI show vanish infection with little reduction in size in compare with control in same animal, and control group at 7th day. The scab was seen still cover the incisions. On the 14th day PI, the treated wounds show more reduction in size in compare with 7th day of same group, while both incisions (control and treated) in this time (14 days) appears in same size. Complete epithelialization, scar tissue formation, and no scab cover the incisions also seen. Cranial wounds of G4 at the 7th day PI demonstrate inexistent of infection, more reduction in size in compare with day 0, and little reduction in size in compare with wounds of control in same animal, while the scab still cover the both wounds. On 14th day PI the treated wounds show more reduction in size in compare with control wounds, also there was complete epithelialization of both incisions reach hard to distinguished from the normal skin, scar tissue formation, and no scab cover the incisions, cranial wounds show more reduction in size in compare with caudal of same animal. The shape of incisions sometimes remains circular and other times change to liner. Caudal wounds in treated groups (G2, G3, and G4) at 7th and 14th day exhibit no development of infection, while the infection was involving the caudal wound at 14th day in most animals of control group. The edges of the wound initially at early hours of day 0 were seen clean, marked sharp, then all the wound become swell. Primarily all wound areas increased in size within 4 hours after creation of wound and continued in swelling and increase in size for the next 24 hrs. post wounding (P.W.) with exaggeration of the inflammatory signs. The whole wound seen swollen, and the edges of the wounds were elevated, red in color, and from the second day P.W. show a thick scab upon the wound persisting more than the 7th day P.W. The wounds exhibit normal healing processes and gradually decreased in size till the 14th days were become small scar tissue as circular or liner in shape in treated groups, while become worse, and pus discharging in (G1) control group (Fig. 1).

Histologically: The cranial wounds 7 days (PI) in G1, show absence of re-epithelization and substantial infiltration of inflammatory cells PMNC (neutrophils) in the wound site, randomly oriented collagen fibers, and sometimes shows horizontally oriented collagen fibers, also there was an accumulation of fibroblast in the wound area (Fig.2a). In G2, re-epithelialization was occur in most of wound surface, and healing of the underlying dermis was nearly complete; slight hemorrhage, edema, and infiltration of the inflammatory cells in the epidermis was

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www.qu.edu.iq/journalvm

notified. The prominent feature in this group was completely normal skin layers epidermis and dermis (Fig. 3a). G3 show incomplete regenerated epidermal layers and presence of thick crust containing inflammatory cells has adhered to the epithelium. In the dermal layer, edema was notified beneath the regenerated epidermal layers, also there was a severe inflammatory infiltration of different poly nucleated and mono nucleated inflammatory cells, and randomly arranged collagen fibers (Fig. 4a).

G4 shows formation of an epithelized epidermis bridging between the wound edges, covered by a thin scab, inflammatory cells in the epithelized epidermis were also notified. Newly formed hair follicle, and newly formed blood vessels are notified, and infiltration of inflammatory cells in the dermis layer were present (Fig. 5a).

The cranial wounds 14 days (PI) in G1 shows massive infiltration of neutrophils (PMNC), and debris tissue, no epithelial layers, no signs of process of wound healing, except tiny lines of the early stage of re-epithelialization, categorized by migration of epithelial cells from the periphery of wound underneath small crust (scab) containing inflammatory cells adheres to the edge of the unhealed wound surface. The dermis was gotten randomly oriented arrangement collagen fibers, massively infiltrated with inflammatory cells, fibroblast, and newly formed blood vessel was also present (Fig. 2c).

The cranial wounds 14 days (PI) in G2 show well-developed and complete re-epithelization of entire epidermis. In the regenerated dermis, there was mild edema between the dermis and epidermis, few inflammatory cells and sometimes no infiltration of inflammatory cells were seen, and fibroblast accumulation, newly formed blood vessels and newly formed hair follicles were also seen. The dominant feature of the field was completely normal skin layers and cells (normal skin show) (Fig. 3c). G3 display complete regenerated epidermal layers covered by scab and slight edema between regenerated epidermis and dermis also notified. In dermis, newly formed blood vessels and infiltration of mononuclear inflammatory cells also present. Often normal skin structure shows and newly formed hair follicle also seen (Fig. 4c). G4 show complete re-epithelization of the epidermal layers bridging between the wound edges where covered by thick scab; infiltration of inflammatory cells, mild edema were also notified. The dominant feature of the field was appear as completely normal skin layers and cells (normal skin show) (Fig. 5c).

The caudal wounds 7 days (PI of cranial wounds), in G1 shows the initial stage of reepithelization and no scab were found upon the wound. There was infiltration and severe accumulation of inflammatory cells (PMNC), as well as accumulation of fibroblasts in the wound site (Fig. 2b). G2 exhibit presence of scab shielded the wound. The epidermis shows abundant re-epithelization process elucidated by the migration of epithelial cells from the periphery toward the center of the wound. Sometimes nearly complete healing of the epidermal layers were seen (thick layer of the epidermis) beneath thick scab forming, and infiltration of inflammatory cells in the dermis layer including infiltration of neutrophils, and few macrophages. Nearly completely regular healing skin layers (epidermis and dermis) and completely normal epidermis layers of skin (Fig. 3b). G3 exhibit presence of thick epithelial crust cover the wound, an initial stage of re-epithelization informed by formation of epithelial tongue from the periphery to the center of wound, and infiltration and accumulation of inflammatory

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cells underneath the regenerated area (Fig. 4b). G4 exhibit presence of thick scab over the epithelized epidermal layer, and the epithelial tongue also notified in the dermis layer. The epithelial tongue consists of newly formed blood vessels and chains of fibroblasts. Accumulation of evenly arranged collagen fibers in the edge of the skin wound was seen. Also inflammatory cells in the wound surface were present, and accumulation of inflammatory cells in the dermis layer also (Fig. 5b).

The caudal wounds 14 days (PI of cranial wounds), in G2 show re-epithelization of the epidermis layer. In the dermis, collagen fiber accumulated beneath the regenerated epidermis, and forming new hair follicles was also notified. Healing of the underlying dermis is nearly complete (Fig. 3d). G3 show good re-epithelization of the epidermal layers covering the wound; there is edema in the dermis layer and absence of inflammatory cells. Some fields show large crust sheltering the skin wound who shows incomplete regenerated epidermal layer, and there was a migrated tongue that is mainly composed of inflammatory cells, thin collagen fibers and fibroblasts, and presence of edema beneath the regenerated epidermal layer, with infiltration of different inflammatory cells (poly nucleated and mono nucleated) inflammatory cells in the dermal layer (Fig. 4d). G4 display absence of scab over the wound. The epithelial layers were regenerated completely, they were composed from 4 layers rested upon basement membrane, including tinny and thin keratinized layer. The dermis layer shows infiltration of many mononuclear and few poly nuclear inflammatory cells and accumulation of many fibroblasts and moderate new blood vessels arranged horizontally with the surface of the wound with no congestion of blood vessels also notified. More collagen fibers arranged horizontally. Edema also present. The field appears like normal skin tissue (Fig. 5d).

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

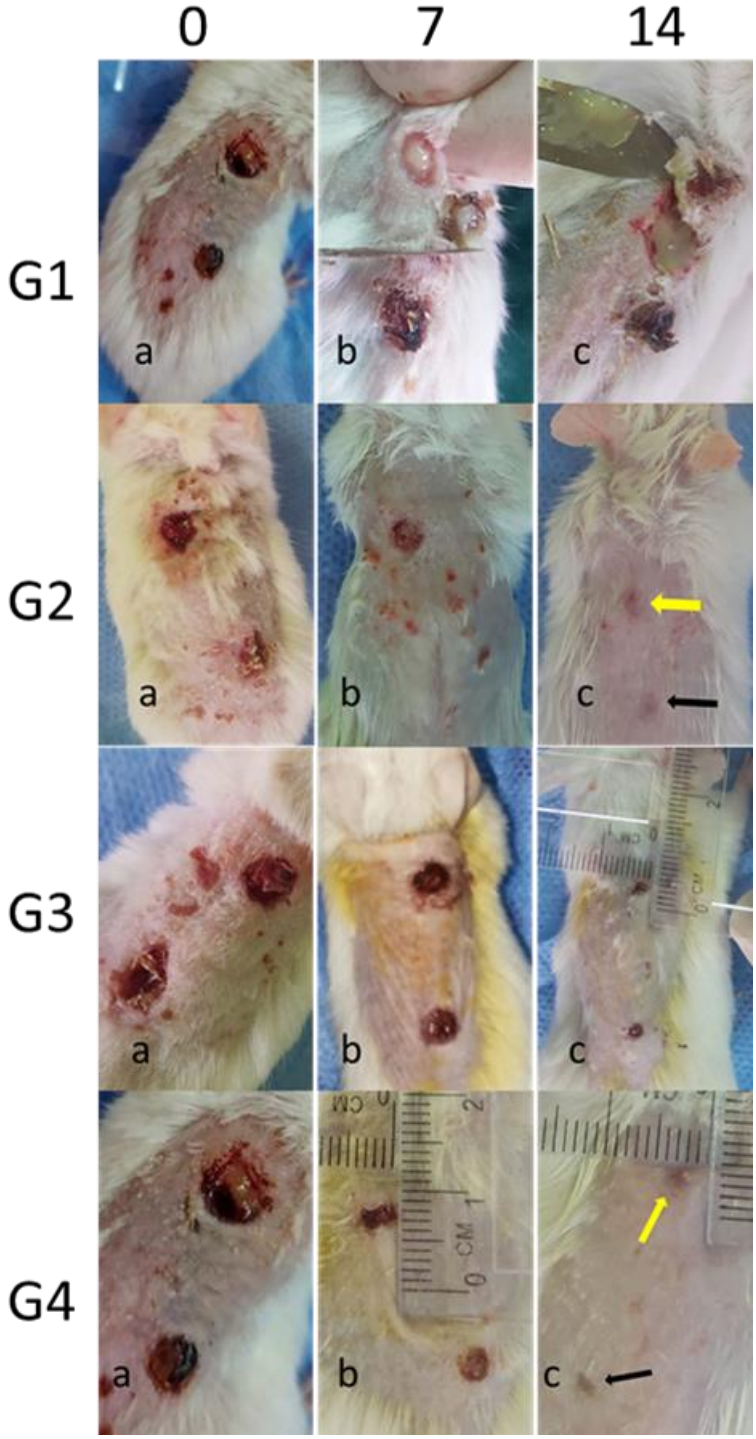

Fig. (1):Macroscopic photographs; G1 (control group); G2; (Laser group); G3 (povidone iodine group); G4 (povidone iodine + laser group): (a)Time zero, (three days after inaculation of *Pseudomonas aeruginosa* in cranial wound), (b) 7 days post infection PI, (c) 14 days post infection. G1(a); see the early signs of infection in cranial wound, the wound become larger, presence of patches of pus bneath the scab. The caudal wound exhibit no signs of infection. G1(b), see the large quantity of pus under the scab in the cranial wound. No signs of infection in the caudal wound. G1(c) see a huge quantity of pus in the cranial wound and on the blade after removing of scab. The caudal wound has no infection but still large and covered with scab. G2(a) see the early signs of infection in cranial wound, the wound become larger, presence of little pus bneath the scab. No signs of infection in the caudal wound. G2(b) The cranial wound, see inexistent of infection, the wound becom less small, dry (no exudate), no scabe, and early epithlialization was seen.The caudal wound

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

become more smaller and more epithelialization. G2(c) see complete epithelili-zation of both incisions, the cranial incision exhibit fleshy raw epithelialization (yellow arrow), while the caudal incision show more advanced epithelialization reach hard to distinguished from the normal skin (black arrow). G3(a) Cranial wound, see the early signs of infection, the wound become larger, presence of little pus bneath the scab. No signs of infection in the caudal wound. G3(b) Cranial wound see vanish infection, the wound becom less small, and coverd with scabe. The caudal wound have same size of the cranial, dry and covered with scab. G3(c) See more reduction in size, more advance epithelilization of both incisions. (G4)(a) Cranial wound see the early signs of infection, the wound become larger, presence of expanses of pus splash the wound bneath the scab. No signs of infection in the distal wound. G4(b) Cranial wound see inexistent of infection, the wound becom less small, dry, and cover with scabe, and early epithlialization was seen.The caudal wound become more smaller and more epithelialization. G4(c) See complete epithelilization of both incisions reach to difficult to distinguished from the normal skin, the cranial incision (treated) exhibit wider area of epithelialization (yellow arrow). The control incision show same degree of epithelialization (black arrow).

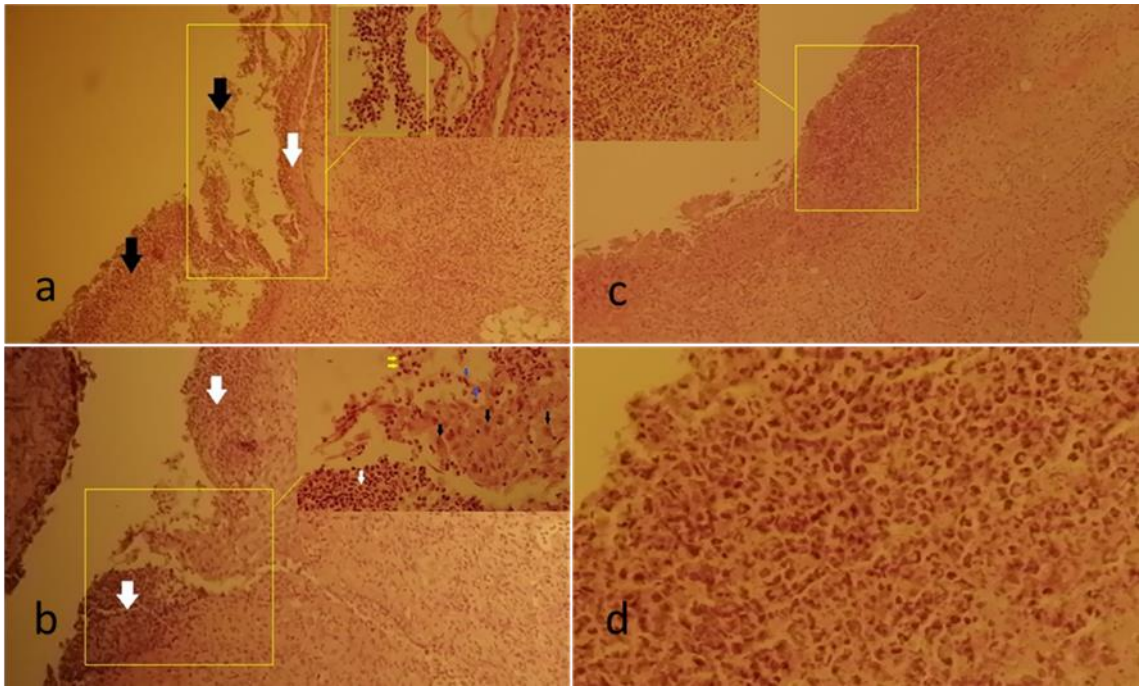

Fig. (2); G1; (a) Cranial wound 7 days PI, shows absence of re-epithelization, horizontally oriented collagen fibers in the wound site (white arrow) and infiltration of inflammatory cells (black arrows). H&E, 10X. (b) Caudal wound 7 days (PI of cranial wound), shows an initial stage of re-epithelization and accumulation of inflammatory cells in the wound area (white arrows). H&E, 10X. (c) Cranial wound 14 days PI, shows infected skin area, no epithelial layers, no process of wound healing is notified. Also shows massive infiltration of neutrophils (polymorphonuclear cells), and debris tissue, H&E, 10X. (d) Cranial wound 14 days PI shows infected skin area, shows massive infiltration of neutrophils (polymorphonuclear cells), and debris tissue, no epithelial layers, no sign of wound healing is present. H&E, 40X.

Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

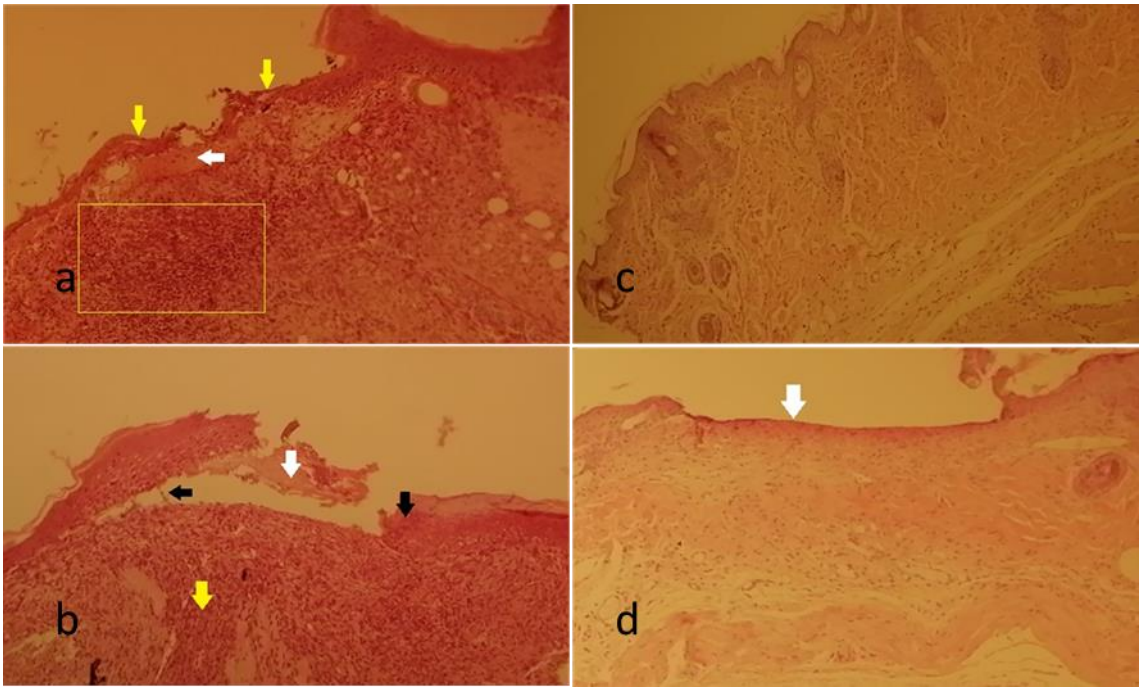
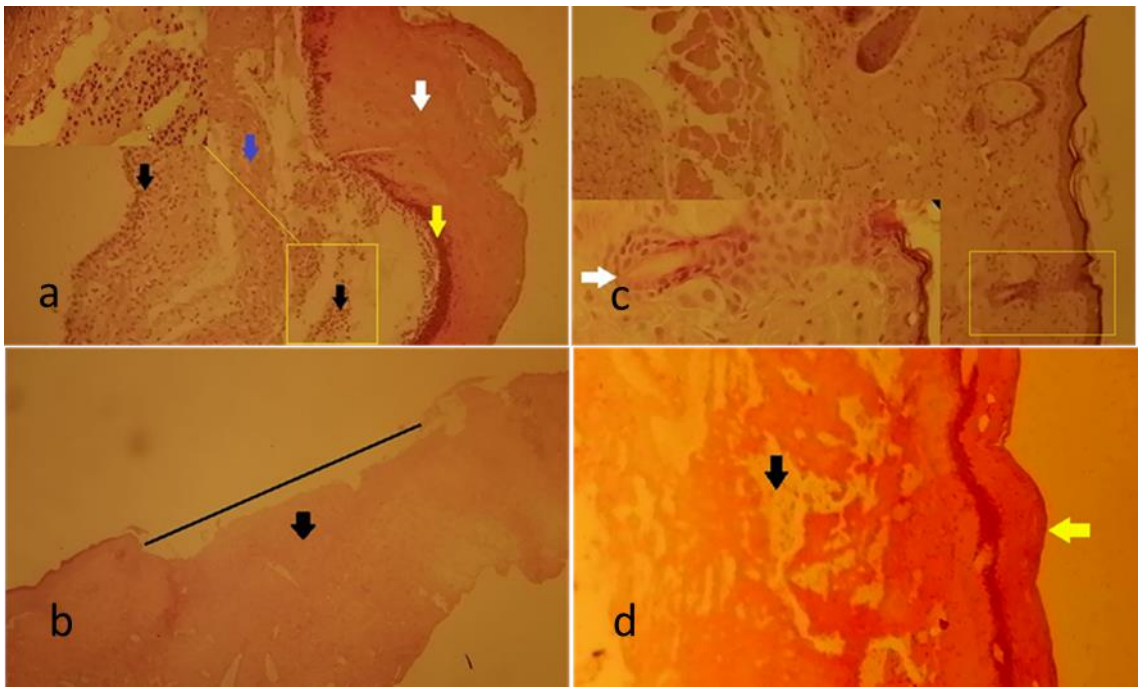


Fig. (3); G2; (a) Cranial wound 7 days PI shows most of the wound surface is re-epithelialized (yellow arrow), and healing of the underlying dermis is nearly complete; slight hemorrhage is present (white arrow), and infiltration of the inflammatory cells (yellow box) in the epidermis is notified. H&E, 10X. (b) Caudal wound 7 days PI shows the migration of epithelial cells (black arrows), scab forming (white arrow), and infiltration of inflammatory cells in the dermis layer (yellow arrow). H&E, 10X. (c) Cranial wound 14 days PI shows completely normal skin layers and cells. H&E, 10X. (d) Caudal wound 14 days PI show most of the wound surface is re-epithelialized (white arrow), and healing of the underlying dermis is nearly complete. H & E, 10X.



Al-Qadisiyah Journal of Veterinary Medicine Sciences
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www.qu.edu.iq/journalvm

Fig. (4); G3; (a) Cranial wound 7 days PI shows an incomplete regenerated epidermal layer (yellow arrow) and a large crust (white arrow); also, edema is notified beneath the regenerated epidermal layer. However, a severe inflammatory infiltration in the dermal layer (black arrows) and randomly arranged collagen fibers is also present in the dermal layers. H&E, 10X. (b) Caudal wound 7 days PI shows complete loss of epithelial layer (black line) with an initial stage of re-epithelization and accumulation of inflammatory cells in the affected area (black arrow). H&E, 4X. (c) Cranial wound 14 days PI shows normal skin structure H&E, 10X., a newly formed hair follicle (white arrow). H&E, 40X. (d) Caudal wound 14 days PI shows a good re-epithelization (yellow arrow) of the epidermal layer covering the wound area; there is edema (black arrow) in the dermis layer and absence of inflammatory cells. H&E, 10X.

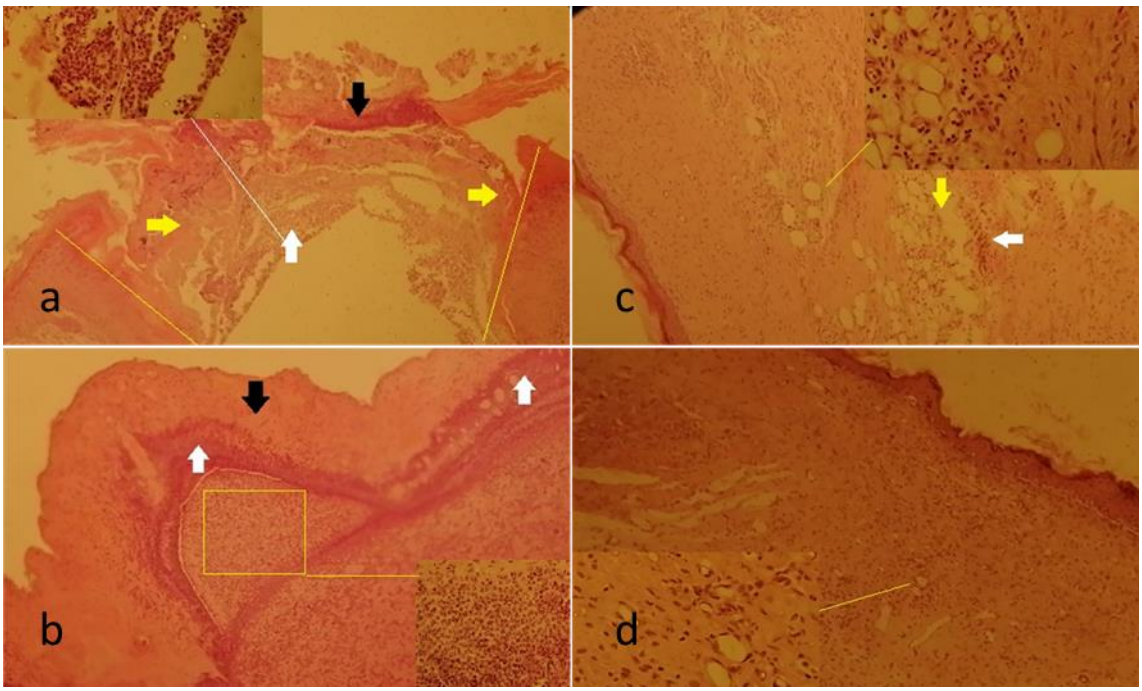

Fig. (5); G4; (a) Cranial wound 7 days PI shows the formation of a re-epithelized bridge between the wound edges (yellow lines). The epithelized epidermis (yellow arrows) covered by a scab (black arrow) and inflammatory cells (white arrow) in the epithelized epidermis are also notified. H&E, 10X. (b) Caudal wound 7 days PI shows presence of thick scab (black arrow) over the epithelized epidermal layer (white arrow). H&E, 10X., infiltration of inflammatory cells (magnified yellow box). H&E, 20X. (c) Cranial wound 14 days PI shows normal skin tissue, several blood vessels are present, and infiltration of few inflammatory cells (white arrow; yellow line) are notified, edema also present (yellow arrow). H&E, 10X. (d) Caudal wound 14 days PI shows normal skin tissue, several blood vessels are present, and few inflammatory cells (yellow line) are notified. H&E, 10X.

Discussion

The cranial wounds (infected wounds) in G1, 7 days (PI) display absence of re-epithelization in the wound site, massive infiltration of inflammatory cells, accumulation of fibroblast, and randomly oriented collagen fibers in the wound site. Infections with bacteria have a particularly deleterious effect on wound healing, especially when the wound already exhibits delayed wound healing (17). Bacterial infections slow down wound healing because they prolong the inflammatory phase (18). Macroscopically the cranial wounds of G2 at 7^{th} day PI show marked drop of signs of infection, the wound dried up and shrank, there was no scab, and early epithelialization developed. The wound shrank significantly by the 14^{th} day, becoming little and disappearing with a small scar. Both incisions had complete epithelialization; the cranial incision had meaty raw epithelialization, whereas the control incision had more progressed

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epithelialization that was difficult to identify from normal skin. Microscopically, the infected and treated wounds show re-epithelialization of most of the wound surface, near-complete healing of the underlying dermis, and infiltration of inflammatory cells in the epidermis. The epidermis and dermis of the skin were seen to be entirely normal in this group. This result is in agreement with (19), who found complete healing of the epidermis with keratin formation, crust and small hair follicles can be seen at the site of injury. Our result indicates inhibition of infection with G2 laser treatment group. Two *in Vitro* studies (8, 9) indicating that LLLT inhibit the growth of bacteria. The growth stimulation and inhibition were largely dependent on bacterial species and wavelength of irradiation and that effects were produced by low rather than high radiant exposures. *P. aeruginosa* appeared to be more affected by laser irradiation than *E. coli* and *S. aureus*, although some similarities were detected in the responses of *P. aeruginosa* and *E. coli* to irradiation with all wavelengths at a radiant exposure of 1 J/cm^2. Of the four wavelengths (630, 660, 810, and 905 nm) used in this study, irradiation with 630 and 905 nm most consistently inhibited and stimulated bacterial growth respectively. However, the effects of 905 nm irradiation were not significantly different when compared with controls for any bacteria. *P. aeruginosa* was inhibited using wavelengths of 630, 660, and 810 nm, and as with *E. coli*, maximum inhibition was achieved with 630-nm irradiation at radiant exposure of 1 J/cm^2. Irradiation using 810 nm (0.015 W/cm2) increased *E. coli* growth (8). Laser irradiation with 810 nm decreased growth of *P. aeruginosa* and increased growth of *E. coli*. Exposure to 810-nm irradiation (0.03 W/cm2) could potentially benefit wounds infected with *P. aeruginosa*. However, increased *E. coli* growth could further delay recovery (9). The dose (6 J/cm^2) used in G2 show had an additional effect on accelerating healing of the infected open skin wound in mice, by reducing inflammation, increasing collagen fiber synthesis and more deposited, rising fibroblast activity, and myofibroblast activity is increased. The wave length of (660 nm) used in our experiment in G2 and G4 is accord with (20) who found the diode laser with a wavelength in the range of 655–980 nm is able to accelerate wound healing by promoting angiogenesis and release of growth factors. At the 7th day PI, the wounds of G3 exhibit minimal reduction in size and are still covered with scab. The cranial wounds are smaller on the 14th day PI than they were on the 7th day, while the control wounds are the same size. The incisions had complete epithelialization, scar tissue development, and no scab. Microscopically At 7 days (PI), the cranial wounds of G3 demonstrate an incompletely regenerated epidermal layer and the presence of a substantial thick crust, indicating the existence of debris foci in the epidermis. Edema was found beneath the regenerated epidermal layers in the dermal layer, as well as a significant inflammatory infiltration of various poly nucleated and mono nucleated inflammatory cells, as well as irregularly oriented collagen fibers. Although we found povidone iodine reduce the signs of infection, but the size of wound little reduced. Povidone iodine in high concentrations is toxic for both the microorganism and the cells of the tissue (21). It inhibits leukocyte migration and fibroblast aggregation in wounds. Povidone iodine used as wound cleaning solution in 10% concentration. Previous studies illustrate that povidone-iodine solutions diluted to concentrations of 0.1% to 5% were more effective in killing common wound contaminants than was the 10% stock

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solution. Concentration greater than 0.05% is toxic to all of the cell types that are essential to the healing process, like granulocytes, monocytes, keratinocytes, and fibroblasts (22). The cranial wounds of G3 at 14 days (PI); Show complete regenerated epidermal layer, and Infiltration of mononuclear inflammatory cells. This result is supported by (23) who showed that topical application of 0.5% PVI promotes acute cutaneous wound healing. The healing process of cutaneous wounds involves numerous cell types, including neutrophils, macrophages, fibroblasts and endothelial cells. At day 1 to 14 post injury povidone-iodine (PVI) treatment enhanced cutaneous wound closure and granulation tissue formation and maturation wounds from a control and PVI treated rat (23). In our study at 14th day newly formed blood vessels in the dermis layer, this result accordance with (23) who found organized formation of granulation tissue, increased growth of new vessels, may be attributed to the increased growth of new vessels. TGF-β stimulates endothelial cell migration, angiogenesis neovascularization, following PVI treatment. G4 cranial wounds have shrunk in size and are covered with scab on the 7th day of PI macroscopically. On the 14th day, the wound had completely epithelialized, making it difficult to identify it from normal skin. Microscopically, the wound shows freshly created hair follicles and blood vessels, as well as inflammatory cell infiltration in the dermis layer. The combination between PVI and laser in G4 group reduce the infection, and accelerate the wound healing reach to earlier show of skin adnexa development. In this study, povidone Iodine and laser interact synergistically or additively against pseudomonas microorganisms, and for healing of wound. PVI here not act as photosensitizer as a part of antimicrobial photodynamic therapy (aPDT). Two studies only were found used PVI as a photosensitizer (24, 25). Antimicrobial photodynamic therapy (APDT) combines the use of light with a photosensitizer (PS) and oxygen to kill microbial cells. Results of G4 also according with (24) who demonstrated that, lower-power laser combined with povidone-iodine as a photosensitizer to kill *P. aeruginosa* causing various infections to man. Our results settled with (25), he was found that povidone iodine were good photosensitizer with different exposure times to sensitive *P. aeruginosa* for killing by low-power diode laser light and there were synergistic effects between laser light and the photosensitizers. Exposure of *P. aeruginosa* isolates to low-power diode laser light in the presence of photosensitizer such as povidone iodine was very effective and led to acceleration of wound healing. Also, (26) suggested that NIR laser light irradiation by itself would also inhibit growth of P. aeruginosa in infected wounds and only the continuous-mode of irradiation was capable of killing *P. aeruginosa* (26).In Conclusion**:** The 660 nm, 6J/cm^2 LLLT used in this experiment effectively inexistent of the wound bacterial infection caused by *Pseudomonas aeruginosa* in mice. Treatment of *Pseudomonas aeruginosa* infected wounds with laser (660 nm, 6J/cm^2) once time for 7 successive days, gives the best results in suppression and eradication of infection and enhancement of open wound healing. Povidone iodine 10% suppress and eradicate the *Pseudomonas aeruginosa* wound infection, with negligible delay in wound healing in compare with laser group. Povidone iodine and laser interact additively or synergistically against *Pseudomonas aeruginosa* microorganisms in this experiment.

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