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The healing effect of Chitosan nanomaterial versus Low Level Laser Therapy (660nm) on Second Degree Burn Wounds in Rats

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Abstract
The goal of the present research is comparing the histological and clinical outcomes regarding the application of chitosan nanomaterial and low level laser on rats with superficial second-degree burns. The 30-adult male Wistar rats, weighing (240 ± 20gm), have been placed into 3 equal groups at random, with the G1/control group receiving no treatment. Topical treatment of ChNMS has been used for treating the G2/Chitosan group (ChNMs) for a total of 7 days. G3/Laser group (LLLT) received 8J/cm2/day treatments for seven days in a row. Following general anesthesia, a stainless-steel bar has been placed into a boiling water bath for 15 minutes, creating one burned circle on the back of each animal's body to cause superficial second-degree burns. Histological and clinical evaluations regarding the wound healing were conducted. According to macroscopic findings, G3 and G2 wound contraction increased considerably ($p \leq 0.05$) in comparison to G1 in all groups. On day 21, an animal (in G2) treated with chitosan nanomaterials showed signs of complete wound healing. It is obvious that wound care in treated rats took place for at least 7 days quicker compared to other groups and that they had the highest rate of lesion contraction. At 7 days, the skin's surface has a substantial crust that is firmly attached to it. In addition to a modest hyperplasia of the stratum basale and an irregular, dense collagen network, adipose tissue can be found in the dermis. There are significant($p<0.05$) amounts of inflammatory cells, primarily macrophages, crust, and purulent exudate. At 14 days, the dermis has inflammatory cells, collagen fibers, new vascularization, macrophage infiltration, and fibrosis. There's also epidermal hyperplasia, visible and significant granulation tissue, a small incision, fibrosis, and bleeding. Full healing, including the complete epithelization as well as emergence of outer keratinized layer on top of epidermis, has occurred after 21 days. Bleeding along with new vascularization, skin fibrosis, and a little infiltration regarding the inflammatory cells, primarily macrophages.The stratum basale has undergone certain stratum basale hyperplasia, the epidermal layer has entirely shed, and G3 at 7 days exhibits a crusty layer above the skin's surface. Although there is an infiltration of inflammatory cells, primarily lymphocytes and macrophages, little granulation tissue actually forms. At 14 days, there is a purulent discharge and thick crust above the surface of the skin, and stratum basale had only incompletely and mildly hyperplized. Hemorrhage, granulation tissue presence, and fresh BV development with the infiltrations of the inflammatory cells in dermis, primarily lymphocytes and macrophages, a fibroblast proliferation, and thin network of the collagen. The layer of the epidermis had completely epithelized and developed keratinized layer following 21 days. In addition to fibroblast proliferation, growth of more blood vessels, RBCs, hair follicles, and thin collagen network, there is also frequent and extensive fibrosis.Tthe use of chitosan nanomaterials in the treatment of second-degree superficial burns in rats resulted in faster connective tissue regeneration and enhanced wound healing.
Keywords: Chitosan, Nanomaterials, Laser, Second Degree Burn, Rat

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Introduction

The largest organ in the body, the skin, is crucial for numerous internal processes, such as excretion, the production of vitamin D, hydration, defense against viruses and pathogens, and temperature regulation. As a result, severe skin injuries could put lives in jeopardy. Skin wound healing reveals a remarkable and distinctive cellular function mechanism. During the healing process, growth factors, cells, and cytokines collaborate to shut the lesion (1). Since they can result in severe wounds that destroy tissues and organs, burns are one of the major public health risks. In the case when the tissue is subjected to chemical, physical, and biological elements, they take place and are directly related to the tissue degeneration, pain, infections, and in some of the cases, even death (2). Skin cells are destroyed by heated solid objects, direct fire exposure, hot liquids, which results in the occurrence of the thermal burns. Because sepsis-related deaths could result from prolonged hospital stays and the treatment of potentially contagious illnesses. Burn injuries are a global health problem that are exacerbated in underdeveloped countries. Thus, they add significantly to the cost of public health systems. According to the WHO, 180,000 individuals died from burns in the year 2017, with 2/3 of those deaths taking place in Africa and Southeast Asia. Mild to severe burns are present in over a million cases from India yearly. According to INEGI, burns are the most frequent injury sustained in the home in Mexico, accounting for 128,000 cases annually, with children making up one-third of those cases (3). The second-most prevalent natural amino polysaccharide worldwide is chitin and chitin derivatives (i.e. the chitosan), whose sources include crustacean shells, like the shrimp, crabs, crayfish, krill, and others. (4) By N-deacetylating chitin, chitosan [(1, 4)-2amino-2deoxy-D-glucan] represents linear poly amino-saccharide with therapeutic qualities that speed up healing, Edema reduction, cell migration, anti-bacterial, pro-inflammatory. Promotes angiogenesis, cell growth factor activation, neovascularization, fibro-proliferation, and granulation tissue(5). Low-level laser therapy (LLLT) or laser therapy was deployed extensively for more than 5 decades (6). Based upon the tissue laser light absorption that will affect the metabolism of the cells, this technology lately showed a positive influence on stimulations of cell activities that are involved in the process of the healing of wounds. Increased ATP synthesis, mitochondrial respiration, and calcium exchange through cell membrane, which results in brief variations in the levels of cytoplasmic calcium. When tissue repair and wound healing occur, such alterations may increase the creation of DNA, RNA, and cell-cycle regulatory proteins that may encourage cell proliferation and aid the regeneration of the connective tissues (7,8).

Materials & methods

Thirty adult male Wistar rats had been obtained from the animal house in the veterinary collage/University of Al-Qadisiyah, weighing(200±30grams)with average age of (8-10 weeks) were used and housed under standard conditions in room temperature and ventilation, those animals had been allowed free access to water as well as food *ad libitum*. After general anesthesia with a mix of xylazine (10mg/kg B.W)and ketamine (50mg/ kg B.W)administered intramuscularly (9). The dorsal portion of all animals were prepared for aseptic surgery. A second degree 1cm diameter burn wound was created by a hot cylindrical stainless steel (rod) with insulated rubber handle warmed five minutes within water boiling and put for ten seconds on the skin with its own weight (160 gm) and pressure for infliction of burns (10) the rod had been lifted of boiled water through pinching the insulating handle with the middle finger and the thumb. The rod had been placed perpendicular onto skin, it rested on its own weight. Animals were individually

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kept in cages in same normal laboratory conditions. All animals were divided randomly into 3 groups (n=10). Group One (G1) control group, in which all rats did not receive any treatments. Group two (G2) (chitosan nanomaterials); burns were treated with a topical application of 10 mg/ml of chitosan nanomaterials. G2 were treated with 1ml of chitosan nanomaterials daily for 7 consecutive days, which was synthesized by dissolving the amount of 500 mg in 50ml of a solution of one percent acetic acid and stirred for 25 minutes at 1000rpm at the temperature of the room until the solution become clear. The resulted solution had been sonicated and tittered after that by adding solution of NaoH or HCL that had been adjusted to pH7 and filtered with the use of 0.20µ filter (11). Group three (G3) Low level laser group, rats were treated with low level laser as a single dose/day for incessant seven days. The diode laser which used in this treatment was Gallium Aluminum Arsenide Laser (GaALAs)(Omga Laser System Limited UK),with wave length (660nm) power (50mw) pulsing rate (146) energy density 8J/cm2. The laser was begun immediately after making skin burn and daily for seven days. Treatment was applied on each rat at the same time of the day. The laser probe is situated closely perpendicular with direct contact on the center of burn**.**The recent study has been achieved according to national guidelines for the care and use of lab animals.

Ethical approval:

The researchers obtained ethical approval from the research Ethical Approval Committee of the College of Veterinary Medicine, University of Al-Qadisiyah.

Results

On the 7th day after a burn injury, the control group (G1) showed histopathological changes that included a thick crust on the skin's surface, a few stratum basale hyperplasia with inflammatory cells, and profuse, irregular thick collagen in dermis (Fig.1-a). At 14 days after the burn injury, G1 burns displayed a wide incision, sloughing of epidermal layers, infiltration of inflammatory cells, and profuse collagen. Additionally, there was mild epidermal hyperplasia in the neighboring region, which was characterized by the development of new blood vessels, granulation tissue, and fibrosis with a thick collagen network and infiltration of inflammatory cells(Fig.1-b). At the 21st day post burn injury, the epidermal layers showed incomplete hyperplasia with narrow incision and profuse granulation tissue where there was new blood vessels' formation and profuse fibrosis with a small number of the collagen and high infiltration of the inflammatory cells (Fig.1-c). Burns of chitosan group(G2) at 7th day post burn injury, the surface of skin showed presence of thin crust with narrow incision complete epidermis sloughing, in dermis there was profuse inflammatory cell infiltration with the granulation tissue and profuse adipose tissue, thick network of collagen(Fig.2-a).At 14th day post burn injury, epidermis showed a complete healing of epithelial layer with presence of keratinized layer. There was an evident thick network of collagen with a profuse of inflammatory cells in dermis. There was also a significant profuse of regular fibrosis with hemorrhage and the development of new blood vessels (Fig.2-b). At day 21 following burn injury, there was narrow scar tissue with full epithelization, the existence of keratinized layer above epidermis, as well as new vascularization with frequent regular fibrosis and few, regular and thin collagen with new hair follicle development (Fig.2-c). Burns of LLLT(G3) at 7th day post burn injury, the skin surface showed presence of crust with complete sloughing of epidermal layer with few hyperplasia of stratum basale, thick and interlocking collagen fibers with inflammatory cell infiltration and few granulation tissue formations (Fig.3 -a). At 14th day post burn injury, the skin surface

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showed thick crust and purulent exudate with moderate and incomplete hyperplasia and stratum basale, there were hemorrhage and presence of granulation tissue and new blood vessels formation with infiltration of inflammatory cells in dermis with thin network of collagen (Fig.3-b). At 21st day post burn injury, the epidermal layer showed there was complete epithelization with presence of keratinized layer also there was regular profused fibrosis with formation of new blood vessels and developed hair follicles (Fig.3-c).

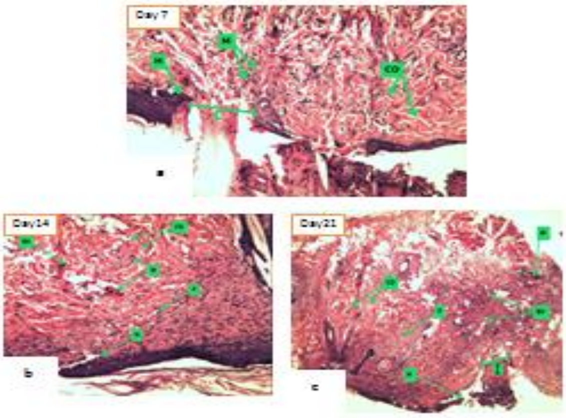

Fig.1 : Microscopic evaluation of burn injury on rats in G1(H&E):**a-** Note few hyperplasia of stratum basale (H) and wide incision (I) infiltration of inflammatory cells (M) and profuse irregular thick collagen (CO). 50X. **b**- Hyperplasia of epidermal layer with presence of granulation tissue which characterized by formation of new blood (BV) and fibrosis (F) with thick network of collagen and infiltration of inflammatory cells 50x**, c**- Incomplete hyperplasia of epidermal layers and narrow incision with profuse granulation tissue in which there were formation of new blood vessels and profuse fibrosis with few collagen and high infiltration of 20x.

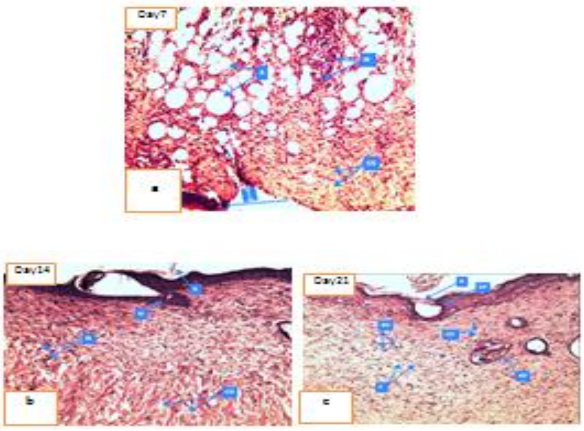

Fig.2 : Microscopic evaluation of burn injury on rats in G2(H&E):**a-** presence of thin crust on the surface of skin with narrow incision (I) with complete sloughing of epidermis. In the dermis, there were profuse infiltration of inflammatory cells (M) with granulation tissue and profuse adipose tissue (A) with profuse and thick network of collagen (CO) 50x**, b-** complete healing of epithelial layers of epidermis (H) with presence of keratinized layer (K) profuse and thick network of collagen with scattered inflammatory cells in the dermis 50x, **c-** Note narrow scar tissue with complete epithelization (EP) and presence of keratinized layer above the epidermis (K). New vascularization (BV) with profuse and regular fibrosis (F) and few, thin and regular collagen with formation of new hair follicles (HF) 50x**.**

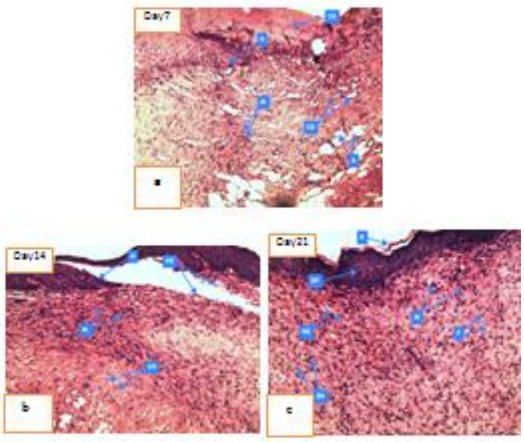

Fig.3 : Microscopic evaluation of burn injury on rats in G3(H&E): **a-** Presence of thick crust (CU) attached closely with the surface of skin. Mild hyperplasia of stratum basale (H) high infiltration of inflammatory cells (M) with irregular thick network of collagen (CO) with present of adipose tissue (A) in the dermis 50x**, b-**There were hyperplasia of epidermal layers with presence of narrow incision, marked and profuse granulation tissue with hemorrhage in the dermis (HE) and presence of collagen fibers with inflammatory cells 20x**, c-** complete healing in which complete epithelization (EP) with formation of outer keratinized layer(K)above the epidermis. Hemorrhage and few infiltration of inflammatory cells with new vascularization (BV) and fibrosis (F) in the dermis. 50x **.**

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Discussion

The goal of the research was to compare the efficacy of LLLT (660 nm) with chitosan NMs for second degree burns. At 7 days, there was a thin crust on skin's surface, a little incision, and the epidermis had completely shed. Inflammatory cells, primarily lymphocytes and macrophages, as well as profuse adipose tissue and an abundant and dense collagen network, were widely infiltrated in the dermis. The epithelial layers regarding the epidermis had fully healed at 14 days, and keratinized layer had appeared. Proliferate and thick collagen network that have dispersed inflammatory cells in the dermis, along with the regular and proliferate fibrosis with the hemorrhage as well as new BV development at day 21, observe the existence of a keratinized layer above the epidermis and the narrow scar tissue with complete epithelization. New vascularization with regular and profuse fibrosis and few, regular and thin collagen with new hair follicle formation with new blood vessels and scattered macrophages' formation. These findings concur with those by (12) who noted the creation of granulation tissue on day 7, as well as fibroblast proliferation and angiogenesis, and full skin regeneration at 21 days and this accord with (13) who found that chitosan nanoparticles have a positive effect on angiogenesis. Additionally, 120 Wistar rats that have second-degree burns were treated by using 1% chitosan (water soluble via carboxymethylation) every 24 hours for 30 days, according to (14). On day 15, the researchers found that the treated rats have more wound retraction. This result was likely caused by chitosan's capacity to accelerate wound healing by initiating fibroblast proliferation and collagen formation in response to N-acetyl-D-glucosamine (15). Furthermore, the activation of macrophages results in phagocytosis and release of mediators like the platelet-derived transforming growth factor (TGF-), which quickens the creation of extracellular matrix and increasing healing rate(16). Chitosan usage causes the development of granulation tissue by enhancing collagen expression and promoting the proliferation of inflammatory cells, including macrophages, leukocytes, and fibroblasts (17).In order to cure this kind of injury, LLLT had become very popular. At 7 days, the skin's surface had a substantial crust that was firmly attached to it. The dermis contain adipose tissue, the stratum basale had modest hyperplasia, a dense collagen network was irregular and present, along with crust and exudate. At 14 days, the dermis has inflammatory cells, collagen fibers, new vascularization, macrophage infiltration, and fibrosis. There is also a small incision, epidermal hyperplasia, visible and significant granulation tissue, fibrosis, and bleeding. Complete healing, including the complete epithelization and emergence of outer keratinized layer on top of epidermis, has occurred after 21 days. Bleeding accompanied with skin fibrosis, new vascularization, and a small amount of inflammatory cell infiltration, primarily macrophages. These findings agreed with those of (18), who employed 32 male Wister rats and find that their research had a beneficial impact on skin burn healing in rats that had been exposed to laser light and have less necrotic tissue, more granulation tissue, and re-epithelialization.Laser therapy can act in improving burn healing by reduce inflammation, pain relief, reduction in the edema production and keeping adjacent tissue safe (19).The laser was used to stimulate of fibroblast and deposition of collagen fibers and increased the activity of ATP synthesis and mitochondrial respiratory chain eventually stimuli cell proliferation and considered as one of the phototherapeutic agents (20). The findings in group 3 were consistent with (21), who discovered that LLLT can speed up burn wound healing, which may be associated with lower levels of proinflammatory cytokines like IL-6 and TNF-α. Additionally, throughout the healing

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www.qu.edu.iq/journalvm

of burns, the laser-treated group had a faster rate of wound contraction than the control group. This is in line with the results of (22,23), who discovered that the LLLT group had lower levels of NF-Kβ and TNF-α, and higher levels of the proteins FGFR-1, vEGF, HSP-90, HSP-60, and matrix metalloproteinases-2 and 9 compared to the control, all of which were able to increase collagen deposition, cellular proliferation, and wound contraction in the repair wounds.

In Conclusions: The second degree burn in rats after application of chitosan nanomaterials revealed there were an improvement in healing process due to increment in wound contraction and acceleration the reformation of connective tissue.

Conflict of Interest: there is no conflict of interest.

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