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The Local Anesthetic Effect of SNL Lidocaine and Conventional Lidocaine on Skin Wound Healing in Rabbits

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ABSTRACT

Introduction: Local anesthesia is a technique used to induce the absence of sensation in a specific vital part of the body. The methods for developing durable controlling term acting of local anesthetics mainly include the continuous pumping of drugs around the nerves. The use of a drug delivery system with controlled-release functions, promises the development of new compounds.

Objective: Several notions in literature denoted the use of lidocaine delayed wound healing, and the therapeutic manoeuvres of open wound gated for more than several days, at this time the patient complains of pain, for this reason, the pain reliever and itching during healing phases one of the properly developed uses developed Nano form carrying lidocaine SNL lidocaine as an alternative to conventional lidocaine maintain superior efficacy during the management of open skin wounds through maintaining local anesthesia as an assistant line for managing to wound healing.

Patients and Methods: Fifty rabbits were divided into four groups. The full excisional wounds were treated with different formulas, conventional lidocaine, SNL lidocaine, and SNL lidocaine carpabol gel, as a daily dressed treatment.

Results: The results of wound healing revealed that Nano lipid loaded lidocaine gel decreased wounds and was faster healed than conventional lidocaine form; higher closure rate and closure velocity and low closure *t*½. conventional lidocaine delays healing more than other treated groups and control.

Conclusion: SNL lidocaine gel turn on accelerated wound healing and overcomes the adverse effect of delaying healing by conventional lidocaine.

Keywords: Nano-Lipid, Lidocaine, wound, healing, local anesthetics.

INTRODUCTION

Lidocaine is an amide-type local anesthetic with high efficacy, rapid onset, and medium duration of action that can be inhibited the sodium ion transport route involved in neuronal initiation and conduction. Lidocaine has been used therapeutically as a local nerve block and for epidural anesthesia since the mid-twentieth century [(1)].

The primary reason for the challenged structural Nano lipid-loaded lidocaine for mild or avert the toxic and side effects of lidocaine in open wounds and systemic complications that faced the patient jeopardy and risky doses as well as minimized the therapeutic dose. Furthermore, the Nano lipid structure biodegradable type accepted lidocaine penetrable and reaches deeply to the target nerves, rendering animals fair precise anesthesia and reducing pain. The success of creating loaded lidocaine in Nano-lipid has maintained the classified chemical functional group and promised a new generation of safe lidocaine and Nano behavior [(2)].

A new approach using conventional lidocaine on skin open wounds may be impaired wound healing was a serious common health problem that raises the need for novel treatments to reduce medical expenses and improve treatment effectiveness. The effectiveness of currently available conventional treatments in permeating the skin at the target region and speeding the healing process is still limited. Nanotechnology had a significant chance to improve presently used medical therapies, standard care, and wound management. It is a potential strategy that can be solved problems including the permeability and bioavailability of medications with limited water solubility or reduced stability[(3)].

This study focuses on the structure of Nano lipid-based drug delivery systems, describing their applications in managing wounds. With highlights, the impact of structural nano lipid loaded lidocaine (SNL lidocaine) on the applicability and enhanced skin penetration in wound healing therapy compared with conventional lidocaine treatments with increased drug loading capacity and enhanced bioavailability [(4)]. The experiment aimed to determine the efficiency of SNL lidocaine on the healing of excisional wounds for reducing the complication of conventional lidocaine.

METHODOLOGY

Experimental design

Fifty rabbits were divided into four major groups as follows: the first group was five animals as a control group, the second group was five animals as blank SNL, the third group was twenty animals as conventional lidocaine treated group, the fourth group was twenty animals as SNLlidocaine treated group. The third and fourth treated groups were divided into four subgroups according to the dose of concentrations infiltrated in the skin (0.1, 0.2, 0.4, and 0.5 %) in both SNL lidocaine and conventional lidocaine.

Preparation of SNL lidocaine: the preparation and standardization were done according to Jabbar and Al-Bayati [(5)] by the cold infusion method.

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The excisional wound induction in rabbit skin: The rabbit was anesthetized with subcutaneous infiltration of lidocaine local anesthetic formulation 2% of each group, according to the ethical as placebo fact **Balbino *et al.*** [6]

The surgical wounds creation: The dorsal region of the rabbits was clipped and shaved with hair remover and a razor blade, the prepared surgical site was disinfected by alcohol 70%, the skin was labeled about 0.8 cm as a circular line and the skin fold at *para*-Medline and the dorsal parts was raised cranially and caudally by using thumbs, the sterile biopsies were removed on half-circular skin fold by the surgical blade to entirely remove whole skin layers and create symmetrical excisional wounds [7].

Wound treatment and dressing: The SNL lidocaine and SNL gel were used through application and dressing topically on the excisional wound, and other treated groups were dropped form of lidocaine and SNL lidocaine. The wound was plastered and draped in the area to prevent contamination [8].

Wound closure monitoring post-excisional surgery: The wound area of monitored rabbits was evaluated until the completion of the wound closure area, the wound area was kinetic driven during closure area. The dimensional diameters of the excisional wound area were measured on days 5, 10, 20, and 30 according to **Bruno *et al.*** [9] as follows**:**

$$wound\ area = \frac{diameter\ A}{2} \times \frac{diameter\ B}{2} \times \pi$$

Furthermore, the Calculation of the percentage of kinetic wound closure was done by the following equation, **Gopinath *et al.*** [10]:

$$wound\ closure = \frac{actual\ wound\ area\ (cm)}{Initial\ area\ of\ wound} \times 100$$

Timeline analysis of wound healing

The morphology of new tissue formation was analyzed and achieved at certain time points of committed healing on the formed cicatricial tissue. Five slices were evaluated to encompass the wound borders of healing (the area of wound boundary between the edge of intact connective tissue and the one of new tissue formation) and the wound center or the forming scar. The five wounds and five slices were analyzed by **Cassini *et al.*** [12]**.** The closure indices were derived according to three parameters:

1. **Closure rate**: the closure area of the wound per unit time during the total growing phase and estimated by the following equation*:*

$$Closure\ rate = \frac{1 - \frac{Intial\ wound\ area - final\ wound\ area}{intial\ wound\ area}}{Time\ (days)}$$

2. **Closure velocity**: the total required time of the wound area closure and estimated by the following equation[13] *:*

$$Closure\ velocity = \frac{\Delta A_{\ i-f(primeter)}}{\Delta t}$$

$* \Delta A_{\ i-f}$ the change between the area of the excisional wound and the final closure area,

3. **T½ of wound healing**: the half-life of closure area time in treated groups and estimated by the following equation[14] *:*

$$t½\ closure = \frac{q}{Dc} : \text{where}\ \ Dc = \frac{\Delta r}{\Delta t}$$

*q is the intercept, Dc continuous linear healing rate, ΔA: change alter in apparent wound area between two consecutive times of measurement, Δt: time between two certain consecutive measurements

Histological assessment of wound healing

The skin wound healing was processed for histological analysis, the harvested healed wound tissue untrained a fragment of tissue on a sandwiched of filter paper avoiding the folding of tissue through the process of fixation process in the fixative cassette of formal saline 10% for 24 hours and processing of paraffin embedding the sectional slice was stained by Massone trichrome stain, **Camila *et al*.**[7] The wound histological qualitative structural score was performed in stained tissues and the core was documented of each existed structure at the certain timeline **Castro *et al***[15]

The inflammatory score was estimated by the following grades: Grad zero included the absence of inflammatory cells and signs, grad two included the existence of limited inflammatory cells and restricted signs, grad three included numerous inflammatory cells and distinguishing signs**,** and grad four included overstated inflammatory cellularity and massive signs[16, 17, 18]**.**

Ethical Consideration

The ethics were adopted on the ACUC protocol "Laboratory Animal Care and Use Guidelines for Research and Education" for handling and management of rabbits, and ethical craft pharmacological dosing and cure maneuvers according to Al-Bayati and Khamas[19]**.**

Statistical analysis

The data of schematic figures was had been subjected to the analysis of variances ANOVA one and two-way analysis (F test), the significance degree level was measured as $p \leq 0.05$, and statistical comparison was achieved with less significant differences LSD.

RESULTS

The wound closure gross photographs.

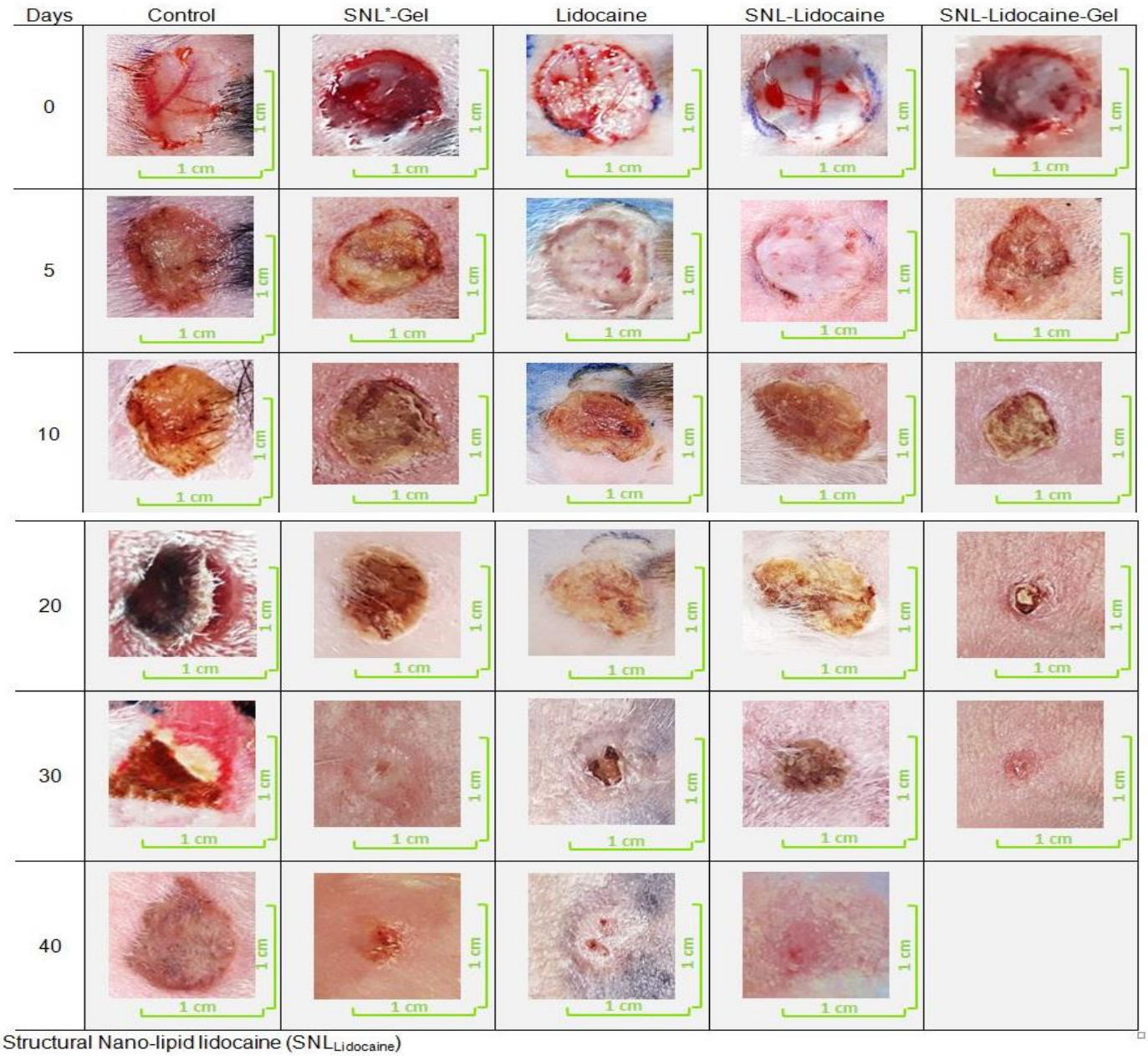
The control gross wound in the dorsolateral area of the rabbit was the circular trimming of bordering skin at day zero. On the 5th day, the control SNL lidocaine displayed an inflammatory superlative layer on the opening area. But the formula of conventional lidocaine and SNLlidocaine were still clear after 5 days of

treatment, the SNLlidocaine Gel was promotion activity by forming the scalp and driving primary closure primitive decreasing the irregular narrowing of the wound area. Whereas the 10th day, the control was slower closure and presented a degree of inflammation with a mild recovery of closure whereas, SNL-Gel was covered by the scalp.

The lidocaine wound-treated groups showed not presented irregular healing mild narrowing, SNL Lidocaine Gel narrowing faster than the lidocaine. the optimized SNL Lidocaine gel dosage form was given transdermally for wound healing. As presented in figure 2 of the gross photograph at day 20 closure wound healing, wounds treated with ordinary lidocaine remained slightly open at day 40. The results presented that wound healing in the SNL Lidocaine 0.2% was higher speed than the ordinary lidocaine during the days after surgery remodeling figure 2 at day 30.

The description of the wound was performed at 0.8 cm in full skin thickness aplasia and the wound picture displayed normality in healing with treatment factor only in lidocaine formulas and set as self-protection the first day of wound creation was bleeding clot was formed the control and other application of conventional and Nano form. The scalp formed on day 10 in the conventional and day 5 in the SNL Lidocaine. Whereas, the description of wound closure during the healing growing time was the Nano form was over the narrowing than conventional in 0.1 and 0.2% of SNL Lidocaine forming tissue fill the wound, but in 0.4 and 0.5% of SNL Lidocaine was delayed and reach to minimized speed forming tissue closed area.

The remodeling wound was closure open set when the sign of regaining normal skin tissue and the normal tissue share the area with hair dotted on the skin and were showed in 0.2 % of SNL Lidocaine faster this sign as compared with control and other concentration on conventional and SNL Lidocaine., the histological appearance was clarifying this fact in days of healing.



Structural Nano-lipid lidocaine ($SNL_{Lidocaine}$)

Figure (1): Represented the wound healing in the treated group as control, SNL gel, lidocaine, and SNL gel Lidocaine during the days after surgery remodeling.

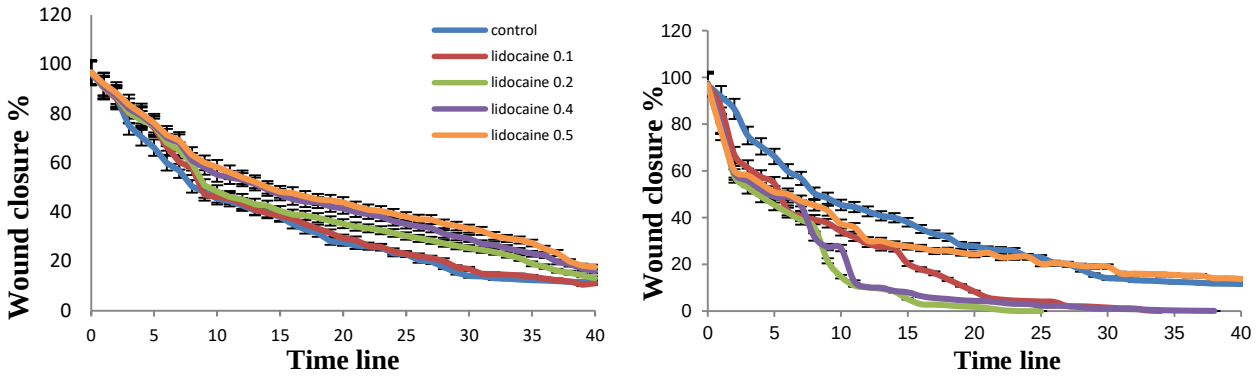


Figure (2): Wound healing curve behavior of in multiple concentrations of ordinary lidocaine; **A** and structural nano-lipid lidocaine; **B** per timeline in growing phase and remodeling phase.
The data presented as mean±SE, the different letters denoted significant $p \leq 0.05$ between lidocaine concentrations.

The Stereometric score of wound healing in conventional lidocaine and SNL$_{Lidocaine}$
The competence of the wound healing process and cellular kinetic in regenerative tissue were scored as the component of skin integrity during wound healing as an index of comparability and interaction of lidocaine and SNL Lidocaine with standardization of the growing and remodeling phases.

Inflammation score:
The leucocyte skin infiltration in the inflammatory phase in the early creation of the wound was scored in high values 2.81, 2.79 in control and lidocaine significantly ($p \leq 0.05$) higher than SNL Lidocaine. The 3rd, 4th, and 5th wound groups negatively decreased leucocytes score as an inflammation phenomenon and increase the time of healing, finally, the inflammatory cells are absent after 28 days of SNL Lidocaine.

Scab score:
The scab score indicated of first closure elements inflammatory phase was shown in figure 3, all groups of the wound decreased their scab score with increased time, on the second day after wound creation the scab tissue was formed in all groups.
Whereas the lower score was a decrease of scab in SNL Lidocaine and as compared with conventional lidocaine and control 0.03 previously was decreased scab score with a time of healing and absence after a complete absence 21 days, the lidocaine scab formation was lower than control at day 4 and still appear until 21 day was significantly ($p \leq 0.05$) decrease as compared with control.

Extracellular matrix deposition:
The fibers tissue distribution is a sign of wound closure and the score of extracellular matrix deposition of fibroblast and collagen fiber as the endpoint consider the second phase of wound healing, The SNL Lidocaine share score than control and lidocaine as well as all groups scored extracellular matrix deposition was increased their values within the time of healing.

Vascularization: The vascularization score presented angiogenesis in the wound matrix was companies with the first and second phases of inflammation and fibroblast, the vascularization share duel distribution stages, first, all groups increased their score at the first half of the healing periods and decrease the second half significantly whereas the maximum vascularization in SNL Lidocaine at day 7 than remodeling after 21 days and the SNLLidocaine vascularization score in the wound healing at the first half periods higher than lidocaine and control and the second half was decrease than lidocaine and control wounds. The lidocaine-treated wound showed vascularization lower than the control in half periods and higher than the control in the second period of the wound.

The Epithelialization Score:
All the epithelialization represented a sign of closure wound and migration of the wound edge to the central wounds. All groups were increased epithelium with increased time of wound healing significantly ($p \leq 0.05$) whereas the SNL Lidocaine was higher than lidocaine and control significantly ($p \leq 0.05$), with a significant difference in day 21 between SNL Lidocaine, lidocaine, and control groups.

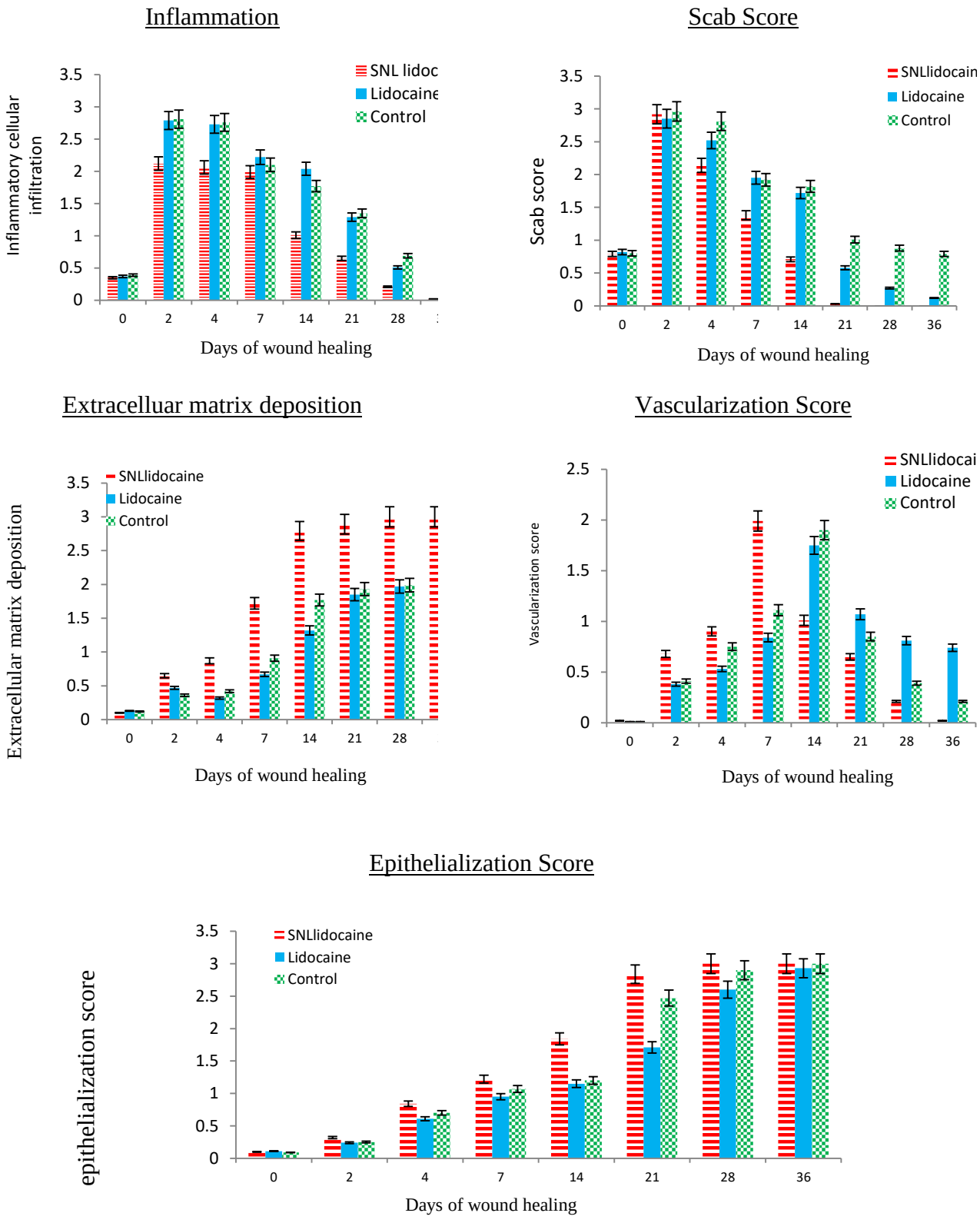


Figure (3): The tissue morphometric score of skin wound healing in conventional lidocaine and Structural Nano-lipid lidocaine ($SNL_{Lidocaine}$) in rabbits.

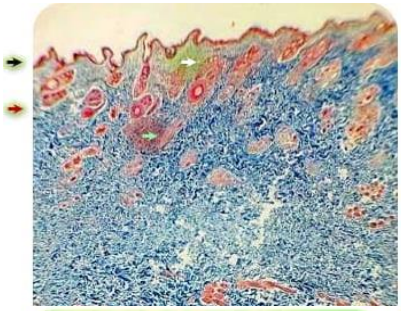


Figure (4): Illustrative photomicrographs of normal skin sections stained with Masson trichrome viewing the normal histologic appearance of the skin is shown the epidermis (black arrow) with thin keratin layer overlies epidermis. Beneath the epidermis is the connective tissue (collagen and elastic fibers) of dermis layers (red arrow) as well as hair follicle (green arrow) and sebaceous glands (yellow arrow).

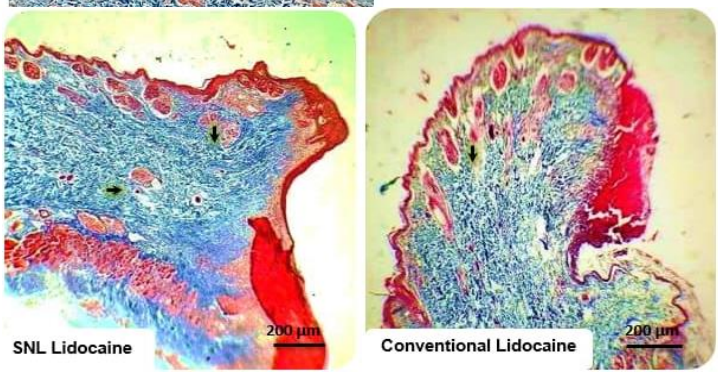


Figure (5): Illustrative photomicrographs of skin sections stained with Masson trichrome viewing the inflammatory cells scoring day of wound 14, infiltration of inflammatory cells denoted by black arrows white blood cells in conventional and SNL lidocaine.

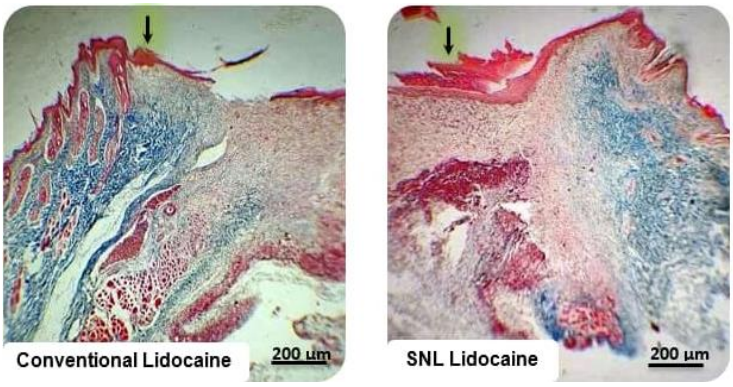


Figure (6): Illustrative photomicrographs of skin sections stained with Masson trichrome viewing the scab scoring day of wound 7, Scab forming density denoted by black arrows in conventional and SNL lidocaine.

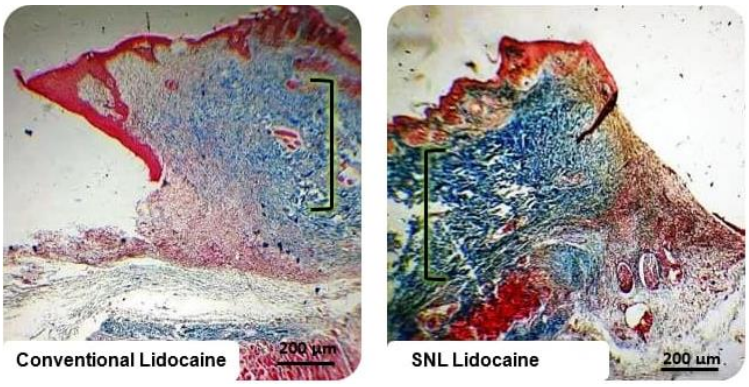


Figure (7): Illustrative photomicrographs of skin sections stained with Masson trichrome viewing the Extracellular matrix scoring day of wound 14, deposition of cellular matrix denoted by a black bracket in conventional and SNL lidocaine.

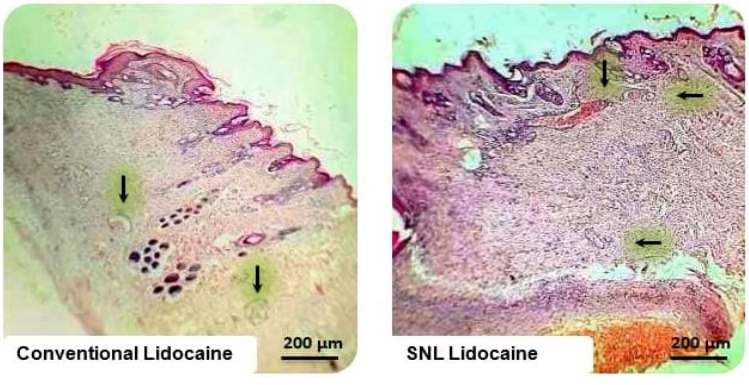


Figure (8): Illustrative photomicrographs of skin sections stained with Masson trichrome viewing the vascularization scoring day of wound 21, newly generated blood microvessels denoted by black arrows in conventional and SNL lidocaine.

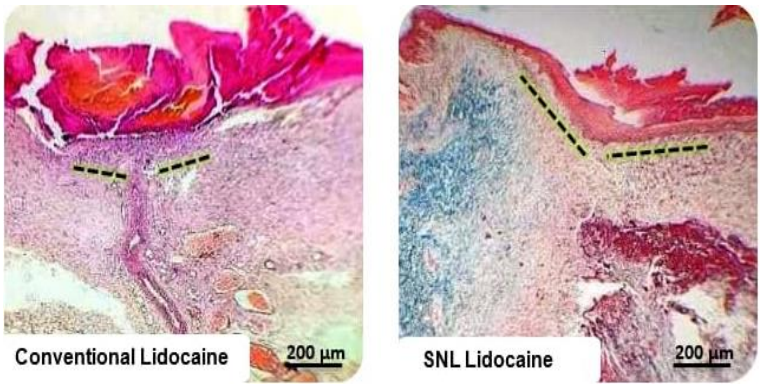


Figure (9): Illustrative photomicrographs of skin sections stained with Masson trichrome viewing the wound epithelialization scoring day of wound 21, wound epithelialized area denoted by the black dashed line in conventional and SNL lidocaine.

DISCUSSION

Skin wounding phases activate a harmonized regular of overlapping responses bringing tissue repair of the injurious or damage sustained. These sequences responses comprise refurbishment of the build-set epidermal barrier endorsed via re-epithelialization derived through migration-proliferation of keratinocytes then reconstitute the dermis via deposition of matrix proteins synthesis. An inflammatory controlling process associated with proteolytic activity, meanwhile; acts as wound cleansing from debris and pathogens [(20)]. Several reports have reported the influence of lidocaine on sequential processes has been probed in a restricted number of animal reports, although lidocaine had been reported to controversy enhance the improvement of wound re-epithelialization [(21)]. The effects of lidocaine exerted on collagen accumulation in wounds had been the source of some contention [(22)].

Collectively, the study presented outcomes that displayed the cutaneous infiltration of relevant dressed concentrations of lidocaine gel considerably delayed wound healing in rabbits. These findings corroborated certain previous annotations and studies by Bagul *et al.* [(23)], but contradict others reported by Dere *et al.* [(22)] and Morris and Tracey [(21)]. However, the disparities may be presumably attributed at least in part, to the delay of wound healing by the doses depended used [(24)], which was agreed by critical differences in experimental protocols with intradermal dosed local anesthetic then found that impaired wound healing applied lidocaine in the jelly drug form. Some events that may be caused a delay of wound healing due to lidocaine that endorsed outcomes, factually the lidocaine derived this adversely affect wound proteolytic activities and neutrophil numbers locally and migrating in the injurious site. Lidocaine has been reported before identified as an inflammatory modulator that primarily reduces neutrophil activation and cytokine secretion [(25)]. The histological profile and morphometric measurements were displayed in conventional lidocaine reduction of inflammatory cells that provoked an expression of attenuation of acute inflammatory response that also documented by Drucker *et al.* [(26)] agreed with this endpoint in outcomes of wounds. Some evidence referred to a trend in the reduction of collagen precipitation in wound areas with lidocaine treatment [(27)]. That may be derived from evidence of reduced breaking strength of wounds due to lidocaine [(21, 28, 29)].

The lidocaine treatment in wounds may be attributed to the inhibition of collagen synthesis due to a partial degree of tissue necrosis locally, which acts as a factor for hindered normal wound healing [(30, 26)]. The differences between the number of collagen fibers and vascularity maybe play an impact on evidence, for this finding in the study result the lidocaine reduced the vascularization score during the term of healing. That may be the causal direction of delayed healing dramatic events the attendance of angiogenesis invasive process of a variety of primary and of mature capillary vessels in the vicinity wound for demined the nourished new generated tissue, the amount of fiber of collagen fibers was directly combined with vascularization that turns on progressive related to efficient promote a cascade of wound healing [(26)].

The lidocaine may presumably weaken the building architecture of collagen fibers associated with the reduction of collagen precipitation as the basic structure of the wound and the histomorphometric appearance of appeared the scalp may be attributed to irregularity and set as a weakness [(28, 31)]. The reports informative referred to the attribution of lidocaine effect OHpyrolinem on decreased collagen α 1 gene expression and presumably decrease collagen forming by fibroblast cells were attributed to the delaying wound healing due to particularly impaired pathway of the mucopolysaccharides synthesis which was presumably

the dived defective irregular collagen forming and exerted sequel impaired wound healing [32].

On the other hand, the previous notions were references of SNL and other polymers donated their loaded drug Nanoscale behavior and achieved overcoming obstacles and reduced toxicity with improved effectiveness and potencies of carrying drugs, lidocaine SNL results were shared biphasic effect on wound healing as dose-dependent firstly was announcement increased potency of in low concentration (0.1 and 0.2%) was as the second phase showed cytotoxicity like effect on closure and intensifying the toxic outcome on healing duration and type of healing [17, 33].

The increased efficiency of lidocaine was presumably achieved due to their behavior of SNL physical properties as high permeability in the skin layer and targeted the active site that may be overcome the harmful effect and facilitated direct concentration in high efficiency to promote proliferative effect, the properties of slow sustained release and increase the duration of action that endorsed the ideas of coupling the small Nano size with a duration of action on promoting direct activation proliferative effect by maintaining anti-inflammatory effect and autacoids agents mediated wound healing. These resulting facts may be causal of many processing of lidocaine-reducing cytokines at the site of wounds, the lidocaine dose-dependency efficiency may be exerted on inhibiting the adhesion of leukocytes with matrix and at the wall of blood vessels. Furthermore, lidocaine was reported as an inducer of prostacyclin releasing that presumably turns on the promotion of liberation and migration of leukocyte and adhered to blood vessel endothelium in high concentration, SNL in low concentrations has direct stimulated phospholipase A2 that increase the proliferation of fibroblast and regular collagen matrix precipitation. Whereas, in the second toxic phase the inhibited phospholipase A2 enzyme delays the sequence cascade of wound healing phases. Dramatically the nonionic surfactants reduced immunological interacted pathways and reduced their mediators, besides getting intermolecular interactive bonding between the SNL and lidocaine at the amide group [34].

For instance, polymerized SNL encapsulated lidocaine may be exerted its slow sustained release through run released via desorption, diffusion, and erosion. Another suggested mechanism arriving on the wound and elaborating the advantages of using Nano-lipid was the biodegradable of this Nano-system this phenomenon was degraded in acid wound media easy to liberated via biodegradation converted to monomers linked with lidocaine and persisted their run function on the accessibility of local anesthetic and enhancement of narrowing opener wounds in the area enrich with lactic and glycolytic acid and get resuming the biochemical mediators' factors enhancement of tissue repair. Otherwise, the SNL-encapsulated lidocaine increases stability via polymer and after biodegradation conjugation with monomer bind drug due to adequate binding via van der Waals, hydrogen bonding, and/or hydrophobic interaction. The final suggestion the nanoparticle was intensifying the response that comparable with the same dose of conventional agreed with our results, despite the share safe mode in vicinity dose and also increase toxic like an overdose, as well as the SNL was manifested safer drug delivery carrier due to minimized dose through delivering the lidocaine to the certain targeted cells receptors in the tissue with stealing distribution, other factors of influence the lidocaine effect on wound healing has an antibacterial effect that was encouraged these activities act as antibiotics in loaded in SNL that may be presumably derived their functional effect on the wound facilitated protection against contamination directly proportion with closure time positively, furthermore, the Nano lipid loaded lidocaine and formulated with gel was prepared suitable condition of hastening the closure due to lipoid and gel forms form occlusion properties which were based on their behavior maybe act on the wound as reduction of loss hydration of skin that increase moisture area of skin that facilitated acceleration of cells building up of the wounds processes. In addition, the gel-formulated lidocaine with SNL was act as a slow-released drug and increased the duration of action, and decrease toxicity [35].

CONCLUSION

The reformulated conventional lidocaine via carrying it in structure Nano lipid and acquired the status of Nanoparticles that SLN-lidocaine has different advantages and this carrier was promised lidocaine delivery systems for wound healing support therapy, the SNL-lidocaine efficiency was superior to conventional lidocaine of dressed the excisional wound, SNL lidocaine gel turn on accelerated wound healing and overcomes the adverse effect of delaying healing indicated by decreased half-life and increase closure velocity and rate.

Conflict of interest: Nano lipid carrying drug and delivery system

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Author contribution: The authors of the study all contributed equally.

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