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EVALUATION THE EFFECT AND EFFICACY OF LIDOCAINE ON HEALING OF SKIN WOUND IN RABBITS

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Abstract
The current study proceeded on six female rabbits weighing 1600 ± 200 g were used to determine the efficacy of 2% lidocaine (most commonly used concentration) in healing of skin wound. After anesthetized a two full thickness surgical incision were made on two sides of midline in back region of all animals, and divided into two groups randomly .In treated group, the right side were infiltrated with 3 ml of 2% lidocaine ; In control group, the left side were infiltrated with 3ml of normal saline. Sites of intervention were estimated by histopathology. This study evidence 2% lidocaine that delay wound healing process and decrease wound strength ,formation of blood vessel and other skin content as hair follicle and there muscle.

Keywords: Wound healing, Skin, Lidocaine, Local anesthesia.

INTRODUCTION

Wound healing stay a defying clinical problem, with early and late complications submitting a frequent cause of morbidity and mortality (1). It is a physiological process that enables tissue restoration through an organized cascade of complex cellular and molecular events (2). In an attempt to reduce the wound burden, much effort has focused on understanding the physiology of healing and wound care with an emphasis on new therapeutic approaches and the continuing development of technologies for acute and long-term wound management (3,4).Wound healing involves multiple cell populations, the extracellular matrix and the action of soluble mediators such as growth factors and cytokines (5). Normal wound healing consists of 4 phases: hemostasis, inflammation, proliferative, and remodeling. Collagen production begin on the 3rd day and continue for 3 weeks. Collagens released from fibroblasts and their cross-linkage enhances wound tension strength (6). When skin is injured, the repair process is initiated immediately by the release of various growth factors, cytokines, chemokines, and low-molecular-weight compounds. Inflammatory cells such as neutrophils, monocytes and lymphocytes, produce growth factors, cytokines and chemokines, which initiate the proliferative phase of wound repair in several hours after injury (Velnar et al,2009; 7). At 3-7 day after injury, migration and proliferation of keratinocytes at the wound edge commence and are followed by proliferation of dermal fibroblasts in the proximal area of the wound (8). Analgesics and anesthetics are widely used in practice, effects of these drugs on wound healing is worth to be studied. In practice, local anesthetic must have efficiency, safety, and operative needs (9).Lidocaine is a drug widely used to numb tissue in a specific area and may be applied directly to the skin for numbing (10). Isolated studies have described some of the effects of the commonly used local anaesthetic (LA) lidocaine on healing skin wounds. In rat models of acute wound repair, doses of lidocaine ranging from 0.5% to 2.0% have been shown to reduce wound breaking strength and to impair healing (11). In this study, we planned to present the effects of local anesthetics which are used widely in clinics such as lidocaine on wound healing, primarily on wound tensile strength and of collagen ultrastructure.

MATERIALS AND METHODS

Experimental Animals:
Sex healthy, adult local breed female rabbits (1600 ± 200 g) were used. Animals underwent a preliminary adjustment period (7 days), when they received a balanced diet and water *ad libitum*; they were individually housed in standard room temperature (22 ± 3C°) throughout the experiment.

Wound procedure and experimental design
After withholding food for six hours and water for three hours, each rabbit intramuscularly injected with 35 mg/kg ketamine (10%), and 5 mg/kg xylazine (2%) as dissociative anesthesia (12). Further, the back of rabbit was washed, shaved and surgical site was prepared & disinfected with Povidone Iodine 10% solution. The animals were randomly divided into 2 experimental main groups (treated and control) 6 animals for each group, Pre-incisional total 3 mL saline was infiltrated to subcutaneously control group (left side) and local anesthetic agent lidocaine 2% with the same volume was infiltrated to treated group(right side)(fig.1). Full thickness incision wound of 3cm was created along the markings using scalp blade and forceps(fig.2) (13). The suturing of incisional wound was done by using of sterile natural non-absorbable, silk suture size 1/0 (fig.3). The animals were examined macroscopically daily along one week. In addition, Biopsies were taken and examined histopathologically at the 7th day.

Histopathological Study:
Macroscopic and microscopic examinations of the healing incisional wounds were performed on all rabbits at 7 days after treatment. On the day of biopsy the animals were subjected again to the anesthetic and surgical procedures as

described before. Approximately 8 mm Specimens were harvested . Macroscopic alterations of tissue repair were evaluated at the moment of skin wound collection. The skin wound specimen was fixed in 10% neutral buffered formalin for 48 hours, then washed, dehydrated in a serial graduated alcohol, cleared in xylol, embedded in paraffin wax, sectioned at 5 microns thickness and stained with Hematoxylin-Eosin (H and E) stain, and examined under light microscope (14). Each segment was morphologically analyzed for healing pattern Several sections were cut from each paraffin-embedded portion, and the sections which appeared to have been least distorted by the preparation procedures were selected for cross-sectional area measurement, and each section was measured. Measurements were made using a planimeter on photomicrographs (15).

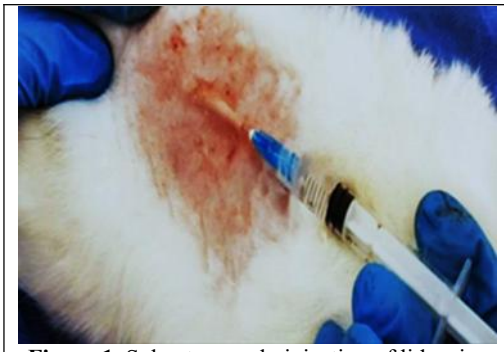

Figure 1. Subcutaneously injection of lidocaine in the right side of rabbit body

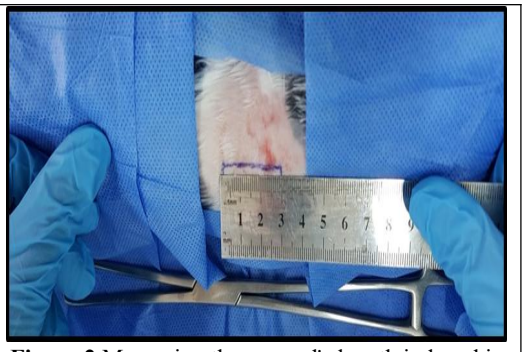

Figure 2. Measuring the wound's length induced in skin

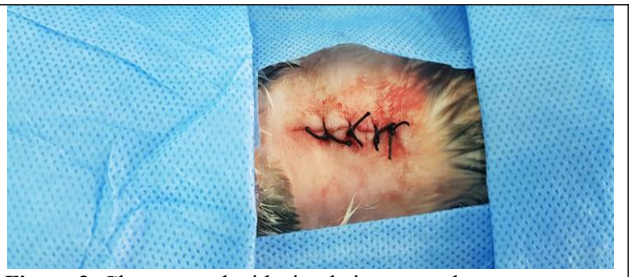

Figure 3. Close wound with simple interrupted suture pattern using non-absorbable suture material, silk No. 1/0 .

RESULT

Clinical Observations:

Initially the cardinal signs of inflammation (i.e. swelling, heat, pain and redness) were seen locally at the site of wound at some hours post induced wounding. Gross examination of the wounds showed extensive rash in site of wound in treated group and some rabbit the wound openesd again(fig.4)

wounds in control group showed complete closure with good adhesions between the wound edges within the same period (fig.5). Wound infection was not recorded in these animals were excluded from the experiment.

Vital signs measurements (body temperature, heart rate and respiratory rate) showed no significant changes.

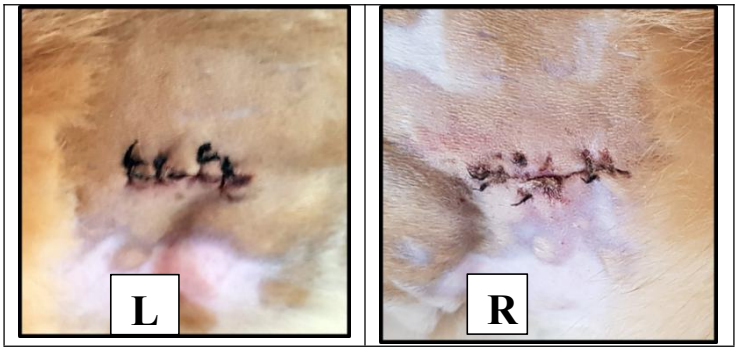

Figure 4. left side (L) control group show less or no rash in site of wound compared with right side (R) treated group show extensive rash

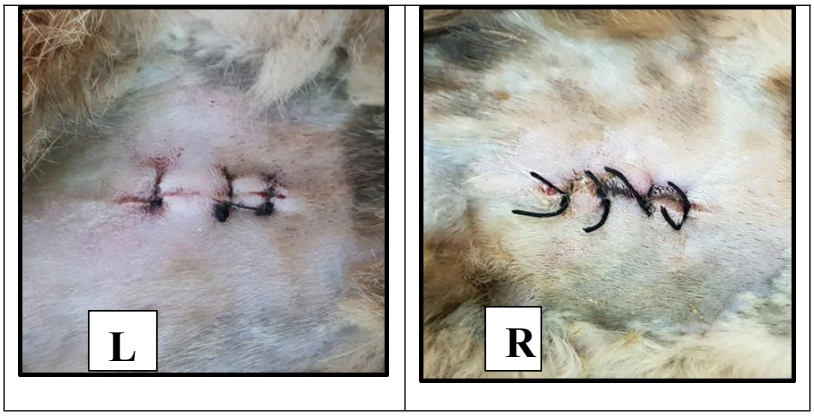

Figure 5. left side (L) control group show complete closure with good adhesions between the wound edges compared with right side (R) treated group.

Macroscopic evaluation:
The macroscopic evaluation showed delayed healing process and wound dehiscence especially in the midway of the wound on the 7th day post wound induction (fig.6) .
Grossly, the wound healing was analogous in all rabbits at the 14th day post wound induction.

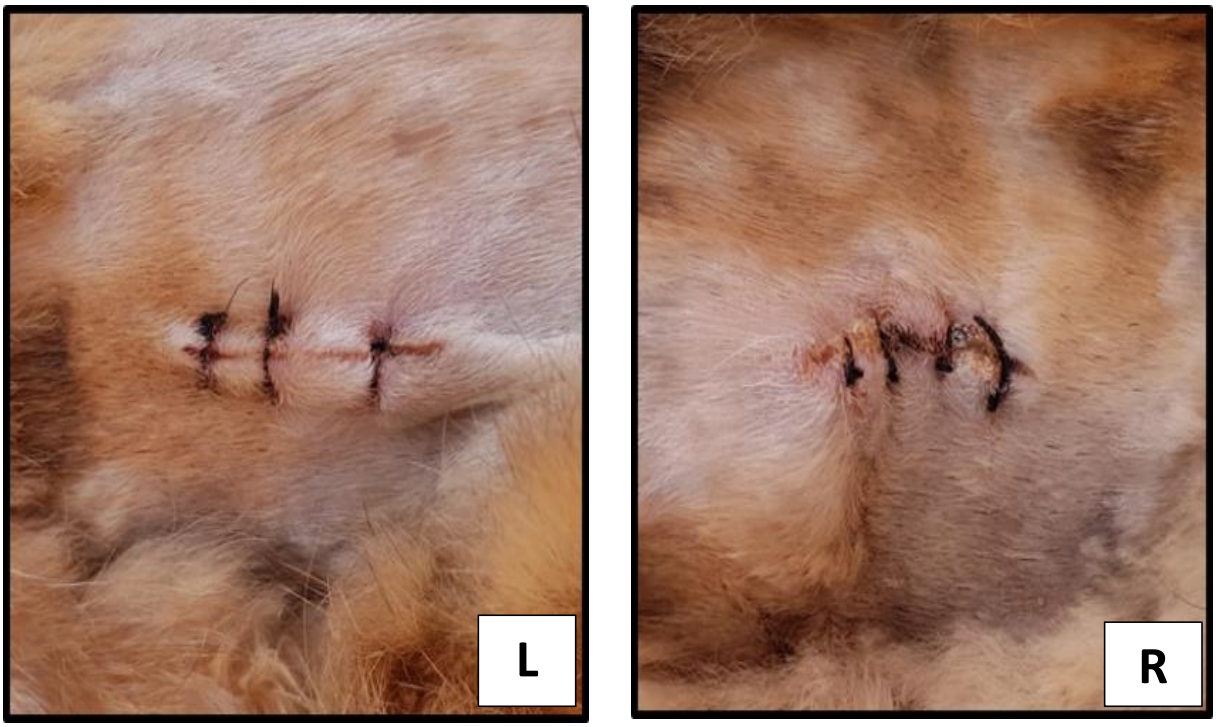

Figure 6. left side(L) control group compared to right side(R) treated group show delayed healing process and wound dehiscence.

Microscopic evaluation :
Control group (left side):
Microscopic examination showed many sings that means good healing such as good collagenase for wound strength and vascularity was significantly higher and less edema and inflammation .
So that, the left side section of skin reveals the present of highly corrugated epidermis with numerous papillae and the keratin appeared very thin on surface of epidermis, the dermis was containing dense connective tissue of collagen bundle, and long shaft of hair follicle extend along the hypodermis, dermis and arrived to the surface of epidermis (fig.7).
Each hair follicle in dermis was covered by cortical sheath cell and surrounded by WBCs and new blood vessel formation also found Arrector pili hair muscle (fig.8) and (fig.9).
The dermis formed by dense connective tissue of collagen bundle with aggregation of WBCs the deepest layer of hypodermis was occupied by skeletal muscle fiber of parallel direction (fig.10).

Treated group(Right side)
But in right side with lidocaine injection show the epidermis was not demonstrated well and its epithelium was densely stained . the dermis was formed by loose connective tissue with fibroblasts and different kinds of WBCs, also new small sized of hair follicle were seen present in group (fig.11) . The dermis was containing few bundle of collagen fiber and loss connective tissue infiltrated with many WBCs and in region, these are forming lymphocyte nodule (fig.12).

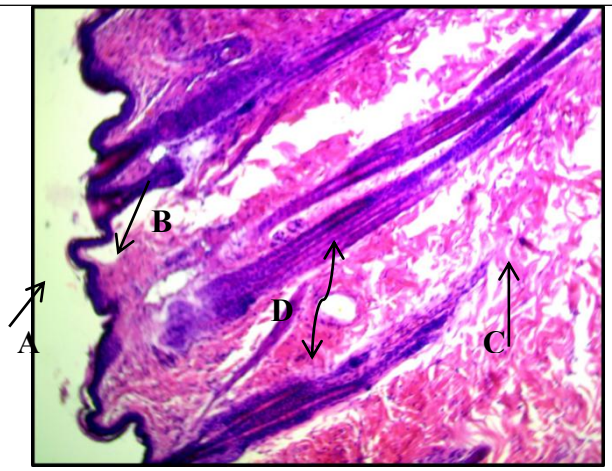

Figure 7. Microscopical picture of control group showing, Corrugated epidermis (A), Dermis (B), Hypodermis (C), Hair follicle traversing of the epidermis (D) (H&E X100).

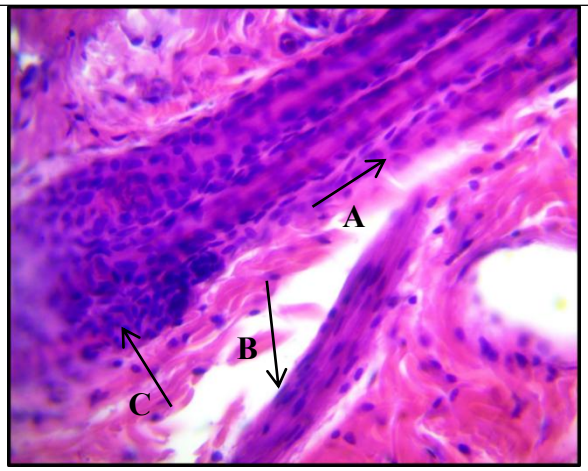

Figure 8. Microscopical picture of control group showing ,Dermis with hair follicle surrounded by cortical cuticle cell (A), Arrector pili hair muscle attached to hair follicle (B), hair bully (C) (H&E X100).

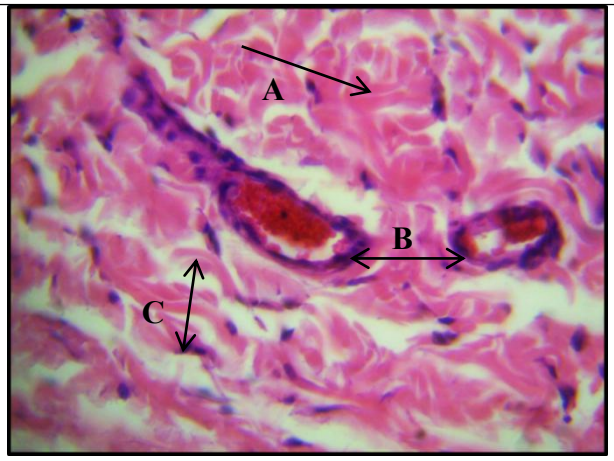

Figure 9. Microscopical picture of control group showing, Dense connective tissue (collagen) (A), New blood vessel with blood (B), fibroblast (C) (H&E X100).

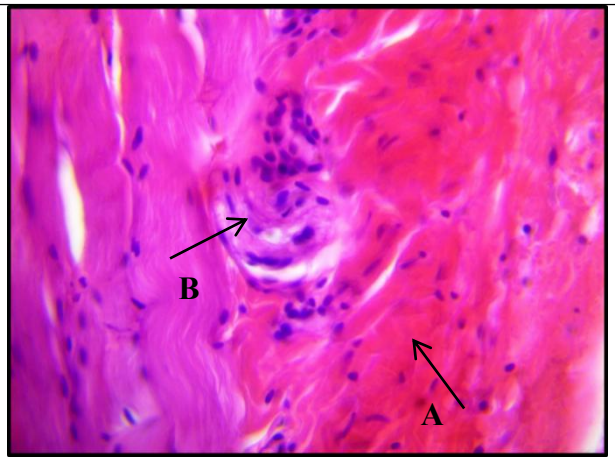

Figure 10. Microscopical picture of control group showing ,Dense connective tissue (collagen) (A), with lymphatic foci (B) (H&E X100).

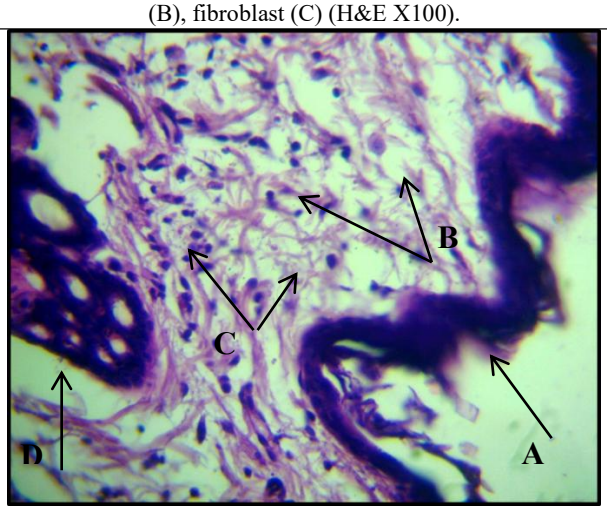

Figure 11. Microscopical picture of treated group showing, Epidermis was irrigated keratin deposited (A), loose connective tissue of dermis (B), lymphocyte & fibroblast (C), group of hair follicle (D) (H&E X100).

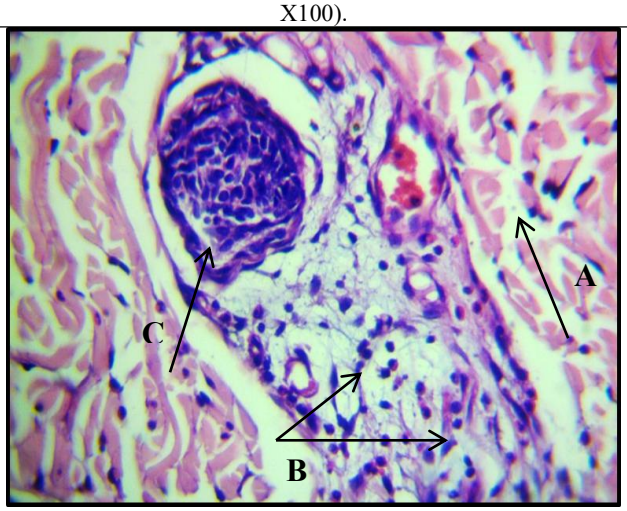

Figure 12. Microscopical picture of treated group showing ,Dermis with collagen bundle group (A), loose connective tissue in the dermis with lymphocyte aggregates (B), lymphocyte nodule (C) (H&E X100).

DISCUSSION

In our experimental study , the histopathological findings through comparing between control group with the groups where lidocaine were used for wound infiltration, collagen production was lower, breaking-strength measurements showed reduced resistance and significantly high edema, vascularity and inflammation scores were found and no infection . However our finding were in agreement with Stratford, *et al* (2002)(16) who reported that infiltration of the wounds with lidocaine 2% had antibacterial effect and decreased the bacterial count (Staphylocoocus aureus) in such wounds . They attributed this to the hypoxia secondary

to the vasoconstricting effect of epinephrine that may interfere with the host defense mechanisms accessing the wound site.

In another study, vassiliadis, (2008)(17) has been recorded that lidocaine has been associated with poor wound healing and increased risk of wound infection especially when combined with adrenaline.

On the other hand our results were with disagreement with Waite, *et al* (2010)(11) and Vasseur, *et al* (1984)(18) who recorded that lidocaine did not alter substantially wound healing.

Morris and Tracey ,(1977)(19) have been found that lidocaine at various concentrations (0.5-2%) had an adverse effect on wound healing and adrenaline (1:100000) potentiated this effect.

Impairment of wound healing in lidocaine groups may be attributed to that lidocaine affects collagenization, reduces the level of collagen as well as increases the activity of the collagen-degrading enzyme MMP-2 and decreased the initial quantity of mast cells at the wound site (20 , 11).

In vitro studies on human skin, Wilmink (2014)(21) suggested that lignocaine depressed the synthesis of mucopolysaccharides and hence possibly that of collagen, tending to impair wound healing.

Drucker, *et al*(1998)(22) have been found that lidocaine 1% decreased collagen fibers number by morphometry as well as vascularity was significantly lower in lidocaine treated animals .

In our experimental study the results showed, 2% lidocaine that delay wound healing process and decrease wound strength ,formation of blood vessel and other skin content as hair follicle and there muscle.

CONCLUSIONS:

The results obtained from this study and other studies showed, wound infiltration in rabbit found lidocaine reduced collagen bundle production, reduced wound breaking strength , retard or delay re-epithelization ,delay formation blood vessel and grossly reduce adhesion of wound edge and rash at the site of injection.

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