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Research Article

Assessing Wound Healing Diameter and Morphological Change in Rabbits: The Impact of Lidocaine, Remifentanil (With and Without Adjuvants), and Tramadol in Burn Wound Healing

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Abstract | This study aimed to evaluate the effects of tramadol and remifentanil on burn wound healing in rabbits. The research involved 30 rabbits divided into five groups: G1 (Lidocaine), G2 (Tramadol), G3 (Remifentanil), and two groups that received combinations of remifentanil with epinephrine. Additionally, one group received remifentanil in a gel formulation. After local administration of these substances by infiltration (s/c) and compared with the control group. the severity of the burn injury and subsequent healing were assessed from first-degree burns. The study results revealed that tramadol and remifentanil with epinephrine significantly slowed down the wound-healing process in rabbit burn models compared to the other groups studied. This study investigated the effects of different medications on wound healing in rabbits. The results at various time points, including 7 days, 14 days, and 21 days, were analyzed. At 7 days post-treatment, Tramadol showed a reduction in wound diameter compared to the control group, suggesting potential wound healing effects. The combination of Remifentanil with epinephrine further reduced the wound diameter, indicating enhanced wound healing effects. The addition of gel to Remifentanil did not significantly impact wound healing compared to the Remifentanil and epinephrine combination. At 14 days, Lidocaine resulted in a more significant reduction in wound size compared to other groups. Tramadol showed a relatively larger wound size, suggesting less effectiveness in promoting wound healing. Remifentanil alone did not significantly reduce wound size. Remifentanil with gel and Remifentanil with epinephrine had a more pronounced effect on wound healing. At 21 days, Tramadol contributed to the complete healing of burn wounds. Lidocaine led to a relatively smaller wound size, while Remifentanil alone did not result in complete healing. The combination of Remifentanil with gel did not lead to full wound closure. Remifentanil with epinephrine resulted in the complete healing of burn wounds. The examination revealed that the outermost layer of the skin, known as the epidermis, appeared normal in thickness and structure, indicating the restoration of turnover and regeneration of epidermal cells to normal levels. The study concluded that tramadol and remifentanil with epinephrine impede burn wound healing in rabbits, highlighting the need for caution in their use.

Keywords | Lidocaine, Remifentanil, Tramadol, Wound healing, Rabbits, Adjuvants

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INTRODUCTION

Burns are categorized based on their depth and severity, which determines the appropriate treatment approach. The American Burn Association uses a classification system consisting of four degrees. Let's begin with first-degree burns, also known as superficial burns. These burns affect the outermost layer of the skin, called the epidermis.

They are characterized by redness, minor swelling, and pain that typically subsides within 48 to 72 hours. Peeling of the damaged epidermis occurs after 5 to 10 days, with no visible scarring resulting from this type of burn. Sunburn is a common cause of first-degree burns (Harkins, 1945).

Partial-thickness superficial burns, also known as second-degree burns, involve damage to both the epidermis and the dermis. This type of burn can be further classified into two subtypes: IIA and IIB. In IIA burns, the epidermis and the superficial layers of the dermis are affected. Blisters may form as a result of the separation of the epidermis from the basement membrane. The healing process for IIA burns typically takes around 14 to 21 days. On the other hand, IIB burns involve damage to the epidermis and layers of the dermis at varying depths. However, these burns do not extend to the islets of the epidermis found within hair follicles and sweat glands (Markiewicz-Gospodarek *et al*., 2022).

The skin affected by partial-thickness superficial burns typically appears red, moist, and painful. The burn injury may result in necrosis of the epidermis within the wound, disrupting the normal epithelialization process. Scarring is possible following these burns. The healing period for this type of burn usually ranges from 21 to 35 days (Chung *et al*., 2020). Full-thickness deep burns, also known as third-degree burns, involve destruction of the epidermis and dermis, extending into deeper tissues. The burned skin appears dry and leathery, with a color that can range from brown and bronze to red. Notably, third-degree burns are characterized by the absence of pain due to nerve damage (Greenhalgh, 2019). Full-thickness burns that extend beyond the third degree are classified as fourth-degree burns. These burns encompass the characteristics of both second-degree and third-degree burns. They penetrate through the epidermis, dermis, and subcutaneous tissue layers, and in severe cases, they may even involve underlying muscles or bones, causing localized tissue necrosis (Markiewicz-Gospodarek *et al*., 2022). The healing process for burns involves various methods depending on the type and severity of the burn. For superficial burns, supportive therapy is typically sufficient. These burns heal within approximately one week through the regeneration of undamaged keratinocytes found within the skin's adnexal structures. In the case of superficial partial-thickness burns, treatment often involves the use of antimicrobial creams and occlusive dressings. These measures create a moist environment that supports epithelialization, the process by which new epithelial cells cover the wound (Cuttle *et al*., 2009). In the case of deep partial-thickness burns, it is important to maintain a warm, moist, and infection-free environment for the wound. The optimal management approach for most deep partial-thickness burns involves the surgical removal (excision) of the burned tissue, followed by skin grafting. This procedure involves transplanting healthy skin from another area of the body or a donor source onto the burn site to facilitate healing (Cuttle *et al*., 2009). Pain management in burn patients presents significant challenges. In addition to pharmacological agents, non-pharmacological techniques can play a valuable role in controlling pain. Methods such as distraction, guided imagery, hypnosis, and virtual reality are effectives and can be used in conjunction with medications (Sawada *et al*., 1997).

Pain is a complex experience that encompasses both sensory and emotional components. It is influenced by a variety of factors, including physiological, sensory, affective, cognitive, socio-cultural, and behavioral elements (Al-Neema, 2008). Lidocaine is a commonly used local anesthetic known for its safety, quick metabolism, and short-lasting effects. It is widely favored in medical procedures due to its efficacy in numbing specific areas. The addition of adrenaline (epinephrine) to lidocaine significantly enhances its effectiveness by constricting blood vessels at the injection site, reducing bleeding, and prolonging the anesthetic effect (Davies and Cashman, 2000; O'Brien *et al*., 2002). Local anesthetics can cause a slight decrease in heart rate (HR) without significant changes in arterial pressure (Gauchan *et al*., 2011). Maintaining hemodynamic stability is crucial during the induction of general anesthesia in surgical procedures. Therefore, when selecting an anesthetic agent for general anesthesia, it is preferable to choose one that has minimal effects on heart rate (HR) and blood pressure (BP) (Andropoulos and Mossad, 2015). Adequate preoperative, intraoperative, and postoperative anesthesia and analgesia can be achieved through the use of local anesthetics, which are considered safer than general anesthetics (Weiniger *et al*., 2010; Al-Rubai *et al*., 2012). Local anesthetics provide effective pain control and can be used to achieve anesthesia locally. However, one of the challenges associated with their use is their relatively short duration of action at the site of administration (Sabri, 2018; Khalil, 2019). Wounds are physical injuries that result in a disruption or break in the skin, leading to a disturbance in the normal structure and function of the skin (Athieu *et al*., 2006). The process of wound healing involves several phases, including coagulation, epithelialization, granulation, collagen formation, and tissue remodeling (Bejon *et al*., 2006). Collagen, a major component of the extracellular matrix, plays a crucial role in providing strength and support to the tissues. Hydroxyproline found abundantly in collagen, has been used as a biochemical marker for tissue collagen (Cherry *et al*., 2000). Understanding the various factors that influence wound healing is essential for developing improved therapeutic options for wound treatment (Chung *et al*., 2015). In the field of surgery, the use of local anesthesia has a long history and has evolved with advancements in anesthetic techniques and knowledge.

Local anesthesia allows for many diagnostic procedures and surgical operations to be performed without the need for general anesthesia (Ogle and Mahjoubi, 2012; Abdeshahi *et al*., 2013).

Local anesthetic agents are commonly used in surgical operations to provide reversible interference with neural conduction and effective pain control (Strichartz, 2009; Caryn *et al*., 2022). The selection of an ideal local anesthetic solution should consider the systemic condition of the individual, aiming to achieve the best fit for optimal surgical intervention. Over time, numerous chemical agents have been developed for local anesthetic purposes, but only a select number of these substances are currently employed (Greisheimer, 1949; Hall and Clark, 1991). Tramadol HCL, for instance, exhibits local anesthetic action on peripheral nerves and is utilized to manage acute and chronic pain of moderate to severe intensity, minimizing side effects, particularly after surgical recovery (John, 2018; Passavanti *et al*., 2020). The use of local anesthesia can help reduce the risks and costs associated with general anesthesia (Barakzai and Perkins, 2005; Baraka *et al*., 2008; Brain and Gliger, 2022). Careful anesthetic and perioperative management of burn patients are of particular importance, given the often lengthy recovery and rehabilitation process they undergo (Bittner *et al*., 2015).

MATERIALS AND METHODS

The experiment was conducted according to scientific controls and high-precision standards to avoid harming animals as much as possible, especially since the research intends to use painkillers in the first place. The humanitarian aspect and consideration for animals are among the basic priorities. All animal experiments were conducted under a protocol approved by the institutional ethics committee and complied with all requirements of the Animal Welfare Act. The study has obtained approval from the Institutional Animal Care and Use Committee (IACUC) or a similar regulatory body responsible for evaluating and overseeing animal care in research. The animals are provided with appropriate housing, veterinary care, and measures to minimize pain and distress throughout the study. And follow the principles of the 3Rs (Replacement, Reduction, and Refinement) by using non-animal alternatives whenever possible, minimizing the number of animals used, and improving handling and care practices to reduce potential pain and stress. committed to upholding the highest ethical standards in animal research and the integrity and validity of the study while prioritizing the welfare of the animals involved.

Animals of the experiment

All animal experiments were conducted under a protocol approved by the institutional ethics committee and complied with all requirements of the Animal Welfare Act. The experiment was conducted at the Department of Physiology Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad. 30 healthy adult male rabbits weighted (1.5±0.08) Kg. Rabbits were anesthetized by intramuscular injection of ketamine (60 mg/kg) and xylazine (5 mg/kg) and their backs were shaved with razors. Were acquired from local markets and housed within animal houses. The rabbits stood kept below controlled environmental situations by heat ranging from 20 to 25°C, in an air-conditioned room with a 12-hour light cycle. The animals were accommodated in metal cages with dimensions of 20 × 50 × 75 cm. A freshly prepared pellet ration was provided as part of their diet. A minimum adaptation period of two weeks was allowed for all rabbits before the start of the experiment.

Experimental design

In this study, 30 healthy rabbits of a local breed were selected to investigate the healing process of burn wounds (first burn degree). each rabbit was then cleansed with an iodine solution and all the surgery was performed under aseptic conditions.

Determination of burn degree

A preliminary study was conducted to ascertain the degree of burn severity first-degree. The dorsal fur of the rabbits was carefully clipped and shaved. The procedure for inducing skin burns was adapted from Arslan *et al*. (2012), with minor modifications. A brass bar with a circular end measuring 2.5 cm in diameter, preheated in boiling water at 100°C for 10 minutes, was applied to the shaven backs of the rabbits for 15 seconds. No pressure was applied; only the weight of the instrument (350 g) was used (Najim, 2015). Skin samples encompassing the burn area and approximately 5 mm of surrounding healthy tissue were collected for histological analysis at intervals of 7-, 14-, and 21 days post-burn.

Measurement of burn wound diameter

The anesthetic agents-remifentanil, tramadol, and lidocaine-were administered at separate dorsal sites on the rabbits. Post-incision, the diameter of the induced burns was measured using a ruler, following the methods described by Suratman *et al*. (1996). A follow-up assessment was carried out after seven days, at which point tissue samples from the treated areas were taken for histopathological evaluation.

Statistical analysis

Results of the current experiment were examined at a Level of $P<0.05$ via two-way ANOVA and utilized the least significant differences (LSD) to contrast between means values (Snedecor-George and Cochran, 1973).

RESULTS AND DISCUSSION

Wounds healing in rabbits

Comparison: Within-group

Tramadol: Showed significant wound healing with the mean score decreasing from 4.1 ± 0.32 at 7 days to 2.80 ± 0.19 at 14 days, and complete healing by 21 days (score of 0), with the 7 to 14-day change being significant.

Remifentanil: Also demonstrated significant healing, decreasing from 4.3 ± 0.51 at 7 days to 2.6 ± 0.22 at 14 days, and complete healing by 21 days (score of 0), with significant change.

Lidocaine: Had a significant reduction in the wound score from 3.2 ± 0.17 at 7 days to 1.80 ± 0.07 at 14 days, and complete healing by 21 days (score of 0), with the change being significant.

Remifentanil with epinephrine: Exhibited significant healing, with scores reducing from 3.1 ± 0.24 at 7 days to 1.6 ± 0.09 at 14 days, and complete healing by 21 days (score of 0), with a significant change.

Remifentanil with gel: Showed a significant decrease in wound scores from 3.1 ± 0.23 at 7 days to 1.5 ± 0.04 at 14 days, and complete healing by 21 days (score of 0), with the change being significant.

Control group: Showed significant decrease in wound scores and complete healing in 21 days as Figure 3.

Table 1: Effect of drug injection on burn wounds healing measures in rabbits.

Groups/ Drug	Mean ± SE of wounds healing		LSD value
	7 day	14 day	
Tramadol	4.1±0.32ABa	2.80±0.19Ab	1.04 *
Remifentanil	4.3±0.51Aa	2.6±0.22ABb	1.22 *
Lidocaine	3.2±0.17AB a	1.80±0.07ABb	1.19 *
Remifentanil with epinephrine	3.1±0.24Ba	1.6±0.09ABb	1.14 *
Remifentanil with gel	3.1±0.23Ba	1.5 ±0.04Bb	1.30 *
Control group +/_	3±0.10ABa	1.80±0.02ABb	1.9 *
LSD value	1.166 *	1.207 *	---

Means with different big letters in the same column and small letters in the same row are significantly different, * (P≤0.05).

Between-group comparison

On day 7, there were no statistically significant differences between any of the groups. By day 14, all groups had significantly improved with no statistically significant difference in wound healing rates between them. By day 21, all groups had achieved complete wound healing with a score of 0, indicating no difference in the outcome of wound healing among the treatments.

Overall interpretation: All groups showed significant improvement in wound healing from day seven. Based on the results, in Figure 1. We can make the following observations on the 7th days Tramadol treatment (group A) resulted in a reduction in wound diameter compared to the control group, indicating potential wound-healing effects. The Remifentanil group (group B) did not show a significant reduction in wound diameter compared to the control group, suggesting that Remifentanil alone may not have a substantial impact on wound healing. The Lidocaine group (group C) demonstrated a slightly smaller wound diameter compared to both the control and Remifentanil groups, suggesting that Lidocaine may have some beneficial effects on wound healing. The remifentanil with epinephrine group (group D) showed a further reduction in wound diameter compared to the remifentanil group, indicating that the addition of epinephrine may enhance the wound healing effects of remifentanil. The Remifentanil with gel group (group E) demonstrated the same average diameter as the remifentanil with epinephrine group, suggesting that the gel formulation did not have a significant impact on wound healing compared to the combination of remifentanil and epinephrine.

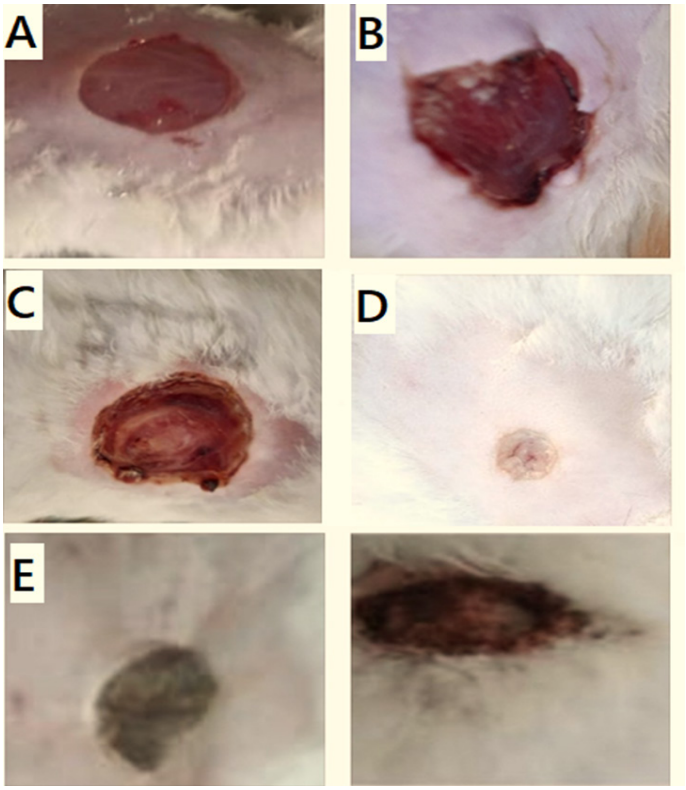

Figure 1: Assessment of wound healing in circular wounds with a 5 cm diameter in various experimental groups of rabbits. treated with (A) Tramadol on 7th day after burn. The diameter was 4.1cm (B) Remifentanil group, had an average diameter of 4.3 cm. (C) Lidocaine group, the average burn diameter was 3.3 cm (D) Remifentanil with epinephrine average burn diameter was 3.1 cm (E) Remifentanil with gel. The average diameter of the burned skin was 3.1 cm.

Overall, these results indicate that Tramadol and Lidocaine might need potential aids trendy promoting wound healing, while the combination of Remifentanil and epinephrine may also enhance twisted curative effects. However, further studies are needed to confirm these findings and explore the underlying mechanisms of action. Tramadol and Lidocaine are medications used for pain relief. Remifentanil is an opioid analgesic, and epinephrine is a hormone that can constrict blood vessels. Based on the wound healing process in rabbits at the 14-day mark, the following results in Figure 2 showed.

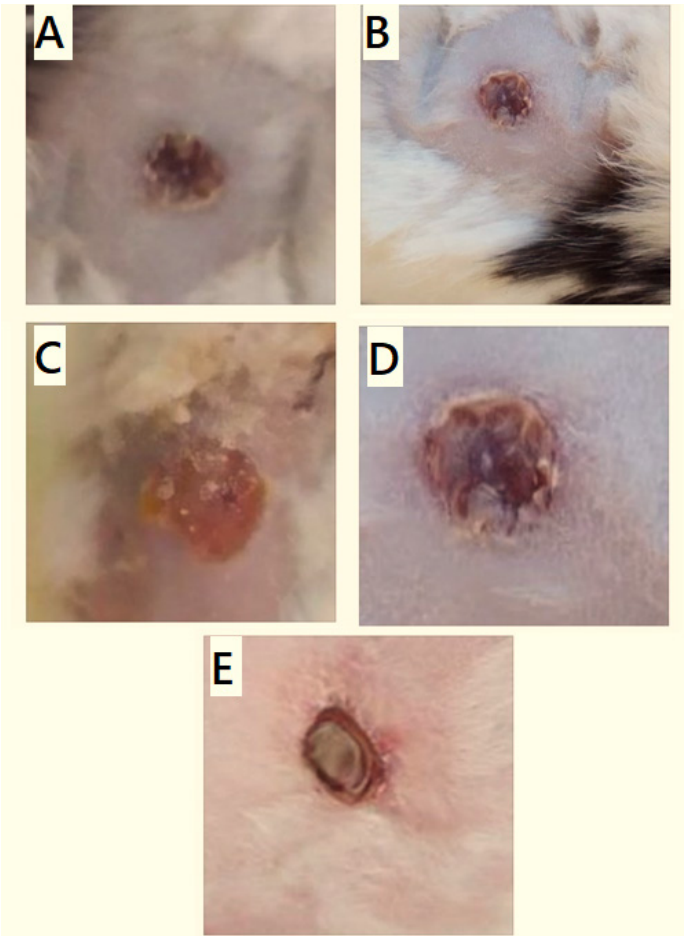

Figure 2: Wound healing process in rabbits at the 14-day. The (A) Lidocaine group the average burn diameter was 1.80cm (B) Tramadol group, resulting in an average burn diameter of 2.80cm. (C) Remifentanil group ,the average burn diameter was 2.6 cm (D) Remifentanil with gel group , resulting in an average burn diameter of 1.5cm (E) Remifentanil with epinephrine the burned skin had an average diameter of 1.6 cm.

Lidocaine group (group a): The average burn diameter of 1.80 cm suggests that Lidocaine may have contributed to a more significant reduction in wound size compared to the other groups at the 14-day mark. This indicates that Lidocaine might have beneficial effects on wound healing, potentially through its local anesthetic and anti-inflammatory properties. Tramadol group (group b): The average burn diameter of 2.80 cm indicates that Tramadol treatment resulted in a relatively larger wound size compared to the Lidocaine group. This suggests that Tramadol may not have been as effective in promoting wound healing at this stage of the process. Remifentanil group (group c): The average burn diameter of 2.6 cm indicates that Remifentanil alone did not lead to a significant reduction in wound size compared to the Lidocaine group. This suggests that Remifentanil may not have a substantial impact on wound healing at the 14-day mark. Remifentanil with gel group (group d): The average burn diameter of 1.5 cm suggests that the combination of Remifentanil with gel had a more pronounced effect on wound healing compared to the other groups. The gel formulation used in this group may have contributed to the observed reduction in wound size. Remifentanil with epinephrine group (group e): The burned skin had an average diameter of 1.6 cm, which is similar to the Remifentanil with gel group. This suggests that the addition of epinephrine to Remifentanil may have enhanced the wound healing effects, potentially by improving blood flow to the wound site and promoting tissue repair. Overall, these results indicate that Lidocaine, the combination of Remifentanil with gel, and the combination of Remifentanil with epinephrine may have potential benefits in promoting wound healing in rabbits at the 14-day mark. However, further studies are needed to confirm these findings and investigate the underlying mechanisms of action. Based on the results in Figure 3 at 21 days.

Tramadol group (group a): The average burn diameter of 0 cm suggests that Tramadol may have contributed to the complete healing of the burn wounds by the 21-day mark. This indicates that Tramadol might have beneficial effects on wound healing, potentially through its analgesic properties and potential modulation of the inflammatory response. Lidocaine group (group b): The burn diameter of 0.5 cm suggests that Lidocaine treatment resulted in a relatively smaller wound size compared to the other groups at the 21-day mark. This indicates that Lidocaine may have played a role in promoting wound healing and reducing inflammation. Remifentanil group (group c): The average burn diameter of 0.7 cm indicates that Remifentanil alone did not lead to complete healing of the burn wounds by the 21-day mark. This suggests that Remifentanil may not have a significant impact on wound healing within this timeframe. Remifentanil with gel group (group d): The average burn diameter of 0.8 cm suggests that the combination of Remifentanil with gel did not result in complete healing of the burn wounds by the 21-day mark. The gel formulation used in this group may have had some therapeutic effects but did not lead to full wound closure within this timeframe. Remifentanil with epinephrine group (group e): The burn showing an average diameter of 0 cm indicates that the combination of Remifentanil

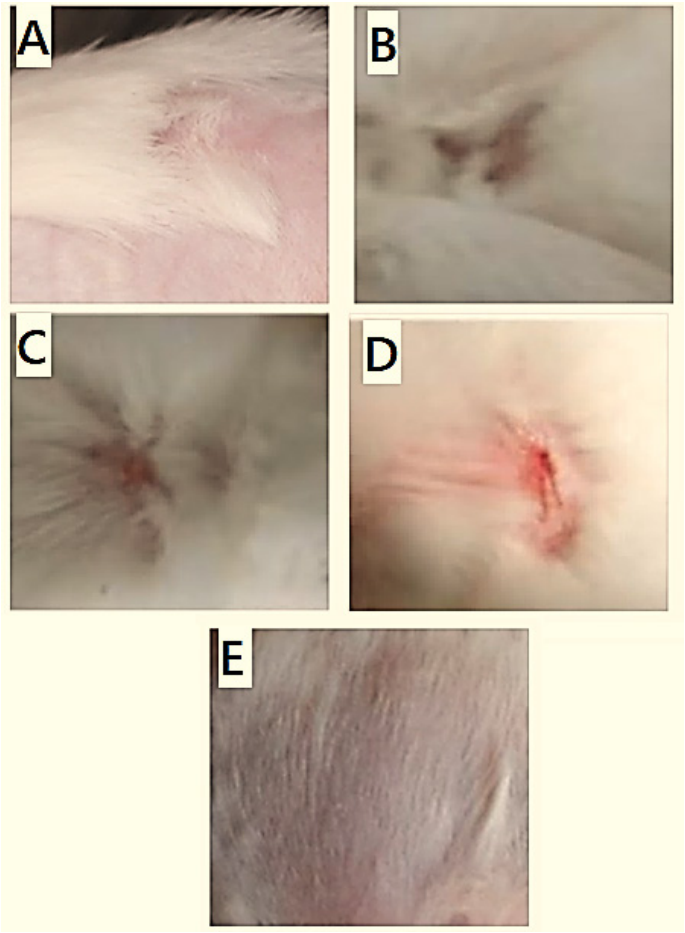

Figure 3: Wound healing progress in rabbits over a period of 21 days. (A) Tramadol group, the average burn diameter measured .0cm. (B) Lidocaine group, burn diameter was 0.5 cm. (C) Remifentanil group. The average burn diameter measured 0.7 cm.(D) Remifentanil with gel group. The average burn diameter was 0.8 cm. (E) Remifentanil with epinephrine group, the burn showed an average burn diameter of 0.cm

with epinephrine led to complete healing of the burn wounds by the 21-day mark. The addition of epinephrine to Remifentanil may have enhanced wound healing, potentially by improving blood flow to the wound site and promoting tissue repair. Overall, these results suggest that Tramadol and Lidocaine may have positive effects on wound healing, with Tramadol potentially leading to complete wound closure by 21 days. The combination of Remifentanil with epinephrine also appears to have beneficial effects, resulting in complete healing of the burn wounds within the given timeframe.

Histopathological results

The histopathological examination of the rabbit's skin at 7 days post-treatment with Normal epidermis epithelium (Black arrow). The epidermis, which is the outermost layer of the skin, appeared normal in structure and did not show any significant abnormalities or damage. Little infiltration of mononuclear cells (MNCs) (Red arrows):

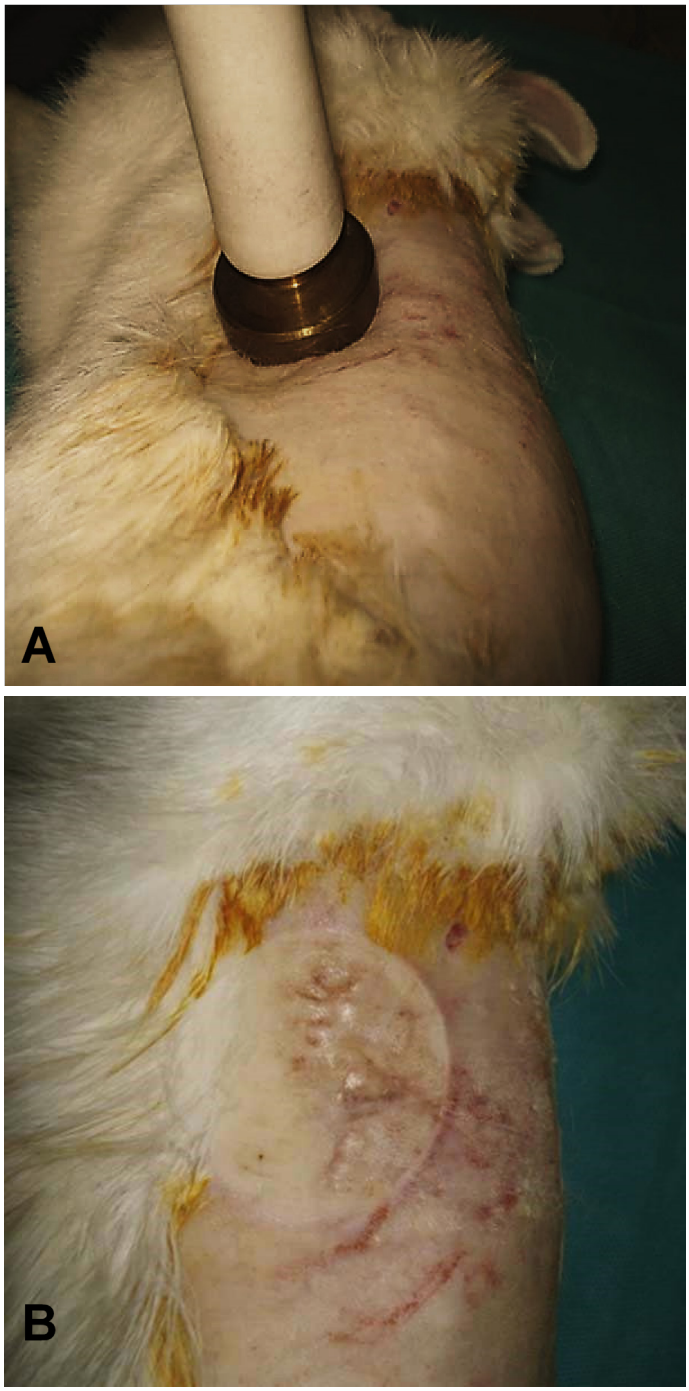

Figure 4: Assessment of wound healing in circular wounds with a 5 cm diameter in control groups of rabbits. (A) On 7th day after burn. The diameter was3.0cm (B) after burn wounds done (control groups).

A few mononuclear cells were observed in the dermis, indicating a mild inflammatory response. Mononuclear cells are a type of white blood cells involved in immune responses. Mild degeneration of dermal collagen fibers (Asterisk): There was evidence of mild degeneration in the collagen fibers of the dermis. Collagen fibers provide structural support to the skin. The observed degeneration may suggest an early stage of tissue repair or remodeling. Newly formed hair follicles (Blue arrows): The presence of newly formed hair follicles indicated an ongoing process

of skin regeneration and healing. The histopathological examination of the rabbit's skin in at 7 days post-treatment with Remifentanil, Remifentanil with gel, and Remifentanil with epinephrine revealed the following findings: Remifentanil: Normal epidermis (Black arrow): The epidermis, the outermost layer of the skin, appeared normal in structure without any notable abnormalities or damage Remifentanil with gel: Normal epidermis (Black arrow): Similar to the Remifentanil group, the epidermis showed normally structure without significant changes. Remifentanil with epinephrine. Normal epidermis (Black arrow). The epidermis displayed a normal structure, similar to the other treatment groups. These results indicate that at 7 days post-treatment, all three groups (Remifentanil, Remifentanil with gel, and Remifentanil with epinephrine) exhibited normal epidermal structures. This suggests that this treatment did not adversely affect on epidermis. It is important that the histopathological findings alone may not provide a comprehensive understanding of the overall effects of these treatments on wound healing. The histopathological examination of the rabbits. Post-treatment with Remifentanil, Remifentanil with gel, and remifentanil with epinephrine receptacle review the following findings: Remifentanil, very thin epidermis epithelium (Black arrow). The epidermis outer layer of the skin appears significantly thinner than normal this indicates distributed normal turnover and regeneration of epidermal cells and Moderate infiltration of polymorph nuclear (PMCS) and mononuclear cells (MNCs)in the dermis (Red arrows).the dermis showed Moderate presence of both PMCs and MNCs suggesting. Suggests that inflammatory response. PMCs are typical of white blood cells involved in intimate stages of inflammation, while MNCs include lymphocytes and monocytes degeneration with depletion of dermal collagen fibers (Asterisk): The collagen fibers in the dermis displayed signs of degeneration and reduction in quantity. Collagen fibers provide structural support to the skin, and their degeneration may indicate impaired healing and tissue repair. Hemorrhage (Blue arrows): Areas of hemorrhage or bleeding were observed in extreme sections of the skin. This suggests vascular damage or increased fragility of blood vessels in treatment areas. Remifentanil with gel. Similar to the Remifentanil group, findings of Remifentanil with gel group included a very thin epidermis epithelium Moderate infiltration of PMCs and MNCs in the dermis, degeneration with depletion of dermal collagen fibers, and hemorrhage. Remifentanil with epinephrine. Similarly, remifentanil with the epinephrine group had very thin epidermis epithelium and modulation filtration of PMCs and MNCs in the dermis, degeneration with depletion. This suggests indicate at day 7 post-treatment all three groups (Remifentanil, Remifentanil with gel, and Remifentanil with epinephrine) exhibited detrimental effects on the skin as Figure 5A, B, C, D. This suggests thinning of epithelium, inflammation, degeneration loss of collagen fibers, and hemorrhage. These findings suggest that the treatments have adverse effects on wound healing and tissue repair processes in the skin. It is important to note that these results are specific to the rabbit model and further research is needed to determine the efficacy of remifentanil, remifentanil-epinephrine, and remifentanil with gel on skin. The histopathological examination of the rabbit's skin at 14 days post-treatment with Remifentanil with gel group showed the flowing findings. Normal epidermis (Black arrow). the outer layer of the skin, known as the epidermis appearance normal skin in the thick and structure. This indicates that turnover and regeneration of epidermal cells to normal levels. Moderate inflammatory cell infiltration, particularly mononuclear cells in the dermis (Red arrows): The deeper layer of the skin, called the dermis, showed Moderate presence of inflammatory cells, specifically mononuclear cells. This suggests an ongoing inflammatory response in the dermis, which could be indicative of the body's attempt to repair any damage caused by the treatment. Depletion of collagen fibers (Asterisk): The collagen fibers in the dermis displayed signs of depletion, indicating a reduction in their quantity. Collagen fibers are essential for developing and providing structural support to the skin, and their depletion suggests a delay or impairment in the healing and tissue repair processes. New formed hair follicles (Blue arrows) era of new synthesized hair follicles were observed in the section of skin. This indicates that the regeneration capacity of the skin has been activated. Potential as part of their healing process. These results suggest at 14 days post-treatment with Remifentanil with gel, the skin of the rabbit undergoes a healing process as Figure 5E, F, G, H, I. The normal epidermis, moderate inflammatory cell infiltration, depletion of collagen fibers, and newly formed hair follicles indicate a complex interplay between tissue damage and repair mechanisms. The histopathological examination of the rabbit's skin at 21 days post-treatment with Tramadol revealed the following findings. Showed normal epidermis epithelium (Black arrow): The outermost layer of the skin, known as the epidermis, appeared to be normal in thickness and structure as Figure 5J, K, L, M. This indicates that the turnover and regeneration of epidermal cells have returned to normal. Little infiltration of mononuclear cells (MNCs) in the dermis and around hair follicles (Red arrows and Blue arrows, respectively. The deeper layer of the skin, called the dermis, showed a small presence of mononuclear cells. This suggests a mild inflammatory response in the dermis and around the hair follicles. Mononuclear cells include lymphocytes and monocytes, which are involved in the immune response as in Figure 5. Post-treatment histopathological examination of rabbit skin revealed varying effects depending on the treatment used.

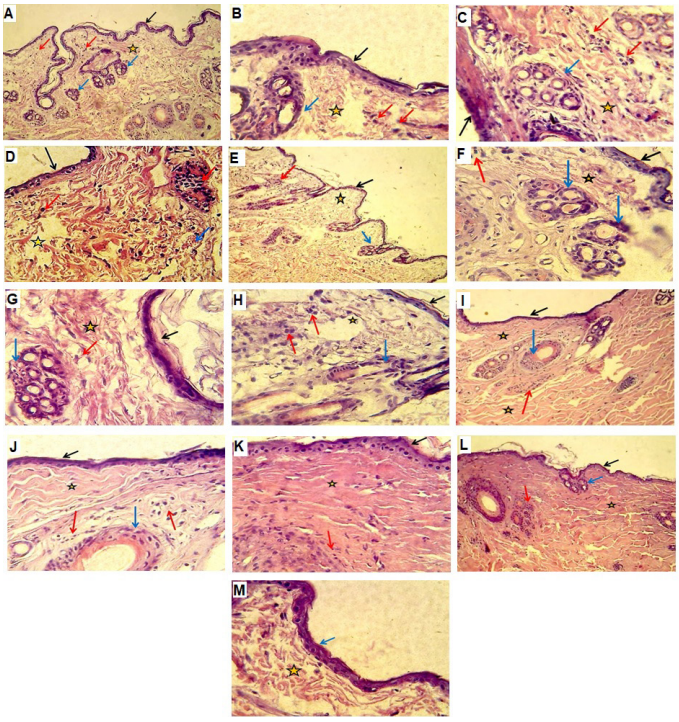

Figure 5: Post-treatment histopathological examination of rabbit skin revealed varying effects depending on the treatment used. At 7 days, treatments with R and Lidocaine resulted in minimal changes, with mild degeneration of dermal collagen fibres and new hair follicle formation. Remifentanil treatments showed little inflammatory cell infiltration but no new hair follicles as A,B,C,D respectively. By 14 days, Remifentanil with gel showed moderate inflammation and collagen fibre depletion, while Lidocaine, Tramadol, and Remifentanil treatments showed minimal changes as in E,F, G,H respectively . At 21 days, Tramadol and Remifentanil treatments showed little inflammation and normal collagen fibers, while Lidocaine treatment showed no inflammatory reaction and normal mature dermal collagen fibers in I, J, K, L, M, respectively.

At 7 days, treatments with Remifentanil and Lidocaine resulted in minimal changes, with mild degeneration of dermal collagen fibers and new hair follicle formation. Remifentanil treatments showed little inflammatory cell infiltration but no new hair follicles as in Figure 5A, B, C, D, respectively. By 14 days, Remifentanil with gel showed moderate inflammation and collagen fiber depletion, while Lidocaine, Tramadol, and Remifentanil treatments showed minimal changes as in (E, F, G and H), respectively. At 21 days, Tramadol and Remifentanil treatments showed little inflammation and normal collagen fibers, while Lidocaine treatment showed no inflammatory reaction and normal mature dermal collagen fibers in (I, J, K, L, M), respectively.

Tramadol: Is central central-acting analgesic that primarily acts as a weak opioid agonist. It binds to mu-opioid receptors in the central nervous system and inhibits reuptake of norepinephrine and serotonin. Tramadol's mechanism of action may contribute to its wound-healing properties by reducing pain and inflammation, which can promote the healing process wound healing (Lewis and Han, 1997). Lidocaine. Is a local anesthetic that blocks sodium channels associated with cell-mediated Its use in wound healing may be attributed to its ability to provide local anesthesia, reduce pain, and potentially modulate inflammation, which can promote the wound healing process (Soto *et al*., 2020).

Remifentanil with epinephrine group's Epinephrine is a sympathomimetic drug that acts as vasoconstriction. When combined with epinephrine it may enhance the analgesic efficacy and provide localized vasoconstriction which can reduce bleeding and improve wound healing (Schnabl *et al*., 2021). Remifentanil with gel group. The results of remifentanil produce a sustainable drug for prolonged analgesia effects. This sustained analgesia may contribute to improved wound healing by reducing pain and inflammation (Moote, 1992).

Studies supporting the mechanisms of action. A study by Barreiro *et al*. (2019) investigated the effects of tramadol on wound healing in rat models. The results showed that tramadol treatment significantly reduced wound size and increased collagen deposition indicating improvement in wound healing (Shiekh *et al*., 2020). The study suggested that tramadol's analgesic and anti-inflammatory effects may contribute to its wound healing properties in a study by Gustorff *et al*. (2001). Remifentanil was administered to patients undergoing surgery and wound healing was assessed. The findings demonstrated that patients receiving remifentanil had reduced pain intensity and improved wound healing compared to the control group. Studies provide that remifentanil analgesic properties may promote wound healing by reducing pain and inflammation. Studies with conflicting findings. A study by Lee (2020). evaluating the effects of lidocaine on wound healing in rabbits. Contributing to the current results, the study reported that lidocaine treatment did not significantly affect wound healing compared to the control group. Others suggest that other factors such as severe pain may have caused or influenced lidocaine treatment. Another study by Chen (2017) investigated the effects of remifentanil on wound healing in rabbits and showed a significant decrease in wound healing in may attempt to immune responses to remifentanil.

The results from the wound healing process in rabbits over 21 days showed interesting findings regarding the direct effects of different medications on wound diameter. The observations made at days the 7th,14th, and 21st days to gain a better understanding as in Figure 4, tramadol group (group a) resulted in a reduction in wound diameter

compared to the control group. This suggests the potential wound-healing effects of Tramadol. Remifentanil (group b): Remifentanil alone did not significantly affect on wound diameter compared to the control group. This suggests remifentanil does not have a substantial impact on wound healing. Lidocaine (group c): demonstrated slightly smaller wound diameter compared to both control and Remifentanil groups. This suggests that lidocaine may have some beneficial effects on wound healing. Remifentanil with epinephrine (group d): The combination of Remifentanil and epinephrine showed a further reduction of wound diameter compared to the Remifentanil group. This implies that epinephrine may enhance the wound-healing effect of remifentanil. Remifentanil with gel (group e) Remifentanil with epinephrine group had a decrease in wound healing compared to Remifentanil and epinephrine at days post-treatment 14 lidocaine in (group a): Lidocaine demonstrated reducing in wound diameter contributed to the wound healing process, potentially through its local anesthetic and anti-inflammatory properties. The combination of Remifentanil with epinephrine led to the complete healing of burn wounds, suggesting enhanced wound healing at days 21-day mark. Research Agreement Tramadol and lidocaine may have contributed potential benefits in promoting wound healing based on the observed reduction in wound diameter as findings in combination of Remifentanil with epinephrine group showed enhanced wound healing effects compared to Remifentanil alone. Research disagreement: The study results are limited to rabbits, and translation to human wound healing may not be straightforward. Further research with human subjects is required to validate these findings. The study did not assess other parameters of wound healing, such as inflammation, granulation tissue formation, or scarring, which are important aspects to consider in wound healing evaluation. The sample size and study design should be taken into consideration when interpreting the results. Larger sample sizes and controlled experimental conditions are necessary for more reliable conclusions.

CONCLUSION AND RECOMMENDATIONS

We concluded of good effects of lidocaine and remifentanil with epinephrine or gel on the burns wound healing process without any complications in healing.

In this study, the effects of tramadol and remifentanil on burn wound healing in rabbits were evaluated. The results demonstrated that tramadol and remifentanil with epinephrine significantly slowed down the wound-healing process in rabbit burn models compared to the other groups studied. Lidocaine showed a more significant reduction in wound size at 14 days, while tramadol contributed to the complete healing of burn wounds at 21 days. Remifentanil alone did not result in complete healing, but when combined with epinephrine, it led to the complete healing of burn wounds.

The examination of the epidermis revealed its normal thickness and structure, indicating the restoration of turnover and regeneration of epidermal cells to normal levels. These findings raise caution regarding the use of tramadol and remifentanil with epinephrine in promoting burn wound healing in rabbits.

Future research directions

- Further investigation is needed to understand the underlying mechanisms by which tramadol and remifentanil with epinephrine impede wound healing in burn injuries.
- Exploring alternative medications or treatment strategies that can effectively promote wound healing in burn injuries without hindering the process.
- Studying the long-term effects of tramadol and remifentanil on burn wound healing and potential complications that may arise.

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NOVELTY STATEMENT

This study aimed to evaluate the effects of tramadol and remifentanil on burn wound healing in rabbits. While previous studies have investigated the use of these drugs individually, this study explores their effects in combination with adjuvants and gel formulations. Additionally, the study assesses the impact of these drugs on the severity of burn injury and subsequent healing.

AUTHOR'S CONTRIBUTION

NFA: Contributed to the design and execution of the experiment, data collection, analysis, and interpretation. RAO: Contributed to the design of the study, data analysis, and manuscript preparation.

Ethical statement

Before starting this study, Ethical approval was granted through the local animal care committee and use, collage of Veterinary Medicine University of Baghdad (number P.G. 115).

Conflict of interest

The authors have declared no conflict of interest.

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