

A Comparative Study of Efficacy of Tramadol and Diclofenac on Healing of Full-thickness Cutaneous Wounds in Rabbits

دراسة مقارنه لكفاه عقار الترامادول وديكلوفيناك على شفاء الجروح الجلدية في الارانب

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ABSTRACT

The present study was investigate to compare the effects of tramadol and Diclofenac on healing rates of skin wounds in rabbits based on histopathological evaluation. Twenty four adult clinically healthy rabbits from both sexes weighing from (1.1- 1.9 kg) were used in this study. All animals were subjected to creation of full-thickness wound (4cm²) in abdominal region. The treatment schedule was twice daily with injection of normal saline (group A) (control group), tramadol 10mg/kg b.w. (group B) and diclofenac 1.5mg/kg b.w. (group C) intramuscularly each group contain eight rabbits. On day 3, 7, 10 and 14 post wounding skin biopsies were collected for histopathological two rabbits/period examination. The result revealed, Clinically, the rate of wound healing was same in all groups, histopathological result was show: In 3rd day of skin wound healing group (A) show normal epidermis, dermis and stratum spinoseum, group (B) show moderate hyperplasia of stratum spinoseum and group (C) show severe hyperplasia of stratum spinoseum. In 7th day of skin wound healing group (A) show similar findings of day three, group (B) revealed moderate hyperplasia of stratum spinoseum and abundant keratinocytes while group (C) show severe hyperplasia of stratum spinoseum and reduce of stratum corneum. In 10th day of skin wound group of (A) reflected epidermis and dermis with moderate hyperplasia of stratum spinoseum, in contrast group (B) show moderate hyperplasia of stratum spinoseum with increase in stratum corneum and group (C) revealed reduce in stratum spinoseum and stratum corneum. In 14th day the wound of group (A) show abundant amount of keratin and increase of fibroblasts in dermis, while group (B) show reduce of stratum spinoseum's hyperplasia and in group (C) there were disappearance of stratum spinoseum's hyperplasia and reduce of stratum corneum.

المستخلص

هدفت الدراسة الحالية الى المقارنة بين تأثيرات الترامادول و ديكلوفيناك على معدلات الشفاء النسيجي- المرضي لجروح الجلد في الارانب. استخدمت أربعة وعشرون ارنبا بالغاً تراوحت اوزانها بين (1.1- 1.9 كغم). احدث جرح شمل جميع طبقات الجلد بمساحة (4 سم²) في منطقة البطن لجمع حيوانات التجربة، العلاج كان مرتين يومياً بالحقن عضلياً المجموعة (A) مجموعة السيطرة بالمحلول المتعادل، المجموعة (B) حقنت بالترامادول بجرعة 10مل/كغم من وزن الجسم والمجموعة (C) حقنت بالديكلوفيناك وجرعة 1.5 مل /كغم من وزن الجسم. اخذت عينات الجلد في 3، 7، 10، و14 يوم بعد العملية لغرض اجراء الفحص النسيجي- المرضي لها ولكل وقت ارنبين. اظهرت النتائج سريرياً ان معدل شفاء الجروح في كل المجاميع متشابه ولا يوجد اي اختلافات. اما الفحص النسيجي- المرضي فكانت النتائج كما يلي : في اليوم الثالث من شفاء الجروح البشرة طبيعية والادمة والطبقة الشوكية طبيعية في مجموعة (A) ، المجموعة (B) تضخم معتدل في الطبقة الشوكية ومجموعة (C) زيادة حادة في الطبقة الشوكية. في اليوم السابع من شفاء الجروح البشرة طبيعية والادمة والطبقة الشوكية طبيعية في مجموعة (A)، المجموعة (B) تضخم معتدل في الطبقة الشوكية وغازارة في الخلايا المولدة للكيراتين ومجموعة (C) تضخم حاد في الطبقة الشوكية. في اليوم العاشر من شفاء الجروح تضخم معتدل في البشرة، الادمة والطبقة الشوكية في مجموعة (A)، المجموعة (B) تضخم معتدل في الطبقة الشوكية والمجموعة (C) اختزال في الطبقة الشوكية. في اليوم الرابع عشر من شفاء الجروح غازارة في كمية الكيراتين وخلايا الارومات الليفية في المجموعة (A)، المجموعة (B) اختزال في تضخم الطبقة الشوكية والمجموعة (C) اختفاء تضخم الطبقة الشوكية.

INTRODUCTION

Wound healing represents a dynamic physiological process initiated and influenced by many factors. Collagen synthesis and its maturation play an important role in the healing process and cross linked mature collagen is essential for platelet adhesion and subsequent aggregation [1,2].

Mechanical properties of skin have so long seek the attentions of dermatologists and bioengineers, as biomechanical skin parameters at various sites change by age and in different disease conditions. Therefore, an objective functional assessment of the skin mechanics was necessary to correlate the mechanical properties of skin with anatomical and biomechanical findings [3].

Diclofenac sodium is probably one of the most common non-steroidal compounds with analgesic, anti-inflammatory, antirheumatic and antipyretic properties[4]. This drug has an inhibitory effect on prostaglandin synthesis and used as an initial therapy for inflammatory and degenerative rheumatic diseases as well as for pain conditions such as musculoskeletal and post-operative pains and acute attacks of gout and ureteric colic [5]. Diclofenac (daily dose of 1.5 mg/kg body weight) is claimed to be faster acting than Ibuprofen, longer acting than Paracetamol and as safe as Ibuprofen[6]. There are many side effects observed with the use of this drug. It is not recommended for (paediatric uses) while its overdosing is potentially toxic. Diclofenac sodium causes a rare but potentially fetal hepatotoxicity that may be associated with the formation of reactive metabolites and subsequent adverse hepatitis effects may arise in certain individuals[7].

Tramadol an atypical centrally acting analgesic with opioid and nonopioid like properties [8] of the aminocyclohexanol group [9] structurally related to codeine and abstract morphine, consists of two enantiomers both of which contribute to the analgesic activity via different mechanisms [10] (+)-Tramadol and the metabolite (+)-O-desmethyl-tramadol are agonists of the μ opioid receptor. (+)-Tramadol inhibits serotonin reuptake and (-)-Tramadol inhibits norepinephrine reuptake, enhancing inhibitory effects on pain transmission in the spinal cord. The complementary and synergistic actions of the two enantiomers improve the analgesic efficacy and tolerability profile of the racemate [11].

The aim of the present study was to compare the effects of tramadol and Diclofenac on healing rates of full-thickness skin wounds in rabbits.

MATERIALS AND METHODS

Twenty four adult clinically healthy rabbits from both sexes weighing from (1.1- 1.9 kg) were used for this study. The animals were housed in the animal farm of the College of Veterinary Medicine, University of Karbala, maintained in individual cages under normal environment including climate, management and feeding. All animals were subjected to wound creation (4cm²) full-thickness in abdominal region after prepared it for aseptic surgical operation [12].

The general anesthesia was induce with diazepam(Diazepam 10® the ampoule contains 2 ml (10mg/2ml), Aleppo-Pharmaceutical Industries, Aleppo-Syria) as a preanesthetic in dose 1mg/kg b.w. after 10 min. injected with xylazine (Xylazine 2%® Vial contains 50ml (20mg/1ml), Ceva Saute animal, Spain) in dose rate of 10 mg/kg b.w. and ketamine(Ketamine® (10%) Vial contains 10 ml Vet. Injection, kepro pharmaceuticals, Holland) in dose rate of 50 mg/kg b.w. all this drugs injected intramuscularly [13].

On each animal, one square (4cm²) full-thickness skin wounds were created in abdominal region by using a template prepared from an X-ray film for all animals and sutured the wounds with silk suture 3-0 by horizontal interrupted mattress suturing. The treatment schedule was twice daily with injection of normal saline (group A) (control group), tramadol (Neodol® the ampoule contains 2ml (100mg/2ml), Ibn Hayyan Pharm-Homs-Syria) 10mg/kg b.w. (group B) and diclofenac (ampoule contains 3ml (75mg/3ml), Hemofarm, Yugoslavia) 1.5mg/kg b.w. (group C) intramuscularly each group contain eight rabbits. On day 3, 7, 10 and 14 post operations skin biopsies samples were collected for histopathological examination and stained with H&E stain[14] two rabbits/period.

RESULTS AND DISCUSSION

During the post surgical period, the animals remained had on infection. The microscopic observation also confirmed the aseptic conditions during the wounding in all groups.

In 3rd day of skin wound healing group (A) show normal epidermis, dermis and stratum spinosum, group (B) there were moderate hyperplasia of stratum spinosum and group (C) reflected severe hyperplasia of stratum spinosum (Fig 1).

The inflammatory response is initiated very soon after the trauma on wound event, its the first phase of the wound healing. During this response the wound and surrounding tissues become inflamed and cells, particularly neutrophils and monocytes are mobilized to infiltrate the clot and start the processes involved in synthesis of granulation tissue [15].

In 7th day of skin wound healing group (A) show epidermis and dermis with normal stratum spinosum, while group (B) show moderate hyperplasia of stratum spinosum and abundant keratinocytes and group (C) revealed severe hyperplasia of stratum spinosum and reduce of stratum corneum (Fig 2).

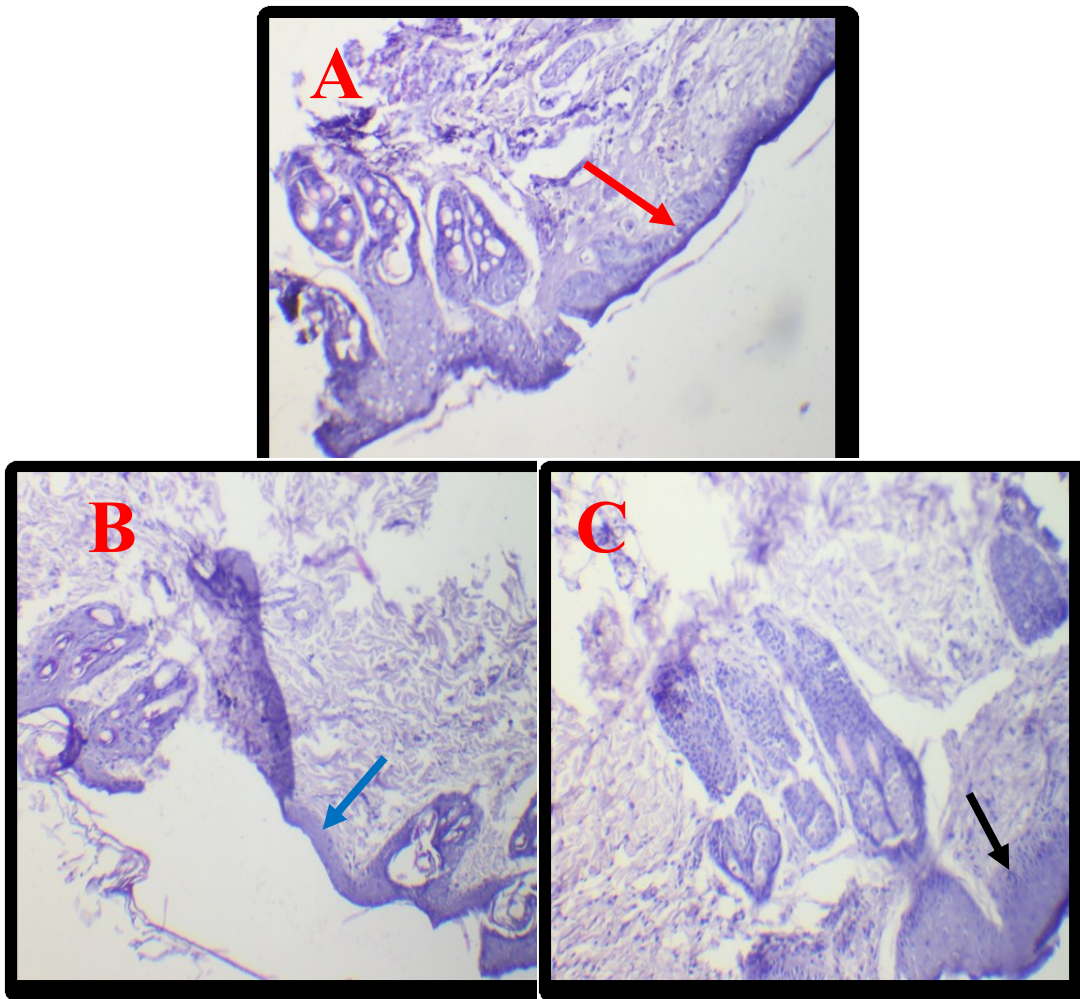
The complete regeneration of the rabbit's epidermis was finished on the day six post surgery, which is comparable to humans [16].

In 10th day the skin wound of group (A) show epidermis and dermis with moderate hyperplasia of stratum spinosum, group (B) show moderate hyperplasia of stratum spinosum with increase in stratum corneum and group (C) show reduce in stratum spinosum and reduce of stratum corneum (Fig.3).

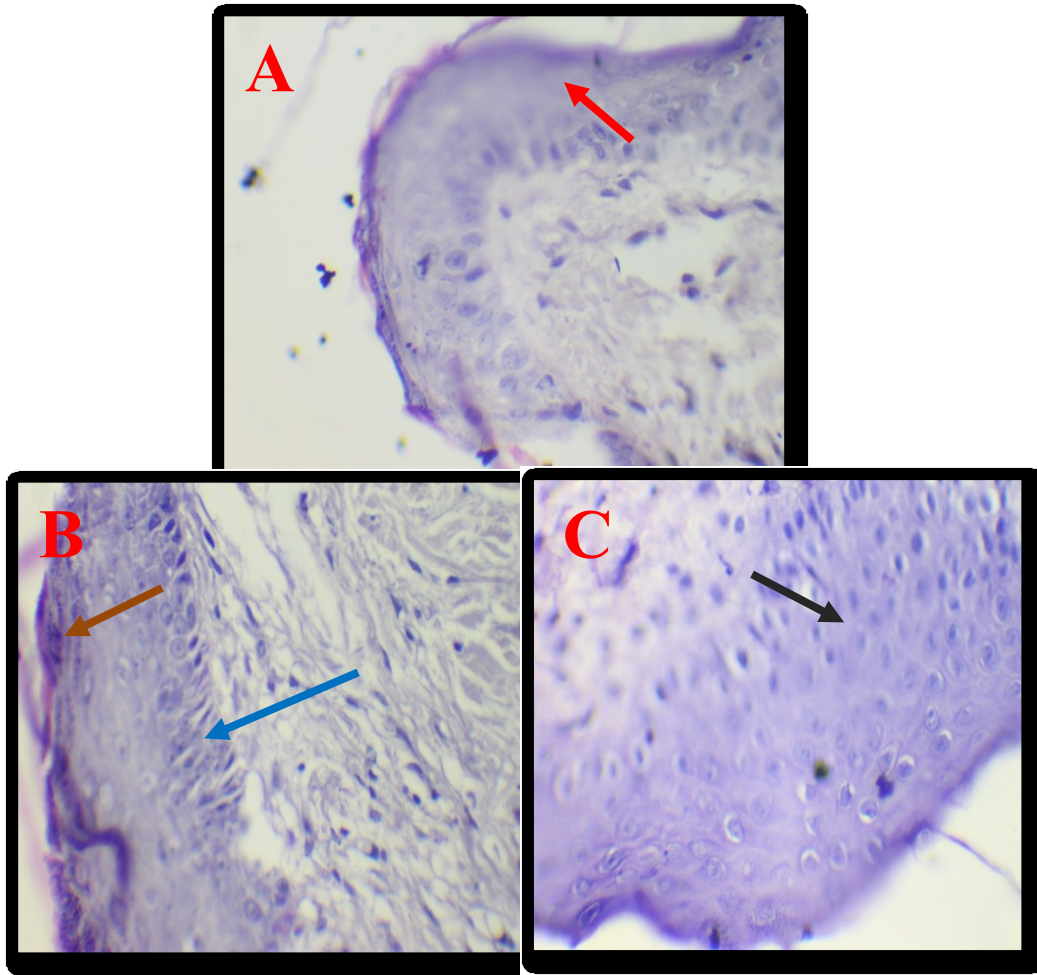
In a study by [17] entitled influence of indomethacin on collagen synthesis during tendon healing in the rabbit, it was shown that indomethacin affected the collagen metabolism differently depending on whether the tendons were involved in wound healing or not.

In 14th day the skin wound of group (A) had abundant amount of keratin and increase of fibroblast in dermis, group (B) show reduce of stratum spinosum's hyperplasia and group (C) show disappear in stratum spinosum's hyperplasia and reduce of stratum corneum (Fig. 5).

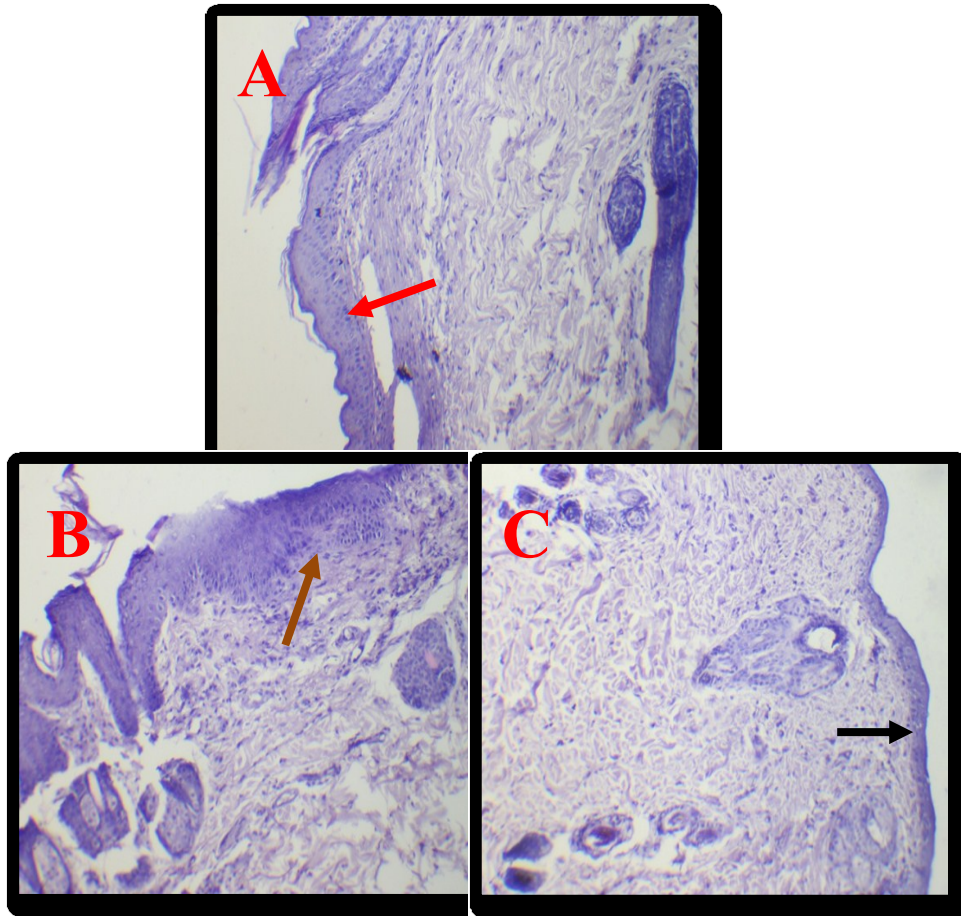
Small amount of voltage produced as long as the collagen bundle was subjected to stress was arrangement and absorption of collagen [18].



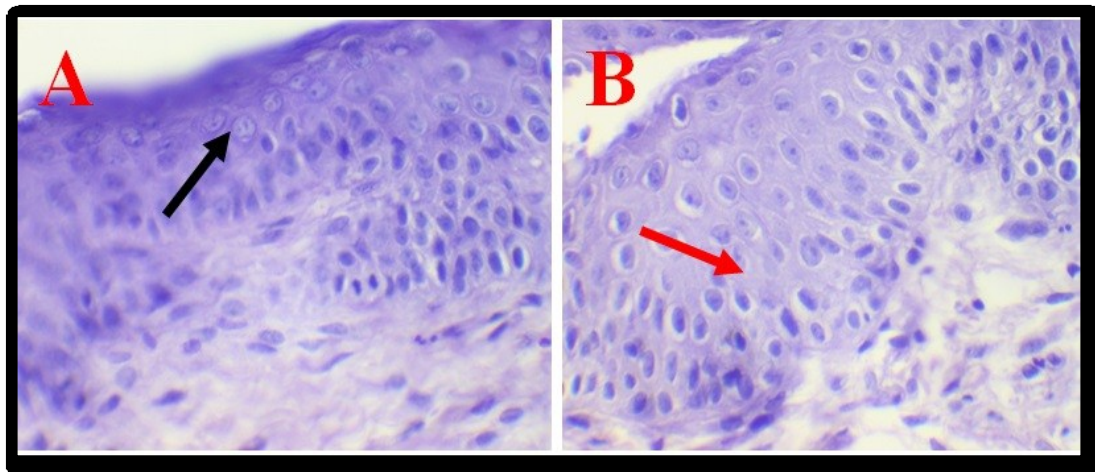
Fig(1) histological cross section of skin show: (A) 3rd day wound reflected normal epidermis and dermis with normal stratum spinosum (**→**), (B) 3rd day wound healing treated with tramadol moderate hyperplasia of stratum spinosum(**→**), (C) 3rd day wound healing treated with diaclofenac severe hyperplasia of stratum spinosum (**→**) and enlargement of sebaceous glands. (H &E stain) 10X.



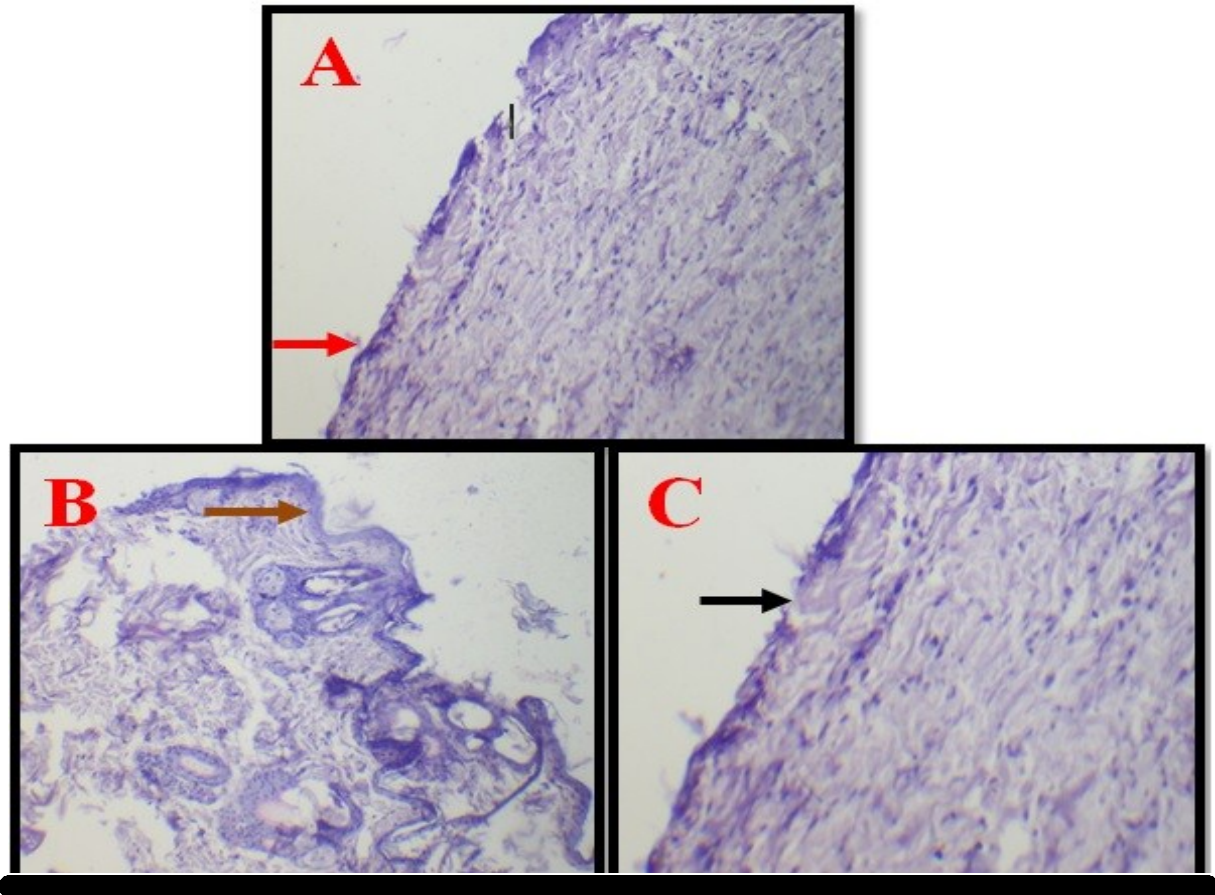
Fig(2) histological cross section of skin show: (A) normal 7th day of wound which epidermis and dermis with normal stratum spinosum (→), (B) 7th day of wound treated with tramadol, moderate hyperplasia of stratum spinosum (→) and abundant keratinocytes (→). (C) 7th day wound healing treated with diaclofenac severe hyperplasia of stratum spinosum (→) and reduce of stratum corneum. (H &E stain) 40X.



Fig(3) histological cross section of skin show: (A) normal 10th day of wound which epidermis and dermis with moderate hyperplasia of stratum spinosum (→), (B) 10th day wound healing treated with tramadol, moderate hyperplasia of stratum spinosum (→) with increase in stratum corneum. (C) 10th day of wound treated with diaclofenac: reduce in stratum spinosum (→) and reduce of stratum corneum. (H &E stain) 10X.



Fig(4) histological cross section 10th day wound treated with tramadol show: (A) increase activity stratum granulozeum with mitotic cells (→). (B) hypertrophy of the stratum spinoseum's cells (→) (H &E stain) 40X.



Fig(5) histological cross section of skin show: (A) normal 14th day of wound with abundant amount of keratin (→) and increase of fibroblasts in dermis. (B) 14th day of wound treated with tramadol, reduce of stratum spinoseum's hyperplasia (→). (C) 14th day of wound treated with diaclofenac which disappear in stratum spinoseum's hyperplasia (→) and reduce of stratum corneum. (H &E stain) 10X.

REFERENCES

- 1- Pierard, G.E.(1986). The liege experience in the assessment of the variability in the mechanical properties of skin, *Bioengineering Skin*, 2:227-229.
- 2- Greven, H.; Zanger, K. and Schwinger, G. (1995). Mechanical properties of the skin of *Xenopus laevis*. *J. Morphology*, 224: 15-22.
- 3- Berardesca, E.; Elsner, P.; Wilhelm, K.P. and Maibach, H.I. (1995). *Bioengineering of the skin: methods and Instrumentation*. 1st. Edn., CRC press, NewYork. Pp: 1-22.
- 4- Miwa, L.J.; Jones, J.K.; Pathiyal, A. and Hatoum, A. (1997). Value of Epidemiological Studies in Determining the True Incidence of Adverse Events. *The Non-steroidal Anti-inflammatory Drug Story*. *Arch. Intern. Med.*, 157(18): 2129-2136.
- 5- Morgan, G. (1999). Beneficial Effects of NSAIDS in the Gastrointestinal Tract. *Eur.J. Gastroenterol. Hepatol.*, 11: 393-400.
- 6- Farag, M.M.; Mikhail, M.; Shehata, R.; Abdel-Maguid, E. and Abdel-Tawab, S.(1996). Assessment of Gentamicin-induced Nephrotoxicity in Rats Treated with Low Doses of Ibuprofen and Diclofenac Sodium. *Clin. Sci. (Cloch)*, 91(2):187-191.
- 7- Tang, W.; Stearns, R. and Banndiera, S.M. (1999). Studies on Cytochrome P-450 Mediated Bioactivation of Diclofenac in Rats and Human Hepatocytes: Identification of Glutathione Conjugated Metabolites. *Drug Metabol. Dispos.*, 27(3):365-372.
- 8- Liu, Y-M; Zhu, S-M ; Wang, K-O; Feng, Z-Y and Chen, Q-L.(2008). Effect of tramadol on immune responses and nociceptive thresholds in a rat model of incisional pain. *Zhejiang Univ. Sci. B*, 9(11):895-902.
- 9- Kaye, K., and Theaker, N. (2001). *Tramadol: A Position Statement of the NSW Therapeutic Assessment Group Inc. An Initiative of NSW Department of Health*. Sydney. Pp:1-15.
- 10- Lewis, K.S., and Han, N.H. (2004). Tramadol: a new centrally acting analgesic. *Am. J. Health Syst. Pharm.*, 54(6):643-652.
- 11- Grond, S. and Sablotki, A. (2004). Clinical pharmacology of tramadol. *Clin Pharmacokin*; 43,: 879-923.
- 12- Bright, C.T. (1994). Fracture Healing in the Rabbits Fibula When subjected to various capacities couple electrical field. *J. Ortho. Res.*, 3: 331-340.
- 13- Albozachri, J.M.K.; Hameed, F.M.; AL-Tomah, H.M. and Muhammid, H.A. (2017). Evaluation of tow general anesthetic regime by use xylazine and ketamine with atropine and diazepam in rabbits. *J. university of kerbala*, 15: 21-30.
- 14- Luna, L.G. (1967). *Manual of histologic staining methods of the armed forces institute of pathology*, 3rd edn. McGraw-Hill, New York, NY.
- 15- Irvin, T.T. (1995). Surgical wound healing in rats. *J.biol.Chem.*; 290: 14854- 14860.
- 16- Rajabi, A.M. and Rajabi, F. (2007). The effect of estrogen on wound healing in rats. *Pakistan J. Med.Scie.*, 23(3): 349-352.
- 17- Carlstedt CA, Madsen K (1986). The influence of indomethacin on collagen synthesis during tendon healing in the rabbit. *Prostaglandins*. 32(3): 8-353.
- 18- Demir, H.; Balay, H. and Kirnap, M. (2004). A comparative study of the effect of electrical stimulation and laser treatment on experimental wound healing in rats. *J Rehbil Res. Dev.*, 41(2): 147-154.