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RADIOLOGICAL AND HISTOPATHOLOGICAL STUDY OF THE EFFECTIVENESS THE NON-STEROIDAL ANTI-INFLAMMATORY DRUG ON THE HEALING OF FRACTURE IN RABBITS

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ABSTRACT

To study the effect of nonsteroidal anti-inflammatory drug on bone fracture healing process. Nonsteroidal anti-inflammatory drug (naproxen 125mg) was used in this work. Eight mature, rabbits, were divided into two groups and subjected to a partial fracture on the lateral aspect of femur by bone drill. The first group of these animals served as control group. The second group was giving naproxen orally 125 mg daily for one month. Results: The present study in radiological examination showed appearance of penetration in both group but in control group showed the callus and not formation of white zone around lesion .in treated group , not callus formation and with slight zone formation around lesion. The histological assessments at day 30 after the fracture of control group showed evidence of new bone growth in penetration of bone associated with laminated osseous tissue which indicated the healing of fracture bone but in nproxen group showed not closed complete of bone penetration and also found growth of bone in penetratio area. Conclusion: nonsteroidal anti-inflammatory drug was delay enhance healing process of a fractured bone.

KEYWORDS: Bone, Fracture Healing, NSAIDs

INTRODUCTION

Bone is a type of hard endoskeleton connect tissue bones support body structures, protect internal organs, and in an attached with muscles facilitate movement. They are also involved within cell formation, calcium metabolism, and mineral storage [1],[2]. Bone is an essential component in the body described as connective tissue, helping to support and bind various part of the body [3] . The three major components of bone are osteogenic cells, organic matrix, and mineral. The osteogenic cells include osteoblasts, osteocytes and osteoclasts , while the matrix cosiss predominantly of collagen and proteoglycans , which constitutes approximately one third of the bone mass. The mineral that makes up approximately two thirds of bone is composed of calcium and phosphate crystals [4] . The femur (thigh bone) is the longest , strongest and heaviest tubular bone in the body [5],[6] ; and one of the principle load-bering bones in the lower extremity. Femoral shaft fractures are

among the most common major injuries that an orthopedic surgeon will be requite to treat [7]. Fracture of bone is an abnormal structure of bone that means discontinuity of the bone tissue , may be broken into two or more parts. And their etiology is either external trauma or pathology , but both types of fractures have same signs [8]. When bones damage or fracture , healing start with hemorrhage at the site , following by formation of hematoma, vascular infiltration, proliferation and aggregation of mesenchymal cells, matrix formation and remodeling of woven bone into lamellar, this complex auto regulated healing process involves bone active cells, protein and hormones [9] . The healing process of fracture bone effect by using some drugs such as Naproxen .The naproxen is Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation in people with chronic musculoskeletal conditions, such as arthritis. People also commonly take NSAIDs after acute injuries, such as fractures – as can frequently occur in people with osteoporosis – or after joint replacement surgery made necessary by advanced arthritis. However, through an Arthritis Foundation-funded project, scientists have determined that taking NSAIDs after suffering a fracture may inhibit your body's ability to heal [10] .

Materials and Methods'

1-Animals:

The experimental performed on 8 rabbits. These rabbits weight in between 1-2 Kg and their ages were more than 6 months. Animals were maintained in individual kennel in animal house, and exposed for the same environment including climate, management , and feeding for week to acclimatize and adaptive on the place . The rabbits were examined clinically to work sure that they were healthy.

2-Experimental design:

The rabbits were divided into two groups:

1-Control group: These groups consist of 4 rabbits, killing after 30 days post operation. These group given only systemic antibiotic (penicillin and streptomycin).

2-Treated group: These groups consist of 4 rabbits, killing after 30 days post operation. Each rabbits in all periods given systemic antibiotic (penicillin and streptomycin)injection, and given orally naproxen drug daily for 30 days.

Surgical procedure: The operations were done under general anesthesia by giving intramuscular injection of 30-40 mg/kg body weight of ketamine hydrochloride and 5-8 mg/kg. B.W of xylazine to anesthesia the animal [11],[12] .Preparation the site of operation

by clipping and shaving of medial and lateral aspect of left thigh , then washing the area with soap and water , and disinfected the site of operation by slices of cotton soaked with % ethyl alcohol. The animal cast on its right side, incision made along the line from greater trochanter to the lateral surface of patella, including the skin, fascia lata and muscle (biceps femurs). When the bone exrosed one cm piece of bone was removed (depth extend to bone marrow)by using modified electrical bone drill , after that the muscle suture by catgut suture and the skin then was closed by simple interrupted suture using surgical silk 3.0 usp and the last step dressing the wound.

3- Radiological and histopathological intake:

Radiological examination was performed on the period 30 post operation in both groups, to investigate the callus formation and degree of fracture healing. Histopathological technique, the rabbits were killed after radiographic picture in the 30 day for all groups (control and treated groups). Histopathological specimens should be taken at the bone fracture site. The specimens were put in 10% neutral buffer formalin for fixation. Bone was cut 2cm including indicated bone gap. Washed with water after that decalcify process by solution of formic acid – sodium citrate for 12 day , during this period the solution should be exchanging and turn off the bones parts in solution [13],[14] .

After 12 days the bone become softening and examined by inserted needle, after that washed with water for 3 days. The bone was longitudinal dissection should be through the induce bony fracture line.

Results

1-Radiographic examination:

A-Control group:

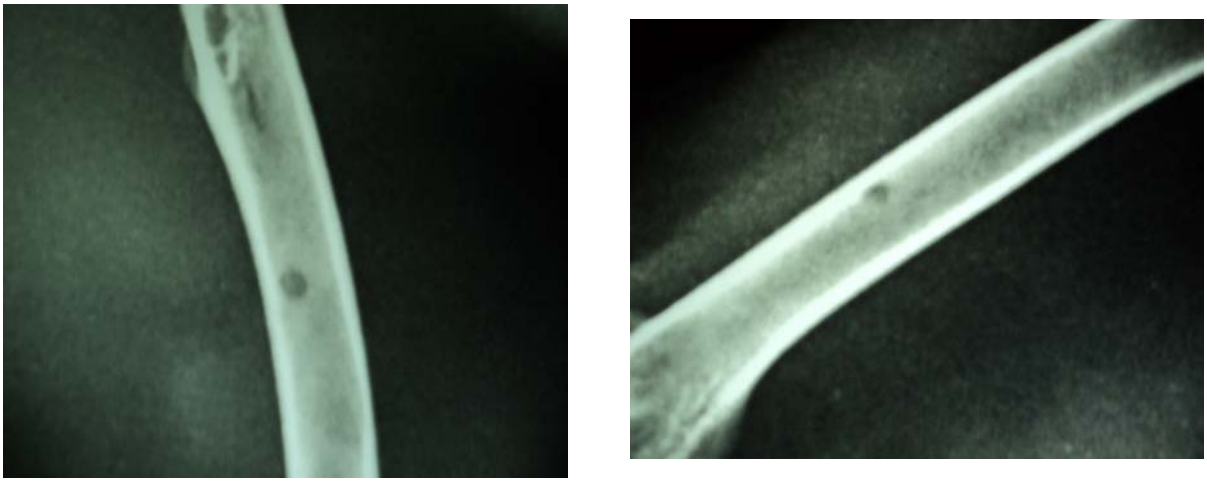
1-There is Callus deposit at the center of the lesion.

2-No white zone formation.

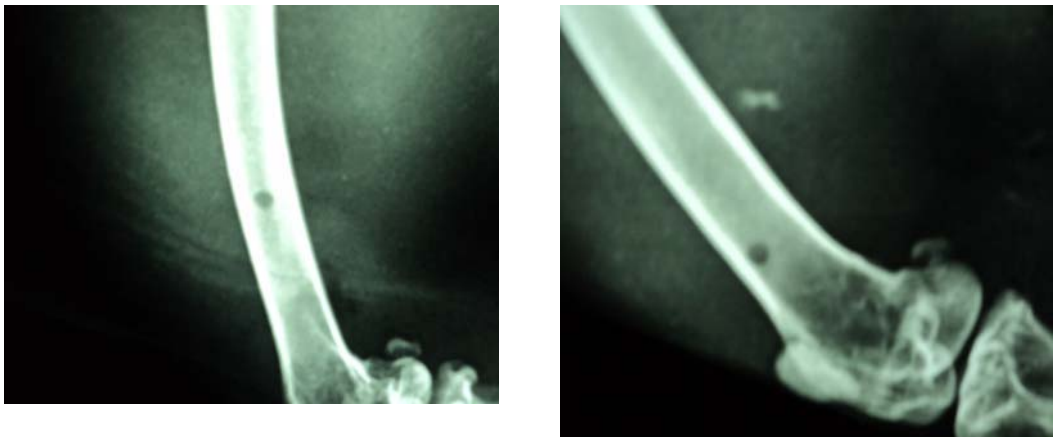
B-Naproxen group:

1-No callus formation , this may attributed to effect of the naproxen .

2-Slight white zone formation all around the lesion.



Fig(1)Control group(callus formation).



Fig(2)Naproxen group(not callus formation)

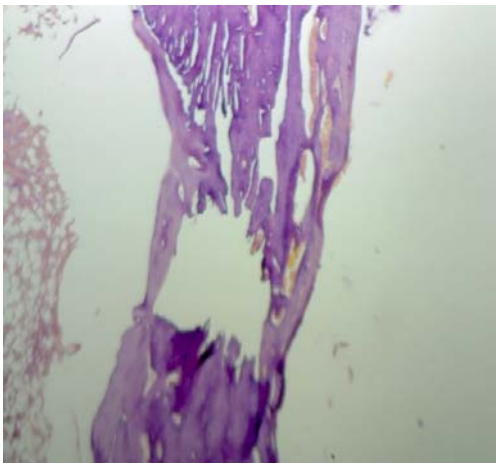
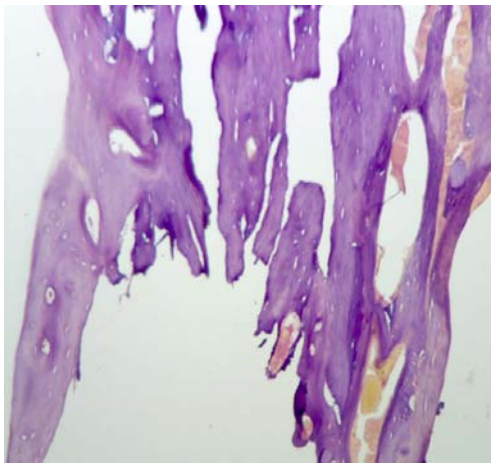
2-Histopathological examination:

A-Control group:

Area of penetration (poring) in the bone Showing evidence of new bone growth formation characterize by new growth of pale bony tissue with some foci of lamination which could indicate evidence of healing of bone fracture.

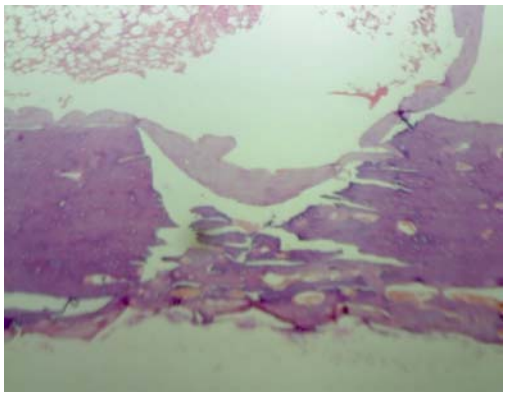
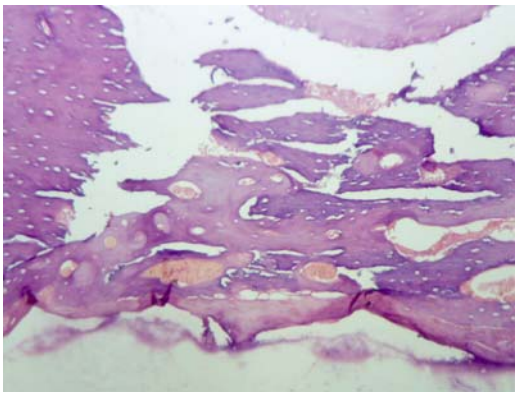
B-Naproxen group:

The opening of penetration is not closed complete yet in there is evidence of few growth of bone associated with laminated osseous tissue. Numerous laminated osseous tissue which indicate attempt for closure of opening.

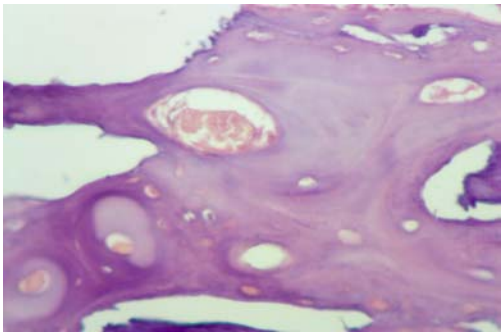
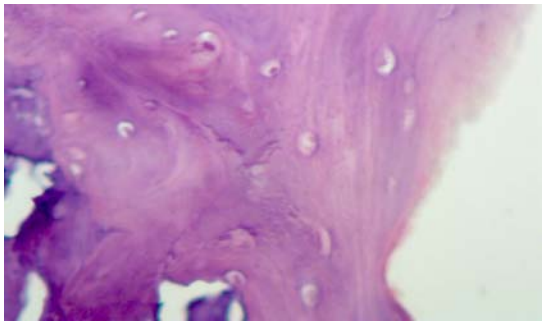



X4 X10

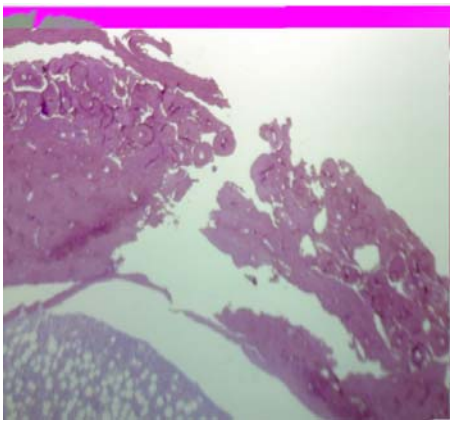
Fig(3)Control group(area of poring penetration and some evidence of repair(growth).

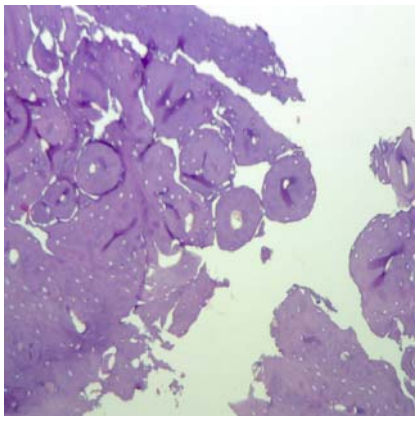



Fig(4)Control group(evidence of new bone)x4, x10

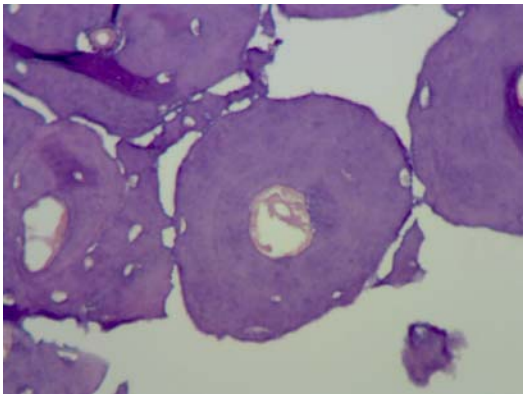



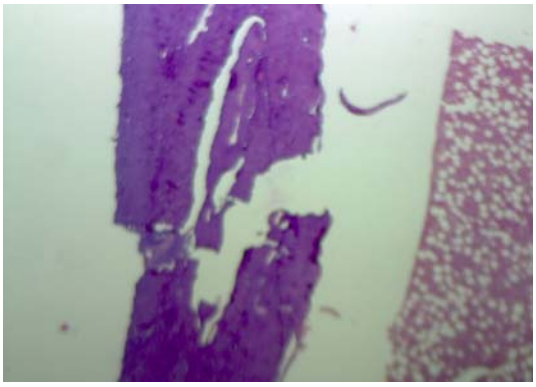
Fig(5)Control group(new growth of pale bony tissue with some foci of lamination)x40


X4

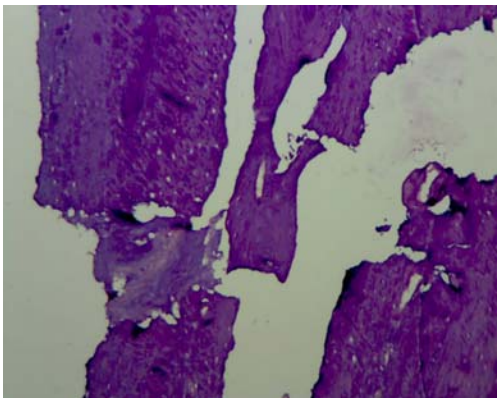

x10

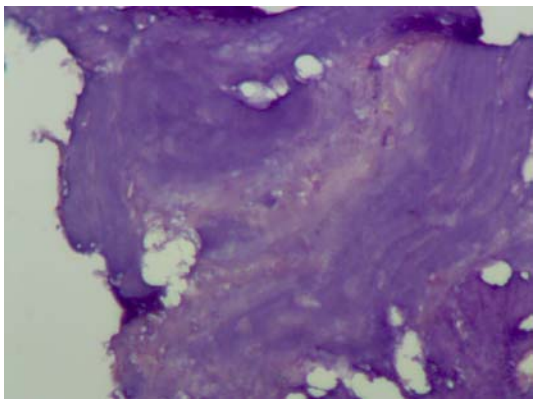
Fig(6)Naproxen group(opening of penetration is not closed complete with evidence).





Fig(7)Naproxen group(enumeras laminated ossieus and attempt for closure of opening).x40 , x4





Fig(8)Naproxen group(few new bone tissue formation).x10, x40.

Discussion

In radioigraphic results showed callus in control group and not callus formation in naproxen grow, this result agreement with Andrew et al 2012 which present when treatment with NSAID during the early stages of fracture repair reduces mechanical properties of the callus at subsequent stages of healing (compared with matched controls) and increase the prevalence of nonunion[15] . Early administration of NSAIDs has also been reported to be associated with greater fracture hematoma volume and with delayed clearance and organization of the hematoma, most likely as a result of COX-1 inhibition. Early NSAID administration has also been reported to be associated with decreased formation of mineralized callus (most likely as a result of COX-2 inhibition)[16],[10],[17]; delayed callus maturation as seen histomorphologically [15].

The histopathological results, in control group showed new bone growth formation characterize by new growth of pale bony tissue ,this result agreement with Ippokratis et al 2012 which present local administration of PGs in rat long bones had stimulatory properties suggesting direct effect on bone by inducing osteogenesis , at a cellular level, PGs have a direct effect on osteoclasts leading to increased bone resorption by a mitogenic effect and increasing their functional activity[18] . On the other hand, PGs can exert an anabolic effect on the bone by increasing the multiplication and differentiation of osteoblasts [19]. Fracture-healing following mechanical injury is an extremely complex process dependent on the coordinated recruitment and action of several cell lineages through a cascade of signal pathways and biochemical events. Broadly, a variety of biological changes follow a fracture, starting with disruption of blood supply, hematoma formation, local hypoxia, and inflammation. The production and activation of pro-inflammatory factors, especially release of cytokines and growth factors, results in increased production of bone-inductive prostaglandins. Prostaglandins then play an important role in the regulation of osteoblast and osteoclast functioning [15]. In naproxen group results showed not closed complete of bone penetration because delay of healing and few growth of bone and lamination. This result agreement with Omer et al 2009 which demonstrated that bone formation was suppressed by oral administration of a nonsteroidal anti-inflammatory drug that contains a COX-2 inhibitor [20].

Numerous animal studies have demonstrated a consistent negative effect of NSAID treatment on endochondral ossification during fracture healing [21]. Cells damaged from the trauma of fracture release large amounts of inflammatory prostaglandins at the site of fracture. The ensuing inflammation causes pain and the natural tendency is to want to block this painful reaction. In this case, non-steroidal anti-inflammatory drugs (COX-1 and COX-2 inhibitors)

might be the medication we reach for to relieve the pain. The use of these COX-1 and COX-2 inhibitors, however, can delay fracture healing. As it turns out, prostaglandin-induced inflammation is an essential component of the fracture healing process, and cyclooxygenase enzymes (COX-1 and COX-2) play important roles in fracture repair. These inflammatory prostaglandins are a natural and essential part of initial tissue repair and the initial inflammatory immune response is crucial to fracture healing. Because of this, the use of non-steroidal anti-inflammatory pain killers (NSAIDs) is not recommended for fracture pain relief. Among the NSAID COX-1 and COX-2 inhibitor drugs to be avoided are aspirin, ibuprofen, indomethacin, etodolac (Lodine), meloxicam (Mobic), nabumetone (Relafen), and naproxen (Anaprox, Naprosyn) [22],[23].

In summary, nonsteroidal anti-inflammatory drugs inhibit osteoblasts in the early phases of bone-healing. COX-2 activity is necessary for normal endochondral ossification during fracture-healing. These drugs exert an inhibitory action on fracture repair in animal models. However, they have been found to cause a delay in fracture-healing. It may be prudent for patients to avoid nonsteroidal anti-inflammatory drugs following osseous injury. This is more important for fractures that are associated with a delay in healing and that are often accompanied by a reduction in blood flow to the fracture site.

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