World Journal of Pharmaceutical, Biological and Chemical Sciences
Vol. 1, No. 1, August 2013, PP: 01-11
Available online at http://acascipub.com/Journals.php

Research article

Effects of Diclofenac Sodium on Serum Calcium , Phosphate, Alkaline phosphatase and Healing of Tooth Extraction Socket in a Rabbit Model

Running Title: Effects of diclofenac on bone markers and healing of tooth extraction socket.

Dr. Tahani A . AL-Sandook, professor
Dr. Ghada Abdul-Rhman Taqa, Assistant professor
Fayhaa AM Al – Mashhadane, Assistant professor

E-mail: amertaqa@hotmail.com; fayhaa6695@yahoo.com

ABSTRACT

Aim of the study : To evaluate the effect of treatment with diclofenac for 30 days on serum level of *alkaline phosphatase , calcium* and *phosphate* ,bone density and it is relation to healing of tooth extraction socket in rabbit .

Methods: This study was carried out on ten healthy male rabbits weighing between 1.0 – 1.5 Kg. They were divided into 2 groups : Group 1(control) consist of 5 rabbits treated with normal saline (0.2 ml) injection, group 2(treatment) consist of 5 rabbits treated by diclofenac sodium injection in dose of 30 mg /kg/day for 30 days . The animals weights were measured at (0,10,20) days of treatment .After 30 days of treatment ,rabbits of both groups were anesthetized by xylazine hydrochloride and ketamine hydrochloride by I.M injection at dose of 0.5 mg/Kg , 50 mg/Kg respectively .Local anesthesia injected opposite to right maxillary incisor, then this tooth was extracted .The surface area of extraction socket wounds were measured at 0,3,6,9, days postoperatively.

All rabbits were sacrificed 10 days after surgery . Venous blood samples(3 ml) were collected from all rabbits via jugular vein during animal sacrificing . Serum *Calcium , phosphate and alkaline phosphatase* were determined.

Statistical analysis of data was done by SPSS program version 15 . Weight of animals was analysed by ANOVA and Duncan's Multiple Range Test . Non parametric test was used to compare scores of healing ,Mann-Whitney test is used to compare the score of extraction wound healing and T- test was used for analysis of serum *Calcium , phosphate , alkaline phosphatase* and number of osteocytes.

Results : Scores of extraction wound healing showed that at day 0 there was no significant differences between control and treatment groups with mean ± SD of 7.0 ± 0.0 for both groups (p = 1.00) while at 3,6, and 9 days of healing period , mean ± SD are 3±0.0 , 0±0.02 ,and 0±0.0 for control group while for treatment group they are 6.75 ± 0.25 , 7.25 ±0.25 and 7.5±0.28 respectively . There were significant differences between 2 groups (p= 0.01 , p=0.008 , p= 0.01) respectively . In comparison between scores of wound healing within one group

,significant differences was found between time 1,2,3 and 4 in control group with mean ± SD of 7.0 ± 0.0 , 3±0.0 , 0±0.02 ,and 0±0.0 respectively, (p=0.002) while a non significant differences was found in treatment group, mean ± SD of 7.0 ± 0.0 , 6.75 ± 0.25 , 7.25 ±0.25 and 7.5±0.28 respectively (p=0.10) .
Statistical analysis for the serum *Calcium* , *phosphate and alkaline phosphatase* showed no significant differences between control and treatment groups with mean±SD of 10.85±3.6 , 4.80±2.10 and 47.83±14.45 (P-value = 0.826, 0.800, 0.720 respectively).
Significant differences between 1st , 2nd and 3rd weight records in control group with mean±SD of 1.28±0.037 , 1.33±0.025, 1.40±0.07 respectively (p=0.022) ,while in treatment group no significant difference was found with mean ±SD of 1.96±0.33 , 2.00±0.49 , 1.80±0.45 respectively (p= 0.792).
Light microscopic examination showed that number of osteocytes was significantly higher in the control group than treatment group with mean±SD of 158.10±59.066 and 96.70±14.667 ($p<0.05$).The micrographs of decalcified rabbit tibia from control group Showed well organized histology. The compact bone of treatment group showed disorganized lamellar architecture of bone matrix which seemed to be more porous with decreased bone marrow cells.
Conclusion : Chronic treatment with diclofenac can delay tooth extraction socket healing without significant effect on serum level of *calcium , phosphate and alkaline phosphatase*.

Key words : diclofenac ,calcium ,alkaline phosphatas ,tooth extraction

---

INTRODUCTION

NSAIDs include some of the most frequently taken medications. These agents share a common mechanism of action, they exert qualitatively similar therapeutic and toxic effects. For the treatment of pain and inflammation that accompany various dental surgical procedures, the short-term use of NSAIDs has generally proved to be highly efficacious and safe[1] .
Diclofenac is a non steroidal anti inflammatory (NSAIDS) presenting significant analgesic and anti – inflammatory activities [2] .There is evidence , however , that NSAIDS may delay the repair of damaged tissues , such as skin , cartilage , fractured bone and new bone formation after tooth extraction[3,4] .
Healing of tooth extraction socket is a complex process involving tissue repair and regeneration, it involve chemotaxis of cells into the wound and differentiation of mesenchymal cells to osteoprogenitor cells , proliferation and differentiation of bone forming cells, maturation and remodeling of bone , all these cellular events are controlled and regulated by specific signaling molecules[5,6] .
The dentist may be the first to detect diseases and clinical problems affecting the bones.Chemical confirmation is dependent on the pateint's serum levaels of *calcium* , *phosphorous* , *and alkaline phosphatase* ,those biochemical markers of bone turnover are currently employed in monitoring therapies used to affect bone tissue and they are used today to reflect underlying bone remodeling process that controlled by these signaling molecules. [7,8]
Alkaline phosphatase is one of the most important Biochemical bone markers ,it is a hydrolase enzyme produced by bones in the body,it is responsible for removing *phosphate* group from many types of molecules by a process of dephosphorylation[9] .Elevation of *Alkaline phosphatase* is readily observed in animals with rapid bone formation[10]
Calcium and *phosphate* are also very important elements of bone markers , although plasma activity of *Alkaline phosphatase* is considered to be more useful in distinguishing between normal and abnormal bone formation [11] .The level of *Calcium* and *phosphate* should be determined whenever there is disturbance in mineral metabolism in bone tissue. Increase in total serum *calcium* level may be seen in osteolytic bone lesion at which *calcium* is librated in the blood stream through the action of bone osteoclasts while serum *phosphate* level may increase with healing process of bone[10] .
In this study the effects of treatment with diclofenac for 30 days on serum level of *alkaline phosphatase* , *calcium* and *phosphate* ,bone density with it is relation to healing of tooth extraction socket in rabbit was investigated.

Materials and methods

The study was conducted at the pharmacology Lab. / College of Dentistry / University of Mosul from 20/10/2012 till 1/12/2012.

It was carried out on ten healthy male rabbits from local market weighing between 1.0 – 1.5 Kg. The animals were kept in standard animal housing condition with the room temperature of 25±2°C.
They were divided into 2 groups as follows: Group 1 (control group) consist of 5 rabbits treated with daily intramuscular injection of normal saline (0.2 ml) ,group 2 (treatment group) consist of 5 rabbits treated with daily intramuscular injection of diclofenac sodium (veterninary and agricultural products Mfg. co ;Jordan) in dose of 30 mg /kg/day for 4 weeks . Animals' weights were evaluated at (0,10,20) days of treatment.
After 4 weeks treatment by diclofenac was stopped and rabbits were anesthetized by intramuscular administration of xylazine hydrochloride (Holland, Castenray, interchemra) and ketamine hydrochloride (Aleppo – Syria, ElSaad) at dose of 0.5 , 50 mg/Kg respectively , then few drops of local anesthesia of lidocaine 2% with adrenaline 1: 80, 000 (Saint –Maur-des-Fosses Cedx ,France) was instilled at the site of right maxillary incisor.
During the surgical stage of general anesthesia, right maxillary incisor have been extracted by stainless steel dental forceps after disconnection of the surrounding gingival tissue using stainless steel Tweezer ,a single dose of oxytetracycline(Norbrook laboratories) in dose 15 mg /kg was given immediately after tooth extraction.

Assessment of extraction wound healing

The surface area of extraction socket wounds were measured by the same operator at 0 ,3 ,6 ,9 days postoperatively. They were categorized with respect to surface area that is calculated by measuring the greatest length and the greatest width (side to side) using digital vernia (Digital Caliper/China) then multiply these two measurements (length in cm × width in cm) to obtain an estimate of surface area in cm^2 ,the scores of wound surface area were as follow: 0 = 0 , 1 = < 0.3 , 2 = 0.3-0.6 ,3 = 0.7-1.0 , 4 = 1.1-2.0 , 5 = 2.1-3.0 , 6 = 3.1- 4.0 , 7 =4.1-8.0 , 8 =8.1-12.0 ,9 =12.1-24.0 , 10 = >24.0.
A comparison of the scores measured over time and the duration of healing in days provide an indication of the improvement or deterioration in wound healing [12] .

Biochemical assessments

All rabbits were sacrificed 10 days after surgery and Venous blood samples(3 ml) were collected from all rabbits via jugular vein during animal sacrificing[13], allowed to clot for 30 min at room tempreture , then centrifuged at 3000 R/min for 10 min .Serum samples were separated and stored at -20C° till time of analysis(within 5 days).

Determination of Serum *Calcium*

The level of serum calcium was determined by Moorehead and Briggs derived CPC (O-Cresol phtalein Complexone) method using kit manufactured by BIOLABO SA ,France In alkaline solution -Cresol phtalein Complexone react with calcium in serum to form a dark red colored complex which absorbance measured at 570 nm by spectrophotometer that is proportional to the amount of calcium in serum.

Determination of Serum *alkaline phosphatase*

Colorimetric determination of alkaline phosphatase activity was done by using alkaline phosphatase kit manufactured by bioMěrieux® sa ,France according to the following reaction:

Phenylphosphate ⟶ phenol +phosphate

The librated phenol is measured in the presence of 4-aminoantipyrine and potassium ferricyanide.The presence of sodium arsenate in the reagent stopped the enzymatic reaction.The color intensity is measured by spectrophotometer at wave length 510nm.

Determination of Serum *phosphate*

The Phosphate Colorimetric Assay Kit manufactured by BioVision ,USA was used .The assay utilizes a proprietary formulation of malachite green and ammonium molybdate which forms a chromogenic complex with phosphate ion giving an intense absorption band around 650 nm.

Histopathological assessment

Also right tibial bones[14,15] from each rabbit were isolated and immersed in 10% formalin for 48 hr harvested and subjected to conventional decalcification by 10% nitric acid embedding in paraffin , longitudinal sectioning (4µm) ,stained by H .E staining and examined by light microscope (type XSZ-510 pro way).
Histological analysis of new bone formation , histological examination of fibrous tissues , , bone trabeculae , blood vessels and bone cells types were assessed . For bone cell number ,osteocytes were counted for control and treatment groups[16] .counting was performed in three randomly selected sites of each section at 40 × magnification.

Statistical analysis

SPSS program version 15 was used to analyze the obtained data .Weight of animals was analysed by ANOVA and Duncan's Multiple Range Test. Non parametric test was used to compare scores of healing ,Mann-Whitney test is used to compare the score of extraction wound healing .Statistical analysis for the serum *Calcium , phosphate , alkaline phosphatase* and number of osteocytes in both groups were done by using T-test.

Results

A comparison of the scores of extraction wound healing between control and treatment groups at 0 ,3 ,6 and 9 days of healing period showed that at day 0 there was no significant differences between control and treatment groups with mean±SD of 7±0.0 for both groups (p = 1.00) while at 3 , 6 and 9 days of healing period there were significant differences between 2 groups with mean±SD are 3±0.0 , 0±0.02 ,and 0±0.0 for control group while for treatment group they are 6.75±0.25 , 7.25±0.25 and 7.5±0.28 , (p= 0.01 , p=0.008 , p= 0.01) respectively . Table (1)
In comparison between scores of wound healing within one group ,significant differences was found between time 1 ,2 , 3 and 4 in control group with mean ± SD of 7.0 ± 0.0 , 3±0.0 , 0±0.02 ,and 0±0.0 respectively, (p=0.002) while a non significant differences was found in treatment group, mean ± SD of 7.0 ± 0.0 , 6.75 ± 0.25 , 7.25 ±0.25 and 7.5±0.28 respectively (p=0.10) . (Figure 1).

Statistical analysis for the serum level of *Calcium , phosphate and alkaline phosphatase* showed no significant differences between control and treatment groups with mean±SD of 10.85±3.6 , 4.80±2.10 and 47.83±14.45 and P-value = 0.826, 0.800, 0.720 respectively (Table 2).
Significant differences between 1st , 2nd and 3rd weight records in control group with mean±SD of 1.28±0.037 , 1.33±0.025, 1.40±0.07 respectively (p=0.022) ,while in treatment group no significant difference was found with mean ±SD of 1.96±0.33 , 2.00±0.49 , 1.80±0.45 respectively (p= 0.792). (Figure 2)
Light microscopic examination of the decalcified sections showed that number of osteocytes was significantly higher in the control group than treatment group with mean±SD of 158.10±59.066 and 96.70±14.667 ($p<0.05$). (Table 3).
The micrographs of decalcified rabbit tibia from control group Showed well organized histology. The section of compact bone showed bone matrix in the form of lamellae with collagen fibers, perforated by the lacunae which housed osteocytes in them.(Figure 3)
After 30 days of drug treatment, the compact bone of treatment group showed disorganized lamellar architecture of bone matrix which seemed to be more porous along with decreased bone marrow cells in central marrow cavity together with areas of necrotic bone and osteoclast cells. Osteocyte number seemed to have been lessened after one month treatment with diclofenac sodium.Tibial bone section in treatmet group also showed osteoclast ,necrotic bone and bone marrow of reduced size.(Figure 4 and 5)

Discussion

This study was designed to investigate the effect of treatment with diclofenac sodium for 30 days on serum levels of *calcium , phosphate and alkaline phosphatase* and its role in bone formation and healing of tooth extraction socket in rabbits.
In this study rabbit was chosen as an animal model since its one of the most commonly used animals for medical research and also due to the similarities between rabbit and human in the bone mineral density [17] .
Diclofenac is a phenylacetic acid derivative that is potent non selective cyclooxygenase inhibitor , the rate limiting enzyme in PG synthesis .PG have been shown to regulate many functions throughout the body including bone healing[2] . Although the exact mechanism for diclofenac induced socket healing inhibition is unknown ,the predominant theory suppose that NSAIDs , by inhibiting PG synthesis , interfere with cell signaling during the inflammatory phase leading to an uncoordinated healing response .Endogenous PGE and

PGF increase locally in the first 7 days of fracture suggesting a signaling function by PG in the early healing period[18] .Inhibition of angiogenesis is another suggested mechanism since that PG is stimulator of angiogenesis and it will be inhibited by diclofenac. [19,20] .This study was shown that treatment of rabbit with diclofenac for 4 weeks in a dose of 30 mg/kg /day which was chosen to obtain sufficient concentration of drug over a large part of the day from only single injection [21] can cause delayed healing of tooth extraction socket as shown by clinical observation and measurements of the socket wound after tooth extraction for this animal. This result was in accordance with other studies stated that short term therapy with diclofenac can cause decreased bone formation during socket healing [22] ,the inhibitory effect of diclofenac on tooth extraction wound healing in rabbit has been attributed to probable interference with inflammatory response ,considering that the presence of optimal inflammation is essential for proper wound healing, Soon after extraction the mineralized socket wall is exposed to bacterial colonization while the body attempts to form a fibrin clot. The fibrin clot becomes filled with inflammatory cells programmed to prevent infection. bone is resorbed in the presence of inflammatory cells. [23]. By inhibiting PG synthesis ,diclofenac interfere with this inflammatory phase of healing process , leading to an uncoordinated response or could be due to angiogenesis inhibition at site of healing[18,19,20].NSAIDs, by decreasing the level of PGE2 may bring about a reduction of vascular endothelial growth factor (VEGF) which important growth factor in osseous repair . Callus maturation in vivo was also impaired after long term application of diclofenac which corresponds to effect of NSAID on osteoblast proliferation[24 , 25]
The state of skeleton can be evaluated by a variety of techniques including biochemical markers of bone remodeling which provide a non invasive and more rapid responding mean for direct information about bone density . [26].
According to the results of this study a non significant differences were found between control and treatment groups in relation to serum level of *calcium ,phosohate and alkaline phosphatase* , this was in agreement with studies considering that the unfavorable effect of NSAIDs was limited to the initial stages of cellular differentiation without altering the serum level of *calcium , phosphate and alkaline phosphatase* [27,28,29].Evans and Butcher performed a study using cells obtained from human trabecular bone at which culture of osteoblasts with indomethacin resulted in a reduction of osteoblast number without significant change in *alkaline phosphatase* activity [30] .
In this study ,delayed wound extraction socket healing without significant change in serum level of bone markers could be related to dose and /or duration of treatment with diclofenac [31,32].
In disagreement with results of this study , Daluiski et al reported that effect of celecoxib ,a non steroidal anti-inflammatory drug, on human osteoprogenitor cells showed a decrease of *alkaline phosphatase* activity with decreased bone formation [33] .
Bone healing process is either direct (primary) or indirect (secondary) process .There are 3 phases of bone healing : inflammatory , reparative and remodeling phase[18]. The histological analysis demonstrated that osteocytes density was significantly reduced in the treatment group and these changes in osteocyte density correlated with changes in osteoblasts and osteoclasts activity .Depletion of osteocytes could be important factor contributing to bone loss in this model since they may adversely affect intercellular communication between osteoblasts and osteoclasts [34] . Histology of decalcified bone revealed increased amount of cartilage and thinning of trabeculae after diclofenac administration. All these deviations hinted at the decreased rate of bone formation from cartilage after drug treatment .In an agreement with these results ,Shalini Chouhan and Sushma Sharma(2012) .They concluded that diclofenac treatment over a continuous period of 30 days seems to be a matter of clinical concern being an interfering agent with osseous metabolism that affected osteoblastogenesis, osteoclastogenesis and collagen fibers organization. [35]
The presence of necrotic bone indicated loss of bone strength. Similar results were documented in earlier work having lesser amount of trabeculae in diclofenac treated rat bones undergoing repair as compared to control and calcitonin treated bone [36] . Histological observations of the present study were supported by previous works where diclofenac and tenoxicam resulted in numerous chondrocytes and immature bone formation during distraction osteogenesis study in rabbit tibiae [37].
Although increasing evidence from animal studies suggests that non steroidal anti-inflammatory drugs suppresses early wound healing ,in vivo studies involving human subjects have not provided convincing evidence to substantiate this concern .[38]

Conclusion

In summary , treatment with of rabbit with diclofenac for 30 days at dose of 30 mg /kg/day can delay tooth extraction socket healing without significant decrease in serum level of *calcium , phosphate and alkaline phosphatase* ,however it is necessary to determine if the analgesic effect of diclofenac is beneficial in treating pain after tooth extraction and other surgical procedure of bone involvement pain, since it is clear that the

function of cyclooxygenase is critical for bone regeneration . So, defining the risk –beneficial ratio of NSAIDs use is very important.

References

[1] Elliot V , Paul J , Clarence L. (2011): Nonopioid Analgesics, Nonsteroidal Anti-inflammatory Drugs, and Antirheumatic and Antigout Drugs .In PHARMACOLOGY AND THERAPEUTICS FOR DENTISTRY 6th ed Mosby Elsevier : 330

[2] Daniel E., Robert W. , Shraddha P.(2012): Nonsteroid AntiInflammatory Drugs,Disease-ModifyingAntirheumatic Drugs, Nonopioid Analgesics,& Drugs Used in Gout. In :Basic and Clinical Pharmacology 12th ed , The McGraw-Hill Companies : 640

[3] Hammad SM ,El-Hawary .(2012):The use of different analgesics in orthodontic tooth movements .Angle Orthod .

[4] Cağlaroğlu M,Erdem A .(2012):Histopathological investigation of the effect of prostaglandin E2 administered by different methods on tooth movement and bone metabolism .Korean J Orthod ;42(3):118-28.

[5] Zahid SL . (2002):Characterization of healing tissue in a tooth extraction socket in a rabbit model. Texas Medical Center Dissertation; AAI3034645.

[6] Devlin H, Sloan P.(2002): Early bone healing events in the human extraction socket. Int J Oral Maxillofac Surg.;31(6):641-645.

[7] Misch CE(2008):Contemporary Implant Dentistry .3rd ED .Mosby Co.,St.Louis , USA.PP : 130-146, 557-598.

[8] Liesegang A .(2000):Use of bone markers in veterinary medicine. Schweiz Arch Tierheilkd ; 142(11): 613-23.

[9] Tamàs L , Huttovà J . (2002):Effect of carboxymethyl chitin –glucan on the activity of some hydrolytic enzymes in maize plants .Chem .Pap;56(5): 326-329.

[10] Embert H .(1986):Mineral balance and parathyroid function ,In veterinary clinical pathology 4th ed ,W.B.SAUNDERS COMPANY :232-235.

[11] Peach H , Juliet E .(1982):Value of plasma calcium , phosphate , and alkaline phosphatase measurements in the diagnosis of histological osteomalacia .J Clin Pathol;35:625-630.

[12] AL-Mashhadane FA. (2012):The clinical effect of phenytoin on oral wound . AL-Rafidain dental journal;12 (1):165-170.

[13] Parasuraman S , Raveendran R , Kesavan R.(2010):Blood sample collection in small laboratory animals .Journal of pharmacology and pharmacotheraputics ; 1(2): 87-93

[14] BiklE DD , Halloran BP. (1990): Different responses of trabecular and cortical bone to 1,25(OH)2D3 infusion.Am J Physiol Endocrinol Metab;259(5): E715-E722.

[15] Goodman S ,Ma T , Trindade M . (2002): COX2 selective NSAID decreases bone ingrowth in vivo.J Orthop Res.;20(6): 1164 – 9.

[16] Qassim AH .(2004):Histological and histochemical studies of human fetal liver during different stages of intrauterine life .M.Sc Thesis ,College of Medicine ,University of Mosul,Iraq .

[17] Al pearce RG , Milz S .(2007):Animal model for implant biomaterial reaserch in bone:A review . European Cells and materials ;vol 13 : 1-10.

[18] Barry S . (2010):Non steroidal anti-inflammatory drugs inhibit bone healing :A review . Vet Comp Orthop Traumatol,; 23:385-392.

[19] Sahin M , Sahin E ,Gömüslü S .(2009):Cyclooxygenase -2 in cancer and angiogenesis .Angiology ,(60):2421

[20] Hausman MR ,Schaffler MB , Majeska RI .(2001):Prevention of fracture healing in rats by an inhibitor of angiogenesis .Bone ; (29):560-564.

[21] Jacobsson SA , Djerf K.(1994):Effect of diclofenac on fixation of hydroxyapatite –coated implants. J Bone Joint Surg[Br] ;(76-B):831-3.

[22] Luciana Y , Sala MA .(2002):Histometric study of socket healing after tooth extraction in rats treated with diclofenac .Braz Dent J ; 13 (2):92-96.

[23] Coon D, Gulati A, Cowan C .(2007): The role of cyclooxygenase-2 (COX- 2) in inflammatory bone resorption. J Endod.;33(4):432-436

[24] Krischak GD,Augat P,Sorg T. (2007):Effects of diclofenac on periosteal callus maturation in osteotomy healing in an animal model. Arch Orthop Trauma Surg ;127(1):3-9.

[25] Patrick J ,John T.(2009):A comparison of the effects of ibuprofen and rofecoxib on rabbit fibula osteotomy healing .Acta Orthopaedica ; 80 (5):597-605.

[26] Omori K ,Wada S et al .(2012): Prostaglandin E2 increases both osteoblastic and osteoclastic activity in the scales and participate in calcium metabolism in goldfish .Zoolog Sci ; 29(8):499-504.

[27] Mohammad S , Denise D .(2012):Bone turnover markers in peripheral blood and marrow plasma reflect trabecular bone loss but not endocortical expansion in aging mice. Bone ;50(3): 628-637 EE

[28] Ho ML , Chang JK .(1999):Effects of nonsteriodal anti-inflammatory drugs and prostaglandin on osteoblastic functions .Biochem Pharmacol ;58 : 983-990.

[29] Goodman SB , Ma T , Mitsunaga L .(2005):Temporal effect of a COX-2 selective NSAID on bone ingrowth.J Biomed Mater Res; (72):279-287.

[30] Evans CE , Butcher C .(2004):The influence on human osteoblasts in vitro of nonsteroidal anti-inflammatory drugswhich act on different cyclooxygenase enzymes .J Bone Joint Surg Br ;(86):444-449.

[31] Karachaalios T , Boursinos L .(2007):The effect of short term administration of low therapeutic doses of anti COX-2 a gentson the healing of fractures :an experimental study in rabbits. J Bone Joint Surg Br ;(89) : 1253-1260

[32] Demos G , Maria T . (2008): Influence of non steroidal anti – inflammatory drugs on osteointegration .Journal of oral science ; 50(3): 239-246.

[33] Daluiski A , Ramsey KE , Shi Y .(2006):Cyclooxygenase – 2 inhibitors in human skeletal fracture healing . Orthopedics;(29):259-261.

[34] Zarrinkalam MR , Atkins GJ .(2012):Changes in osteocyte density correspond with changes in an osteoporotic sheep model. Osteoporos Int;, 23: 1329-1336

[35] Shalini Chouhan and Sushma Sharma.(2012).Potential risks of aberrations in bone histology and myelotoxicity after continuous diclofenac use in BALB/C mice model . An international quarterly journal of environmental sience,1 :185-191.

[36] Sassioto, M. C., Inouye, C. M., Aydos, R. D. and de Figueiredo, A. S. (2006). Bone repair in rats treated with sodic diclofenac and calcitonin. SHALINI CHOUHAN AND SUSHMA SHARMA Acta Cir. Bras. 21(4): 40-44.

[37] Sen, C., Erdem, M., Gunes, T., Koseoglu, D. and Filiz, N. O. 2007. Effects of diclofenac and tenoxicam on distraction osteogenesis. Arch. Orthop. Trauma Surg. 127: 153-159.

[38] Andrew P . (2012):The effect of Nonsteriodal anti-infammatory drugs administration on acute phase fracture healing :A review .J Bone Joint Surg Am ,94(9):815-823.

Table (1) : A comparison of the scores of extraction wound healing between control and treatment groups at 4 different times of healing.

Days of healing	Control group Mean ±SD	Treatment group Mean ±SD	Mann-Whitney U	P- value
0	**7 ± 0.0**	**7 ± 0.0**	**8.000**	**1.000**
3	**3 ± 0.0**	**6.75 ± 0.25**	**0.000**	**0.011***
6	**0 ± 0.2**	**7.25 ± 0.25**	**0.000**	**0.008***
9	**0 ± 0.0**	**7.5 ± 0.28**	**0.000**	**0.013***

*** significant at p<0.0**

Table(2): Mean ±SD of biochemical bone markers for Control and treatment groups.

Biochemical markers	Control group Mean ±SD	Treatment group Mean ±SD	p-value
calcium	10.85±3.6	10.325±2.7	0.826
phosphate	4.80±2.10	5.175±1.8	0.800
Alkaline phosphatase	47.83±14.45	43.10±20.58	0.720

*** significant at p<0.05**

World Journal of Pharmaceutical, Biological and Chemical Sciences
Vol. 1, No. 1, August 2013, PP: 01-11
Available online at http://acascipub.com/Journals.php

Figure (1): A comparison of the mean scores of extraction wound healing between 4 different times of healing period for both control and treatment groups .

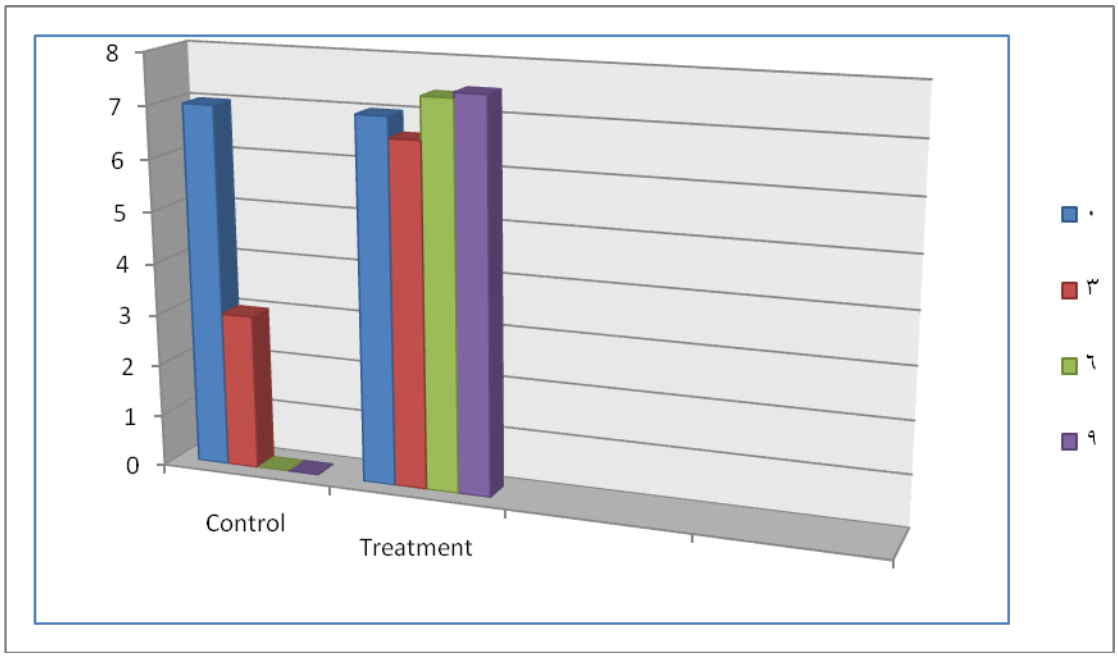
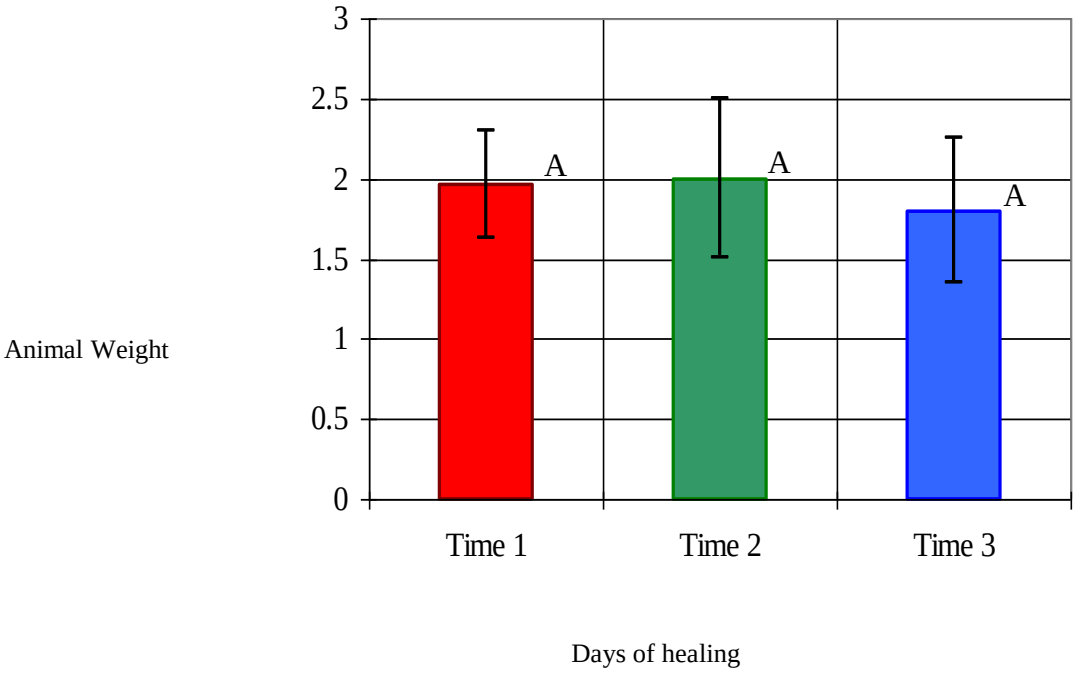


Figure (2): A comparison of the animal weights between control and treatment groups at 0 , 3 , 6 and 9 days of study period.



World Journal of Pharmaceutical, Biological and Chemical Sciences
Vol. 1, No. 1, August 2013, PP: 01-11
Available online at http://acascipub.com/Journals.php

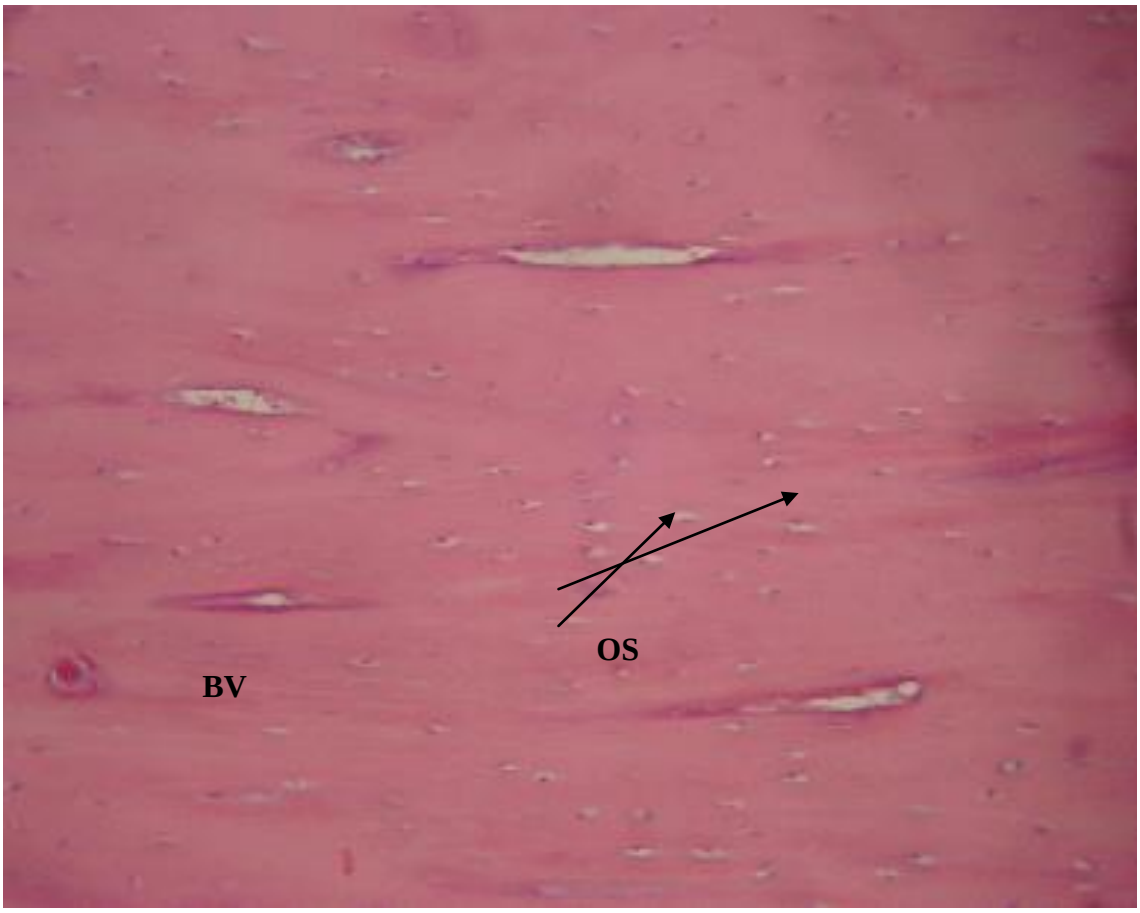


Figure (3) : Light micrograph of normal tibial bone in control group showing osteocytes (OS) and blood vessels (BV).H&E. [X- 2].

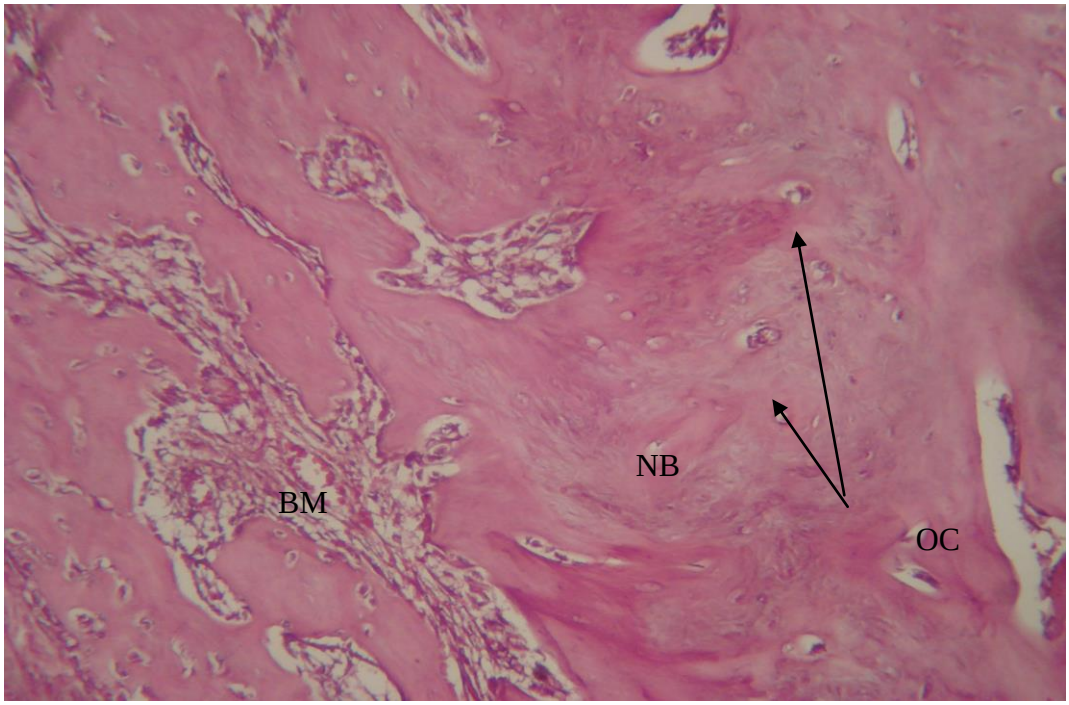


Figure (4) : Light micrograph of tibial bone in treatmet group showing osteoclast (OC) ,necrotic bone (NB) and bone marrow(BM).H&E.
[X- 2].

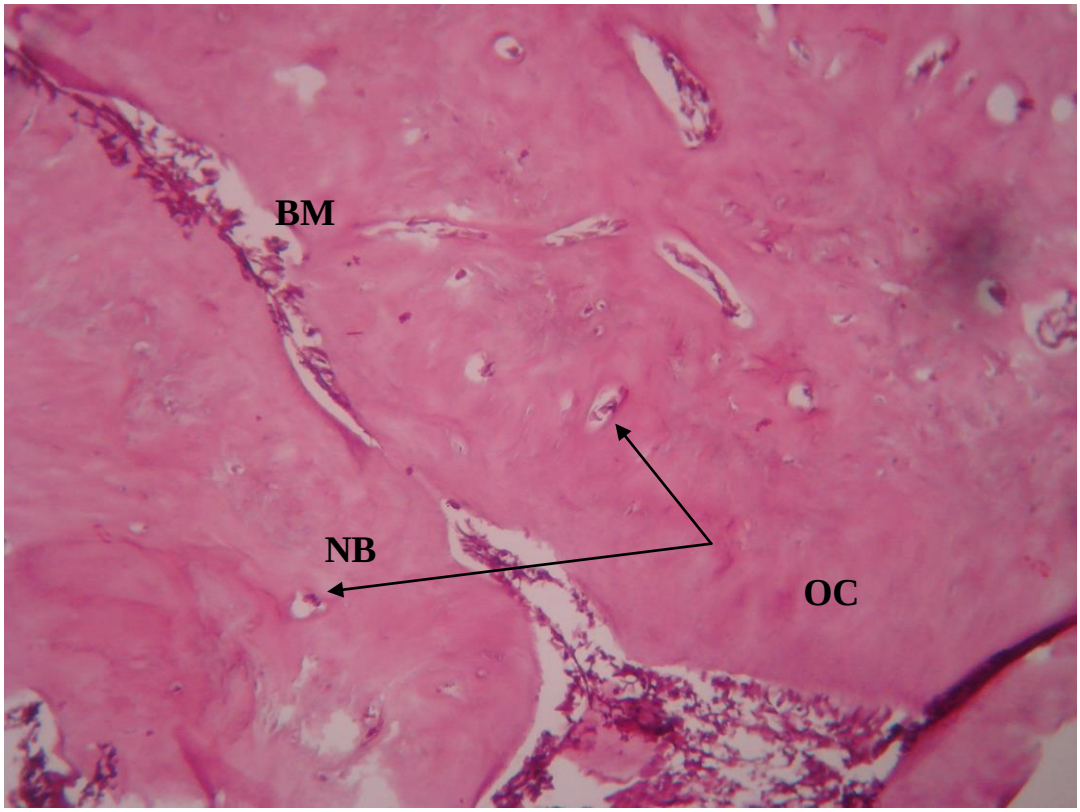


Figure (5) : Light micrograph of tibial bone in treatmet group showing osteoclast (OC) ,necrotic bone (NB) and bone marrow(BM) with reduced size .H&E. [X- 2].