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Effect of Ketoprofen and Paracetamol on Bone Mineral Density in Rabbits

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ABSTRACT

Objective: The aim of this study was to investigate the effect of ketoprofen and paracetamol on bone mineral density (BMD) in rabbits at healing site postoperatively, when given the drug in therapeutic doses for three different follow-up periods (15,30,45) days after operation in mandibular bone.

Materials and Methods: Twenty seven male rabbits with almost same age, weight and circumstance were chosen for this study. All animals were submitted to operation in mandibular bone region. Groove of 2mm diameter and 6mm length was drilled by heavy duty dental engine. After operation rabbits were randomly divided into three groups (Control, Ketoprofen, Paracetamol), each group contain 9 rabbits. Group1(control): received no treatment. Group2: Ketoprofen group received IV(4mg/kg). Group3: Paracetamol group received IV (35 mg/kg) Paracetamol for 15,30, 45 days respectively. BMD were measured after 15,30,45 days respectively for all rabbits at healing site by using densitometric software analysis.

Results: Statistical analysis showed significant differences between all three groups. The estimated BMD showed significant decrease in Ketoprofen group (42.00±1.00) compare to control and paracetamol groups after 15 days of treatment (118.0±01.15)(80.33±0.57) respectively. Also in the present study we found that significant decrease in BMD after 30 days of operation in Ketoprofen group(70.33±0.58) compare to control and paracetamol groups(119.33±0.58) (84.66±0.57) respectively. After 45 day of daily administrated paracetamol no significant difference in BMD between paracetamol and control(144.33±0.52)(131.00±1.00) groups respectively but significant differently with Ketoprofen group **(**96.66±0.57).

Conclusions: We concluded that Ketoprofen and paracetamol administration for long period after fracture bone decrease BMD in rabbits compared to control groups and Ketoprofen groups showed more decline over time in BMD than paracetamol groups.

Key words: BMD (Bone Mineral Density) bone healing. densitometry analysis, Ketoprofen, paracetamol

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INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) comprise a heterogeneous group of pharmacological agents used for the symptomatic treatment of fever, pain, and inflammation [1]. NSAIDs were discovered more than 100 years ago. They remain a key component of the pharmacological management of pain which represent a major public health problem worldwide [2] . NSAIDs are classified as non-selective and COX-2-selective inhibitors (COXIBS) based on their extent of selectivity for COX inhibition [3]. Ketoprofen is a nonsteroidal anti-inflammatory and analgesic agent that non selectively inhibits cyclooxygenase, with both COX-1 and COX-2 inhibition. Recent studies on COX receptor expression in reptiles suggest that nonselective COX inhibitors may be more appropriate than more selective inhibitors in some reptiles [4]. Paracetamol (acetaminophen) is a widely used analgesic/ antipyretic with weak inhibitory effects on

cyclooxygenase compared to non-steroidal anti-inflammatory drugs [5]. Paracetamol displays different characteristics from those of other analgesic NSAIDs. owing to its poor inhibitory effect on COX, it lacks anti-inflammatory effects and gastrointestinal side effects [5,6]. Several studies have been conducted to confirm whether paracetamol affects bone remodeling analogously to NSAIDs, which are suspected to have adverse effects on bone healing. In contrast, the effect of paracetamol on bone healing remains controversial because of its low inhibitory effect on COX enzymatic activity [5,7,8]. NSAIDs and paracetamol are distributed via the over-the-counter sale (OTC) and are extremely popular [9]. Unfortunately, NSAIDs and APAP might have serious side effects involving not only gastrointestinal tract, kidneys, nervous system or hematological complications, but almost every organ, especially when they are used with no respect to safety rules, in an irrational way [10,11,12] . NSAIDs commonly prescribed in orthopedic patients following musculoskeletal injuries and fractures [13,14]. However, there exists uncertain evidence for impaired fracture and tendon healing by both selective and unselective COX inhibitors [13,15,16,17,18]. This evidence is mainly limited to low-quality human studies and animal models using supernormal dosages [16,19] .Variables such as the dose, duration of administration, and selectivity of NSAIDs can impair osseointegration, raising the question of whether they can be used safely for pain relief after dental implant surgery .[14, 20,21] .

The goals of the present study was undertaken to study the effect of ketoprofen and paracetamol because the widespread use of NSAIDs for pain relief and access the effect of these medications on bone fracture healing by evaluate bone mineral density (BMD) using densitometric analysis method.

MATERIALS AND METHODS

Twenty-seven white New Zealand healthy male adult rabbits with a mean weight of 1.5- 2.5 Kg were used in this study. The animals were kept under standard condition with the same circumstance of feeding and housing at 25^0C ±2 room temperature given 12 hours of light and 12 hours of darkness [22] and were given standard diet of (wheat and fresh vegetables) and water. The habitant of the animal during the experiment was in cage in the animal house of the College of Dentistry, University of Mosul. Animals examination were performed by a veterinary physician to check general health and condition of the animal before surgical procedure. This study was performed in accordance with guidelines of the institutional animal research ethics committee of the institution.

Animal preparation and Surgical procedure:

All rabbits were anaesthetized with an intramuscular injection of ketamine (40 mg/kg) and xylazine (5mg/kg) [23] **.** After complete anesthesia had been obtained, the hair was removed from the animal‘s submandibular area using hair removal, washed with soap and water, then cleaned with povidone iodine solution disinfectant and properly covered with sterile towel around surgical area . **(**Figure1)

Complete anesthesia had been obtained within 5min. Animals were placed in left lateral position on the operating table. Surgery was performed using standardized sterile technique. Incision of about 4cm was made in the skin over the submandibular area running with the lower border of the mandible starting from the symphysis area using blade no.12.After flap reflection, the periosteum is dissected bluntly and the bone is exposed. Groove of 2mm diameter and 6mm length [24] was drilled by heavy duty dental engine of low speed hand piece of (2000) rpm and profuse irrigation of normal saline. The bony defect dried from blood, then the wound closed with black silk suture and disinfected by povidone iodine.

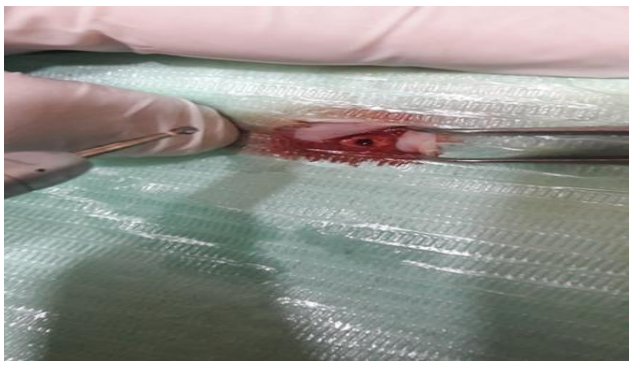


Figure 1: Drilling of the Holes

Immediately after the operation, a mixture veterinary antibiotic containing (0.5g) ox tetracycline had been administered I.M once daily for three days post operatively. The rabbits were isolated after the procedure till they recover from anesthesia. Post-operative monitoring of the operated animals during first 24 hour including observation of their feeding and physical activity.

Experimental protocol:

After complete operation the rabbits were divided into three groups, each group contain 9 rabbits. Each group subdivided according to the medication period into (15, 30, 45 days) to evaluate bone mineral density (BMD) using **Orthopantomograph Device** (Figure 2) as a following:

Group1: (Control) received no treatment over (15, 30, 45 days) respectively.
Group2: (Ketoprofen) received ketoprofen I.V injection via the marginal ear vein at a dose of (4mg/kg) [25] on a daily basis over (15, 30, 45 days) respectively.

Group3: (Paracetamol) received paracetamol I.V injection via the marginal ear vein at a dose of (35mg/kg) [26] on a daily basis over (15, 30, 45 days) respectively.

Figure 2: Orthopantomograph Device

Radiographical Analysis of Bone Densitometry:

Bone density in the region of interest (Mandibular Region at the defect site in right mandible of rabbit) was calculated using the digital Densitometric Kodak software 9000C (kodak9000three-dimensional-3D) Imaging system. The tool allows the measurement between two different points as a linear distance measurement and the reading reveal average value of bone density at any given point [27]. So, we obtained the information on the rate of bone formation and determined the variation in radiodensity between radiographs.

Digital panoramic radiographs were made for groups of rabbits at time (0, 15, 30, 45 days) employing kodak 9000C 3D Extraoral Imaging System , V shape child jaw size, at 60 kv, 2.0 mA, 13sec

After complete anesthesia was obtained, the position of the rabbit‘s head was standardized. Rabbit put on small box and its ears fixed on the system. Laser beam was adjusted in a manner parallel to mandibular plain of mandible bone of the rabbit. (Figure 3)

Linear measurements were made using a digital caliper and a clear plastic acetate sheet superimposed on panoramic radiographs. The test was conducted at AL-RASHEED Specialized Center in Mosul and all measurements were carried out by Specialist Radiologist.

Figure 3: densitometric analysis with OPG for Rabbit

Statistical Analysis:

Statistical analysis All the data has been processed and analyzed by the use of statistical package SPSS ver. 18 (SPSS Inc., Chicago, ILL). ANOVA and Kruskal Wallis test were used to compare the differences in mean bone density among three groups of the study and different follow-up periods. A p-value ≤0.05 was considered statistically significant.

RESULT

Statistical analysis showed significant differences at p≤0.05 in bone mineral density between all groups at different follow-bone density among three groups of the study at different follow-up times as shown in (Table 1) , (Figure 4)

Table 1: Comparison of changes in bone density between control, ketoprofen, paracetamol groups at different follow-up periods.

Group	Follow –up				P-value (follow-up)
	Baseline Mean±SD	Day 15 Mean±SD	Day 30 Mean±SD	Day 45 Mean±SD	
Control	83.00±5.19	118.0±01.15	119.33±0.58	131.00±1.00	0.0001
Ketoprofen	91.66±1.52	42.00±1.00	70.33±0.58	96.66±0.57	0.0001
Paracetamol	97.66±0.57	80.33±0.57	84.66±0.57	144.33±0.52	0.0001
P-value (groups)	0.04	0.0001	0.0001	0.0001	

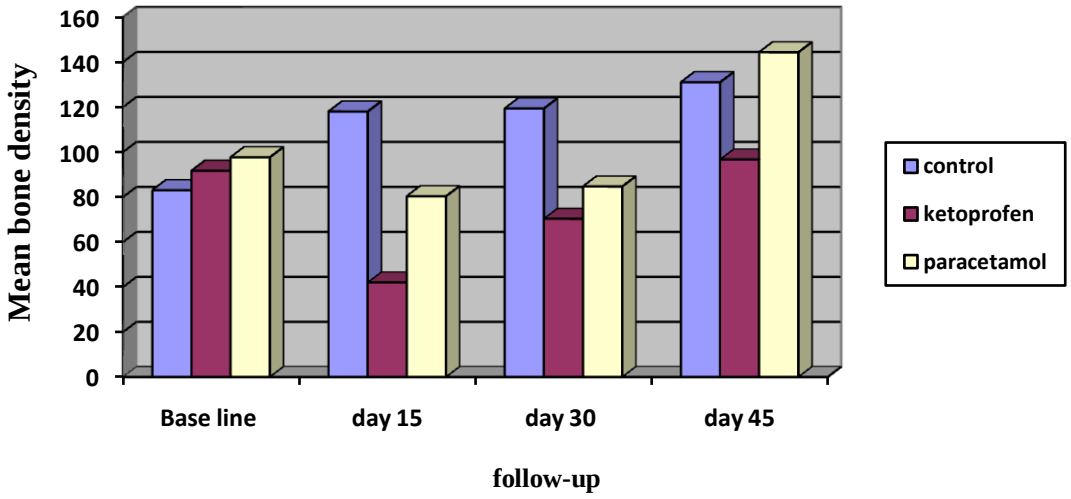


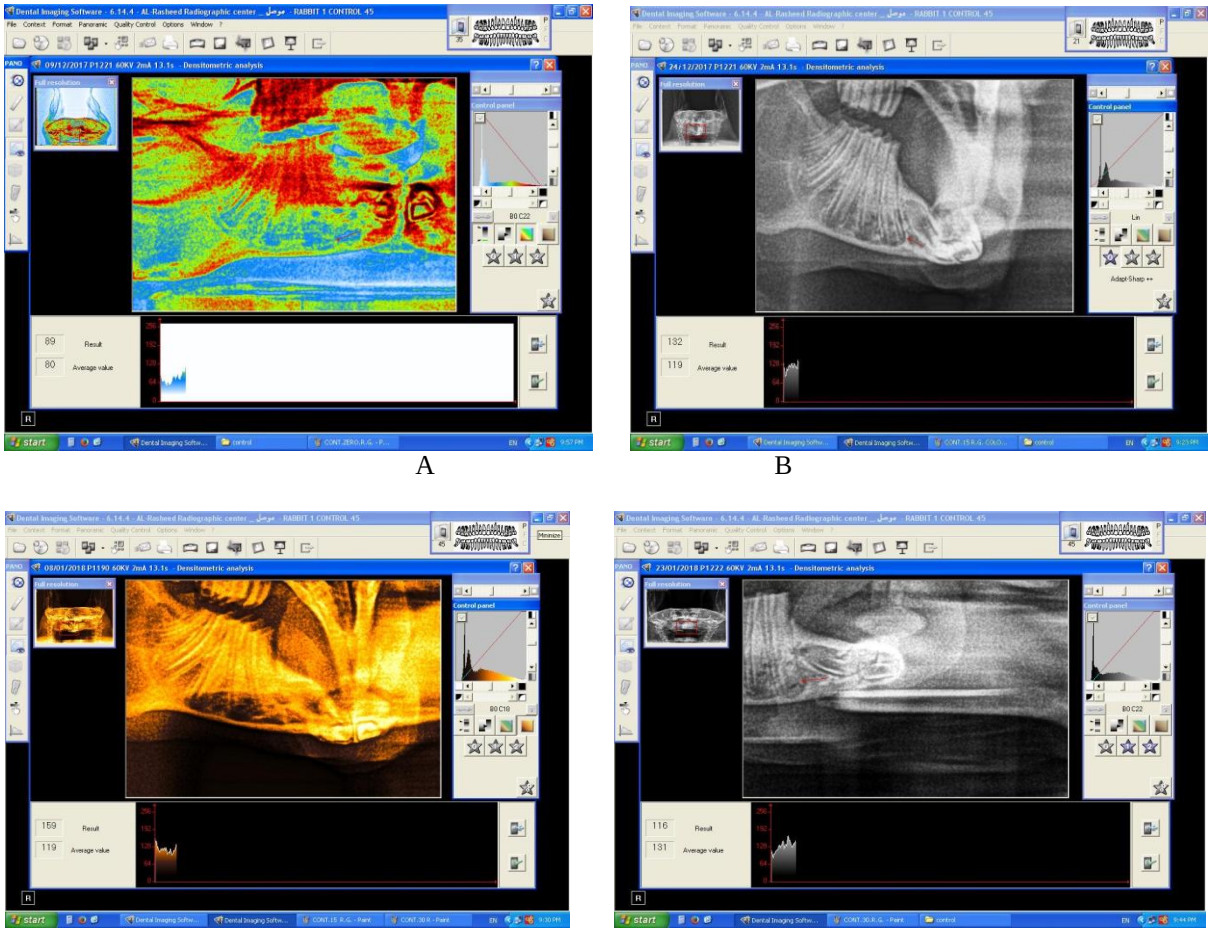
Figure 4: changes in bone density between control, ketoprofen, paracetamol groups at different follow-up periods.

ANOVA and Kruskal Wallis test showed that significant difference comparison changes in bone density between control and ketoprofen groups. The estimated BMD showed significant decrease in Ketoprofen group(42.00±1.00) compare to control groups after 15 days of treatment (118.0±01.15). Also in the present study we found that significant decrease in BMD after 30 days of operation in Ketoprofen group(70.33±0.58) compare to control groups(119.33±0.58) as well as BMD significant decrease after 45 days of ketoprofen treatment (96.66±0.57) compare to control group(131.00±1.00).

In the present study we found that the BMD in control that significant increased from base line (83.00±5.19) at first day of operation to (118.0±01.15)(119.33±0.58)(131.00±1.00) at 15,30,45 days respectively whereas we found that the ketoprofen group significantly decrease the BMD after 15,30 (42.00±1.00) (70.33±0.58) days respectively of operation compare to base line (91.66±1.52) and return the BMD to normal line after 45 day of treatment. As shown in (Table 2) and (figure 4 ,5).

Table 2: Comparison of changes in bone density between control and ketoprofen groups at different follow-up periods

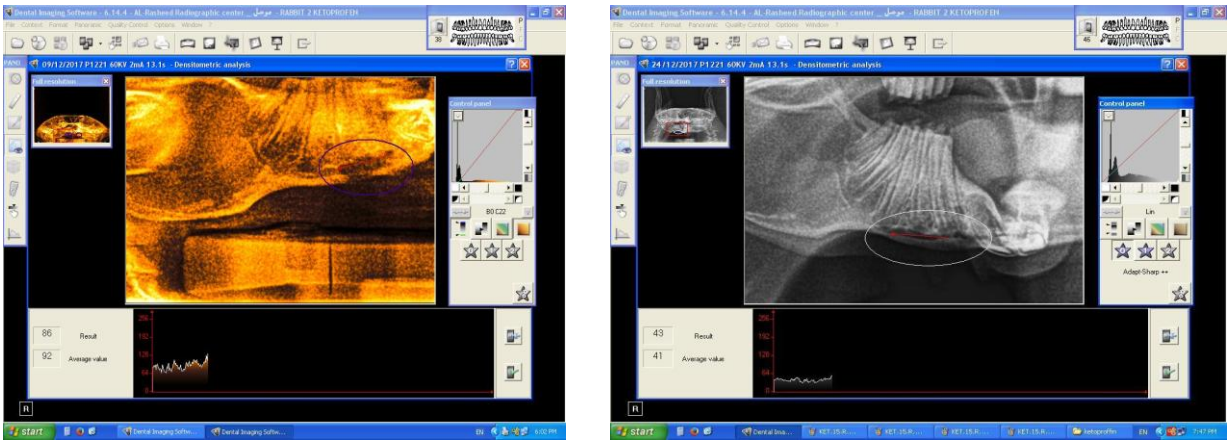
Group	Follow –up				P-value (follow-up)
	Baseline Mean±SD	Day 15 Mean±SD	Day 30 Mean±SD	Day 45 Mean±SD	
Control	83.00±5.19	118.0±01.15	119.33±0.58	131.00±1.00	0.0001
Ketoprofen	91.66±1.52	42.00±1.00	70.33±0.58	96.66±0.57	0.0001
P-value (groups)	0.005	0.0001	0.0001	0.0001	



A B

C D

Figure 4: Densitometric analysis for control rabbit group (A) at time 0.(B)at time 15. (C) at time 30 (D) at time 45.



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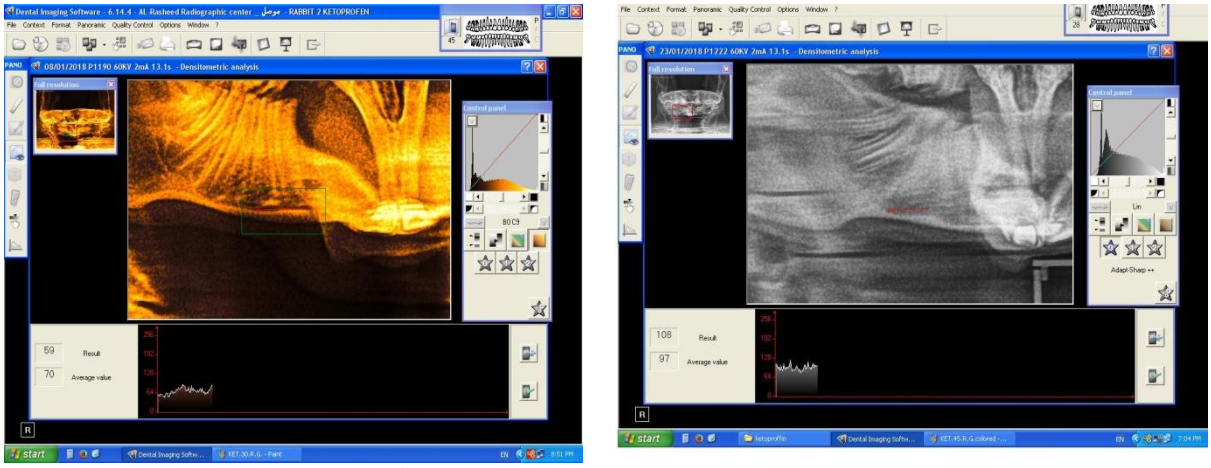
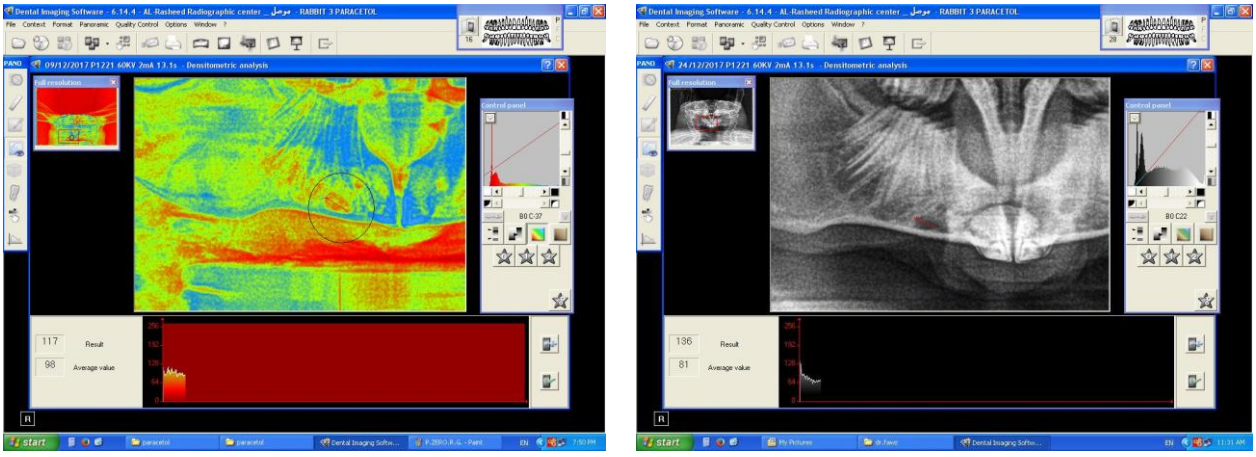
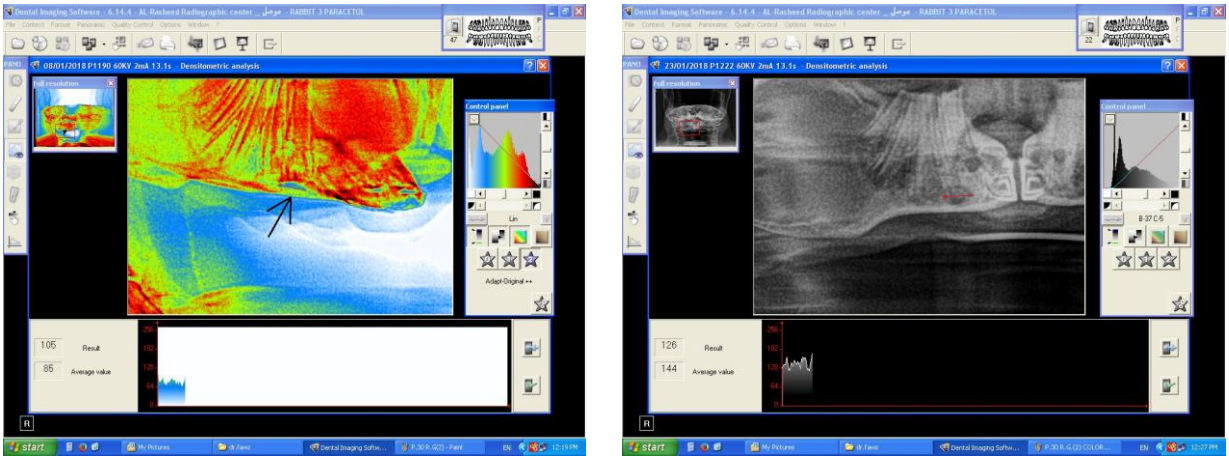


C D

Figure 5: densitometric analysis for Ketoprofen rabbit group (A)at time 0.(B)at time 15. (C) at time 30 (D) at time 45.



A B



C D

Figure 6: densitometric analysis for paracetamol rabbit group (A)at time 0.(B)at time 15. (C)at time 30 (D) at time 45.

ANOVA and Kruskal Wallis test showed that significant difference comparison changes in bone density between control and paracetamol groups. The estimated BMD showed significant decrease in paracetamol group. (80.33±0.57)compare to control groups after 15 and 30 days of treatment (118.0±01.15)(119.33±0.58) respectively. After 45 days of paracetamol administration we found that no significant in BMD in paracetamol group(144.33±0.52) compare to control group (131.00±1.00) as shown in (Table 3) and (figure 4,6).

In the present study we found that the BMD in control that significant increased from base line (83.00±5.19) at first day of operation to (118.0±01.15)(119.33±0.58)(131.00±1.00) at 15,30,45 days respectively whereas we found that the paracetamol group no significantly decrease the BMD after 15,30 **(**80.33±0.57) (84.66±0.57) days respectively of operation compare to base line (97.66±0.57), 15 (80.33±0.57) , 30 days(80.33±0.57) and significantly increased the BMD compare to base line (97.66±0.57) after 45 day of treatment(144.33±0.52). As shown in (Table 2) and (figure 4,6).

Table 3: Comparison of changes in bone density between control and paracetamol groups at different follow-up periods

Group	Follow –up				P-value (follow-up)
	Baseline Mean±SD	Day 15 Mean±SD	Day 30 Mean±SD	Day 45 Mean±SD	
Control	83.00±5.19	118.0±01.15	119.33±0.58	131.00±1.00	0.0001
Paracetamol	97.66±0.57	80.33±0.57	84.66±0.57	144.33±0.52	0.0001
P-value (groups)	0.008	0.001	0.001	0.09	

ANOVA and Kruskal Wallis test showed that significant difference changes in bone density between ketoprofen and paracetamol groups. The estimated BMD showed significant decrease in ketoprofen group (42.00±1.00) (70.33±0.58) (96.66±0.57) compare to paracetamol groups (80.33±0.57) (84.66±0.57) (144.33±0.52) after 15, 30 and 45 days of treatment respectively. as shown in (Table 5) and (figure 5 , 6).

Table 5: Comparison of changes in bone density between ketoprofen and paracetamol groups at different follow-up periods

Group	Follow –up				P-value (follow-up)
	Baseline Mean±SD	Day 15 Mean±SD	Day 30 Mean±SD	Day 45 Mean±SD	
Ketoprofen	91.66±1.52	42.00±1.00	70.33±0.58	96.66±0.57	0.0001
Paracetamol	97.66±0.57	80.33±0.57	84.66±0.57	144.33±0.52	0.0001
P-value (groups)	0.03	0.0001	0.001	0.0001	

DISCUSION

Pain medication such as paracetamol and NSAIDs are widely used.

However, doubts have been reported about their skeletal safety. The analgesic, antipyretic and anti-inflammatory activities of NSAIDs are mediated by their inhibition of prostaglandin synthesis. Prostaglandins (PGs) are multifunctional regulators of bone metabolism that stimulate both bone resoption and formation. PGs have been involved in bone resoption associated with inflammation and metastatic bone disease, and also in bone formation associated with bone healing and heterotopic ossification [28].

PGE2 as downstream product is heavily involved in bone homeostasis [29]. Direct prostaglandin administration causes hyperostosis and an increase in trabecular and cortical mass[13,28]. Bone differs from other tissues due to its remarkable ability to repair and heal without leaving a scar [30].Although the negative effect of NSAIDs on bone healing there is insufficient evidence support withholding NSAID after a fracture or orthopedic procedure in domestic species.

In fact, bone can heal in the face of NSAID administration ,in addition NSAID are important for their potent anti-inflammatory and analgesic effects after bone injury[31].But when the speed and effectiveness of bone healing is of particular concern, such as in nonunion, delayed union, tenuous orthopedic repairs, or in patients with expected delayed bone healing , it is reasonable to consider the evidence when prescribing NSAID. Experimental study suggests that the higher doses and the longer durations of NSAID cause more inhibition in bone healing [32] this result agreement with our study , we found that the administration ketoprofen for long period increase the period time bone healing in comparison to control group.

In our study we found that administration ketoprofen and paracetamol decrease the BMD and delay the mandibular bone healing after 15,30 days after operation, this is evidence to support that NSAID-induced inhibition can be reversible once the NSAID is discontinued [33]. Therefore, when NSAIDs are no longer needed for analgesia, It make sense to discontinue NSAID use after reasonable post-injury period .

Various parameters have been used for measuring bone density. In our study employs the densitometric software with OPG for bone density assessment .Digital OPG scans were performed in Kodak 9000 3D Extaoral imaging system .The unit of BMD measurement was in gr/cm^2.This is the first study done in which m-BMD is measured in a living rabbit at healing site. The results of Radiographical analysis showed significant differences in BMD between all study groups. Ketoprofen group showed minimum BMD while control group showed maximum BMD at all 3 follow- up periods (15,30,45) days. Paracetamol group showed higher BMD than Ketoprofen group and lesser BMD than control group. So, we note obvious negative effect of ketoprofen and paracetamol on bone healing in rabbits. This result agreement with previous study of Nackaerts *et al*. that investigat the accuracy and precision of a digital densitometric tool for the analysis of mandibular bone density. They concluded that digital densitometric tool offers potential for clinical evaluation of bone density and minute bone density changes in the jaw bone [34].

Oliveira *et al*. did a study to evaluate the relationship between trabecular bone pattern and bone mineral density by using digital OPG images. The results showed a significant difference between normal and osteoporotic patients in bone density .The test concluded that mandibular trabecular bone was effective in detecting osteoporotic changes in some regions of the jaws[35]. Taneja *et al*;2015 assessed bone mineral density in pre- and post-menopausal women using densitometric software . They correlate and compare the quality of mandibular bone with the help of densitometric software available in Kodak 9000C and to further substantiate the results, morphometric analysis using orthopantomograph (OPG) and quantitative ultrasound of calcaneus bone. They found out the densitometric software with OPG yielded positive results and could be used independently to measure bone density in future[27].Sun *et al*. also assessed the reliability and accuracy of new software for radiodensitometric evaluations and concluded that this tool has been developed, that is reliable for bone density assessment [36].the result of above study agreement with result of our study that employed densitometric tool to detect the decrease in BMD in mandibular rabbit bone after administration ketoprofen and paracetamol for long time

In our study we found that the administration paracetamol and ketoprofen for long period were decline BMD in mandibular bone this result agreement with other researcher, Yoshida *et al*. study the effect of analgesics on bone mineral density. They measured and compared BMD annually among new users of acetaminophen, NSAIDs, and opioids. They found out the BMD decline over time was similar among the three groups. However, continuous opioids use for 5 years may be associated with greater BMD decline than 5 years on other analgesics[37].and Williams *et al* .assessed the association between paracetamol use , fracture and BMD in women aged ≥ 50 years participating in Geelong Osteoporosis Study (GOS) . Their data suggested that paracetamol use is a risk factor for fracture, despite an mechanism of action remains unclear. Incorporating total body BMD into the model attenuated the association[38].

Vestergaard *et al*. investigated the effect of NSAIDs, paracetamol, acetylsalicylic acid, and opioids on bone mineral density and risk of fracture. BMD was measured by DXA. They reported significant differences between subjects exposed to analgesics and non-users. Users of NSAID show more fractures than expected, although the absence of the effect over time on BMD[39].

The result in our study in agreement with other study on animal experimental studies have indicated that NSAIDs may inhibit bone healing, especially the early phases. These animal experimental studies have shown decreased BMD measured with DXA during NSAID treatment [40,14].but disagreement with other study of Carbone *et al*. reported no differences in BMD for the mixed group of aspirin with relative COX-1 Inhibitor selective NSAID group and controls, whereas for the combination of relative COX-2 selective NSAIDs and aspirin is associated with higher BMD at multiple skeletal sites in women and men [42].While Morton *et al*. concluded that regular daily use of propionic acid NSAIDs, with or without simultaneous use of estrogen, may be helpful in preventing bone loss in older women [43].Bauer *et al*. in their study reported the increase in hip or spine BMD was 1-2.7% with NSAID use for 5-7 times per week, although a non-significant change of 0.3-0.8% was seen with use of 1-4 times per week after multiple adjustment [44].

CONCLUSION

For bone healing to occur, not only adequate bone quantity is required, but adequate bone density is also needed. Results of densitometric software analysis showed significant reduction in BMD and negative effect on bone healing in rabbits in ketoprofen and paracetamol groups compared to control groups. Ketoprofen groups showed more decline over time in BMD than paracetamol groups.

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