

Effect of β -calcium sulphate hemihydrate on mandible healing in dog (radiographical assessment using Image-J Program)

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Abstract

This study was conducted to estimate the bony tissue response to β -calcium sulphate hemihydrate (CSH) as a bone substitute via radiographic assessment using ImageJ software. The extraction sockets in dog mandible were the regions of interest (ROI). Twenty adult (12-24 months), local breed dogs were included in the experiment. All had a complete set of permanent dentition. They were randomly allocated into four groups, each containing 5 animals. Bilateral lower third premolars have been extracted. The right socket was filled with β -calcium sulphate hemihydrate, whereas no material was placed to fill that in the left side to serve as a control. Tissue response in extraction sockets was evaluated using two post-operative intra-oral periapical radiographs for each tooth socket, the first immediately after extraction and the second at the end of each study interval (i.e., after 2 weeks, 4 weeks, 8 weeks, and 12 weeks period for group I, II, III, and IV, respectively). The radiographs were converted from conventional to digital by X-ray scanner, then examined by ImageJ software. Radiographic assessment included the evaluation of differences in extraction sockets densities, bone resorption %, bone formation %, and density of the newly formed bone. The results showed significant differences between the left (control) and right (experimental) sides in all study periods in relation to differences in extraction sockets densities. Meantime, significant differences were noticed between right and left sides during a 12 week period in relation to bone resorption and bone formation %. Concerning density of the newly formed bone, significant differences were noticed during 8 week and 12 week period. In conclusion, the use of β -calcium sulphate hemihydrate as a bone substitute significantly reduced bone resorption and increased the rate of new bone formation. In addition, the density of the newly formed bone in the right (experimental) side was greater than that noticed in the left (control) side.

Keywords: Bone substitute; Calcium sulphate; Dogs; Tooth extraction; ImageJ

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تأثير كبريتات الكالسيوم نصف المائية نوع بيتا على التئام الفك السفلي في الكلب باستخدام (تقييم اشعاعي باستخدام برنامج Image-J)

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الخلاصة

أجريت هذه الدراسة لتقدير استجابة النسيج العظمي لكبريتات الكالسيوم النصف المائية نوع بيتا بوصفه بديل عظمي وذلك بتقييم التصوير الشعاعي عن طريق استخدام برنامج Image-J. تم وقد استخدمت حفرة قلع السن في الفك السفلي للكلب. أختبر عشرون كلباً محلياً بالغ (١٢-٢٤ شهراً) للتجربة. جميع الحيوانات المختارة تمتلك مجموعة كاملة من الاسنان الدائمة. قسمت الحيوانات عشوائياً الى اربعة مجاميع، كل منها يحتوي على خمسة حيوانات. تم قلع السن الضاحك الاسفل الثالث في كلا الجهتين لكل حيوان. في الجهة اليمنى تم ملئت حفرة السن المقلوع باستخدام كبريتات الكالسيوم نصف المائية بينما لم يتم وضع أي مادة في الجهة اليسرى للقلع لكي تبقى كجهة ضابطة (سيطرة). الاستجابة النسيجية في حفر القلع تم تقييمها بأخذ اشعتين بعد الجراحة لمنطقة القلع، الاولى مباشرة بعد القلع و الثانية في نهاية

كل فترة زمنية (بعد اسبوعين، اربعة اسابيع، ثمانية اسابيع، اثني عشر اسبوعاً للمجموعة الاولى، الثانية، الثالثة، الرابعة على التوالي). حولت الأشعات من عادية الى رقمية باستخدام جهاز مسح الأشعة الضوئي لتفحص باستخدام برنامج **Image-J**. شمل التحليل الاشعاعي تقدير الفرق في كثافة الحفرة السنية، النسبة المئوية لارتشاف العظم، النسبة المئوية لتكوين العظم، وكذلك كثافة العظم الجديد. أظهرت النتائج وجود فروقات معنوية بين الجهة الضابطة (اليسار) وجهة التجربة (اليمين) في كل فترات الدراسة بخصوص الفرق في كثافة الحفرة السنية. في الوقت ذاته لوحظت فروق معنوية بين الجهتين خلال الاثني عشر اسبوعاً فيما يخص النسبة المئوية لارتشاف العظم والنسبة المئوية لتكوين العظم. كما لوحظ وجود فرق معنوي خلال مدة الثمانية اسابيع والاثني عشر اسبوعاً بالنسبة لكثافة العظم الجديد. استنتج من هذه الدراسة ان استخدام كيريتات الكالسيوم نصف المائية نوع بيتا بديلاً عظميةً قلل نسبة ارتشاف العظم الى حد كبير وزاد نسبة تكوين العظم الجديد فضلاً على ان كثافة العظم المتكون في جهة اليمين (جهة التجربة) كانت اكبر من كثافة العظم المتكون في جهة اليسار (الجهة الضابطة).

Introduction

Tooth extraction may encourage extensive dimensional changes of the alveolar ridge subsequent to tooth loss, leading to considerable changes in the structure and morphology of the alveolar bone. The percentage of these changes during the first year after tooth loss is almost 10 times greater than that during later years (1). The problem that dentists face is how to undertake tooth extractions without retreating ridge dimensions (2). In dentistry nowadays, the most inventive and rousing treatment modality for substituting missing teeth is the endosseous dental implant (3). That is why bone conservation after extraction plays an important role in achieving proper results in subsequent prosthetic and implant treatments (1). Consequently, bone grafting procedures to augment the alveolar ridge following tooth extraction are frequently performed in modern dentistry (3). Several graft materials have been proposed, although autogenous bone graft has been considered the gold standard. But, the main disadvantages are a limited amount of graft material, the need of an additional surgical site, increased donor-site morbidity, and the need to use general anesthesia for the extraoral bone harvesting (4-6). As an alternative to autogenous bone graft, numerous materials have been successfully employed. Collagen, ceramics, bioglasses, polymers, xenografts, allografts, alloplasts, and synthetic hydroxyapatites are among the materials encompassed in this category (7). Of great benefit to clinicians would be a material that is completely resorbable, safe, inexpensive. The ability able to maintain space, and serve as a reservoir for calcium ions (8). Interestingly, calcium sulphate is one of the first materials reconnoitered as a substitute for bone grafting in many fields of medicine (from dentistry to orthopedics). It possesses an extended history of safe use for over a century (9-12). The first reported case in the modern era where calcium sulphate was used to treat cavities in bone is from 1852 by Mathysen (a Dutch army surgeon) who assimilated plaster into a bandageable form (the form with which we are familiar today). Nowadays, calcium sulphate and its derivatives continue to be the

object of research and interest in dentistry and orthopedics (13). In order to determine whether a newly developed β -calcium sulphate hemihydrate bone filling material conforms to the requirements of biocompatibility, mechanical stability and safety, it must undergo rigorous testing both in vitro and in vivo. Results from in vitro studies can be difficult to extrapolate to the in vivo situation. Recently, analysis of bone texture on radiographs became a common way to investigate bone microarchitecture (14,15). Several researchers stated that in vivo radiographs are typical analytical methods for testing the biocompatibility of such materials. However, conventional radiography presents some limitations due to low sensibility and high inter-examiner disagreement (16). X-ray scanner is used to convert conventional dental radiographs into digital images and save them into computer (17,18), the scanned digital radiographic image can then be displayed by ImageJ program. It is a scientific image processing, freely available java-based public-domain and analysis program (19-21). According to our knowledge, no previous study on the healing procedure in the extraction socket depending on the radiographic analysis using ImageJ program was found in the literatures. So, the aim was to study the bony response to β -calcium sulphate hemihydrate as a bone substitute prepared from Iraqi gypsum rocks (22) via radiographic assessment (using ImageJ software) of the material implanted into the socket immediately following extraction of dog mandibular 3rd premolar.

Materials and methods

Twenty local breed dogs in good general health with an average age (12-24 months) and weighing (13-24 kilograms) were included in the experiment. The selected animals should have a complete set of permanent dentition. The animals were divided randomly into four groups, each containing 5 animals as shown in Table (1).

The animals have been placed in special cages contrived for this purpose in the animal house at the College of Veterinary Medicine, University of Mosul. They were fed

soft food (only fresh meat) and water throughout the period of the study. Before being admitted for surgeries, all the animals were examined by a veterinarian to rule out the presence of any disease, to check general health and condition of the animal before the surgical procedures (23). All of the experimental procedures were performed aseptically and carried out at the surgery theatre at the Department of Veterinary Surgery, College of Veterinary Medicine, University of Mosul. Animals were fasted twelve hours before the operation to avoid aspiration of gastric contents during general anesthesia. Previously prepared β -calcium sulphate hemihydrate powder (22) was packed in special containers (each containing 1 gram), autoclaved to be ready for implantation into the sockets of experimental animals following extraction.

Table (1): Study Groups

Group number	Number of Animals	Period
I	5	2 weeks
II	5	4 weeks
III	5	8 weeks
IV	5	12 weeks

To obtain general anesthesia, an intramuscular injection of Ketamine hydrochloride (40 mg/ Kg) general anesthetic solution (24,25) and xylazine (2mg/Kg) sedative, analgesic solution (26) was accomplished. Complete anesthesia had been obtained within 5 minutes. This dose kept the animal anesthetized for about 40 minutes. Such a time was mostly enough to complete the surgical procedure.

Intra-oral periapical radiographs were acquired using dental X-ray unit (China), operated at 65 kVp, 8 mA, 1 second exposure time and 15 cm focal distance, the distance standardized with the use of film holder. Auto processing intra-oral periapical films (Gulsa Tibbi Comp; Turkey) were used (Figure 1).

A radiograph (bisecting the angle technique) was taken for each right and left mandibular 3rd premolar area immediately before extraction. The area was locally anesthetized using two cartridges (each cartridge contains 1.8 ml of Lidocaine with epinephrine 1:80000; Pharmaceutical Chemistry; Maria Dolly Ospina R. Antioquia-Columbia) to obtain bloodless field. Tooth extraction was performed in the following manner: An intra-sulcular buccal and lingual incisions were made in the crevice region using blade number 15 mounted on scalpel handle no.3. Then, full-thickness flaps were elevated and 3rd premolar was carefully removed. The roots were separated by tooth hemi-sectioning with surgical diamond fissure bur mounted on a surgical hand piece (Strong, South Korea) with a speed of (1500 RPM) under continuous irrigation using cold normal saline. Each root was mobilized with a

straight elevator then extracted using lower anterior root forceps.



Figure 1: A- Dog mouth opened with canine mouth gag (red arrow) and Film holder in place. B- Intra-oral periapical radiograph of dog lower right third premolar.

For the purpose of standardization, each tooth socket was prepared using the dentium implant surgical kit used for implant installation. The length of drills was adjusted at 10mm and the following sequence of drills were used: starting with a pilot drill (Lindermann guide) of 2.2 mm in diameter, then the Lindermann first drill of 2.6mm in diameter, after that the 3.6 mm drill, and finally the 3.8 mm drill diameter was used, so that the final dimensions of each tooth socket were approximately 10 mm in height and 3.8 mm in diameter. Post-operative intra-oral periapical radiograph was taken immediately after extraction, the flaps approximated and the surgical site was closed with 2 stitches of 3.0 black silk suture without placement of any material to serve as the control site. Concerning the lower right 3rd premolar tooth extraction, the same procedure was performed with the exception that each socket was augmented with the tested material after extraction and as follows: 1 gram of β -calcium sulphate hemihydrate was mixed with 0.5 ml distilled water and manipulated to obtain a paste which was then delivered to the tooth socket using spatula layer by layer and condensed with a sterile piece of

gauze mounted on the tip of a tweezers until the socket is completely filled with the graft material. Then flaps were stabilized with two simple interrupted stitches of 3.0 black silk suture. After complete recovery, each animal was kept in its cage with free access to water and fed with soft diet (moistened bread) for 2 days then returned to its normal food before operation, and no antibiotic was given. The animals were daily inspected for clinical signs of complications or adverse reactions. Two post-operative

intra-oral periapical radiographs were taken for each tooth socket. The first was taken immediately after extraction and the second at the end of each study interval (i.e. after 2 weeks, 4 weeks, 8 weeks, and 12 weeks period for group I, II, III, and IV respectively). The X-ray scanner (Super CAM, China) was used to convert the conventional radiograph to digital format, then examined by ImageJ software (ImageJ 1.32j, USA).

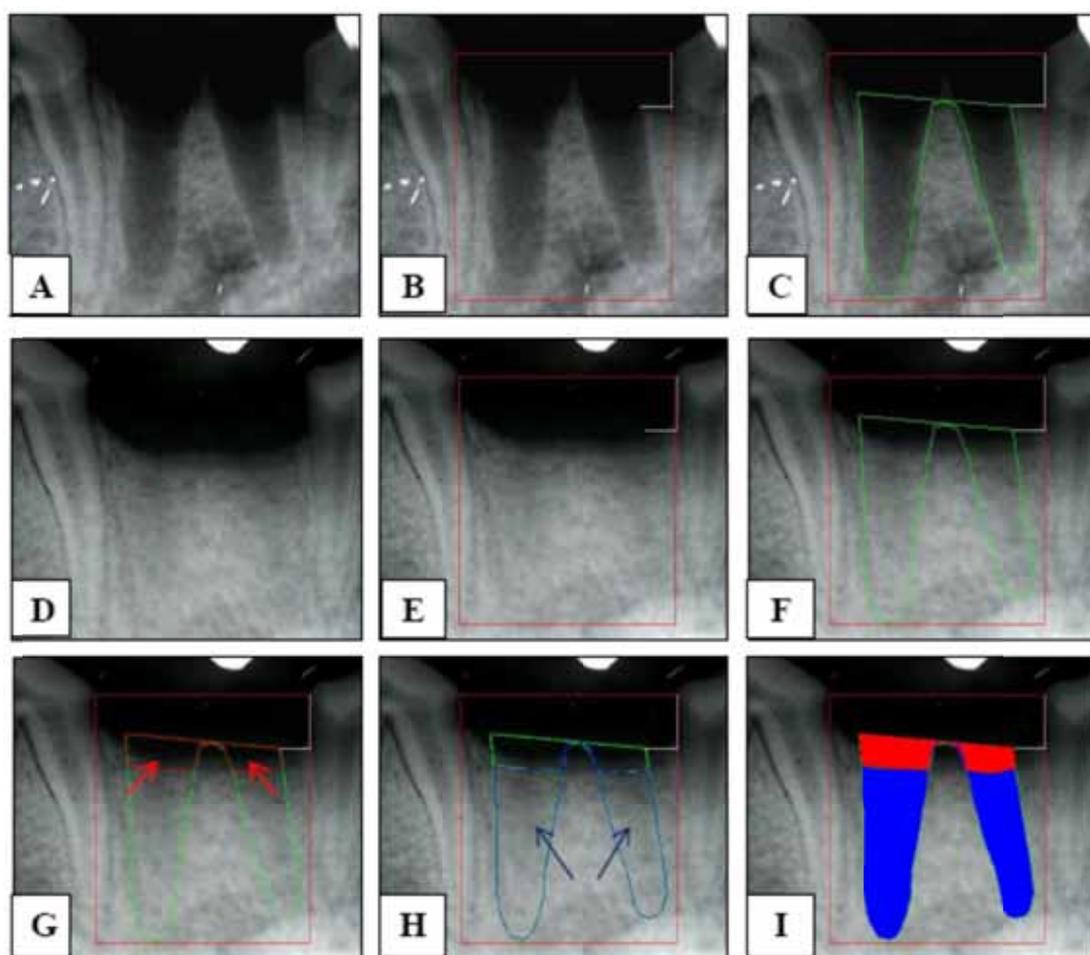


Plate 1: (A) Scale of immediate post extraction intra-oral periapical radiograph (first radiograph) of a dog lower right third premolar socket. (B) Scale of first radiograph, the red square shape delineates the general outline of the area enclosed between the second and forth premolars. Vertical and horizontal white lines represent a guide for drawing the tooth socket. (C) The total tooth socket was outlined starting from the end point of the horizontal line extending along the periphery of the socket to finish at the same point (green color). (D) Scale of delayed post-extraction radiograph (second radiograph) of the same socket taken at the end of each time interval. (E) The red square, vertical and horizontal white lines were drawn according to the dimensions of the first radiograph. (F) A copy of the delineation of the total tooth socket was taken from the first radiograph and pasted on its exact position in the second radiograph. (G) Second radiograph, bone resorption area (red arrows) was demarcated between the highest edge of the newly formed alveolar bone crest and the superior outline of the tooth socket. (H) Bone formation area (blue arrows) sketched on the second radiograph. (I) Second radiograph, the total tooth socket was sketched with red (bone resorption area) and blue (bone formation area) colors.

For standardization purposes, the first radiograph of each tooth socket was scaled in the following manner: it was adjusted to a certain dimension (Plate 1-A), then a red square shape (having definite height and width for each socket) was drawn to delineate the general outline of the area enclosed between the second and fourth premolars. Vertical and horizontal white lines (with a distinct length for each socket) were drawn inside the red square (the vertical line from the right top corner of the red square to the highest point of the alveolar bone crest, the horizontal line drawn from the lowest point of the vertical line to the highest point of the extraction socket) to create a guide for drawing the tooth socket (Plate 1-B). The total tooth socket was outlined starting from the end point of the horizontal line, extending along the periphery of the socket to finish at the same point (green color) as shown in Plate (1-C). In regard to the second radiograph of each tooth socket, it was scaled depending on that of the first radiograph (Plate 1- D & E), then a copy of the delineation of the total tooth socket was taken and pasted on its exact position on the second radiograph (Plate 1-F). Bone resorption area was demarcated between the highest edge of the alveolar bone crest and the superior part of the tooth socket outline using a red line (Plate 1-G, 2-I). The bone formation area (Plate 1-H, 1-I) was delineated by a blue line drawn on the remaining part of the tooth socket outline on the second radiograph.

Pixel was considered as a unit for measuring areas of total teeth sockets, bone resorption areas, and bone formation areas. Gray scale differences among extraction sockets (Region of Interest, ROI) were considered as a value of radiographical density analysis depending on the color of each pixel. Gray scale uses a 256 gray tone scale where zero indicates the most black color and 255 the whitest one (17,27).

Regarding to radiographical evaluation, each extraction socket was evaluated separately and data were recorded. For both first (taken immediately after extraction) and second (taken at the end of each study interval) radiographs, mean density value of each extraction socket was registered in pixels, then the difference in densities between first and second radiographs for each socket in the left and right sides were calculated in the following manner:

Difference in densities in the left side = Density of left extraction socket at the end of each study period – Density of left extraction socket immediately after extraction.

Difference in densities in the right side = Density of right extraction socket at the end of each study period – Density of right extraction socket immediately after extraction.

Bone resorption %, bone formation %, and mean density value of newly formed bone were calculated on

each second radiograph. Density of the newly formed bone at the end of each study period was calculated by measuring the density of the area (determined by gray tones) enclosed by the blue line on each second radiograph.

Data were expressed as mean \pm SD. At first the data were tested for normal distribution using Normality test. Normally distributed data were compared by ANOVA (Two-Way Analysis of Variance). Significant differences were determined by Duncan's Multiple Range Test. All statistical analyses were performed by Sigma Stat (Jandel scientific software V3.1). $P < 0.05$ was considered as statistically significant. Levels of significance in the tables were indicated as follows: * = $P < 0.05$, ** = $P < 0.01$ and *** = $P < 0.001$.

Results

In this study no animal died throughout all periods, healing following extractions progressed. In spite of difficulty in all animals, the extraction sockets healed with no infections or bone exposures in any of the surgical sites. Results of each radiographic analysis variable (differences in extraction sockets densities, bone resorption and bone formation percentage, and density of the newly formed bone) will be listed as follows:

Results related to the differences in the extraction sockets densities (Table 2, Figure 2, Plate 2): The mean and standard deviation values concerning differences in extraction sockets densities in the right and left sides at different time intervals were Summarized in table (2). In the left side, Two-way ANOVA revealed no significant difference between groups I, II, and III, and between groups II, III, and IV. However, a highly significant statistic difference was clear between groups I and IV.

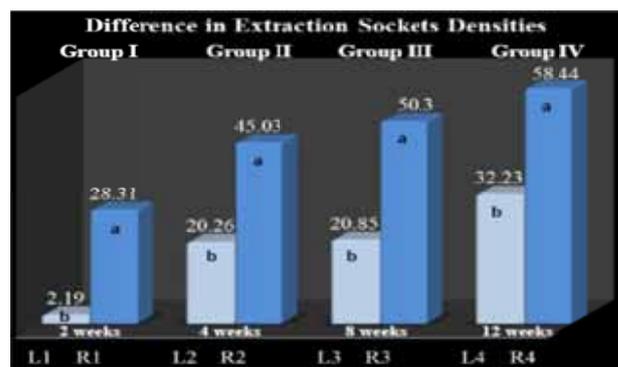


Figure 2: Means of differences in extraction sockets densities. (L= Left side R= Right side).

As for the right (experimental) side, a highly significant difference ($P < 0.001$) was noticed between group I and IV, but there was no significant difference between groups I

and II on one side, and between groups II, III, and IV on the other. Concerning the comparison between the right and left

sides, high significant differences were observed in all study intervals.

Table 2: Radiographic analysis (by Image-J Program) related to differences in extraction sockets densities (Mean ± SD)

Tissue Response Difference in Densities	Group Number and Period			
	Group I (2 weeks)	Group II (4 weeks)	Group III (8 weeks)	Group IV (12 Weeks)
Left side	2.1± 3.67 Bb	20.2±7.49 ABb	20.86±16.9 ABb	32.23±22.31 Ab
Right side	28.32±12.99 Ba	45.04±11.04 ABa	50.3±10.56 Aa	58.44±20.1 Aa

Capital letters mean significant difference between periods (horizontally). Small letters mean significant difference between left and right sides (Vertically). Level of significance horizontally and vertically: *** = P<0.001.

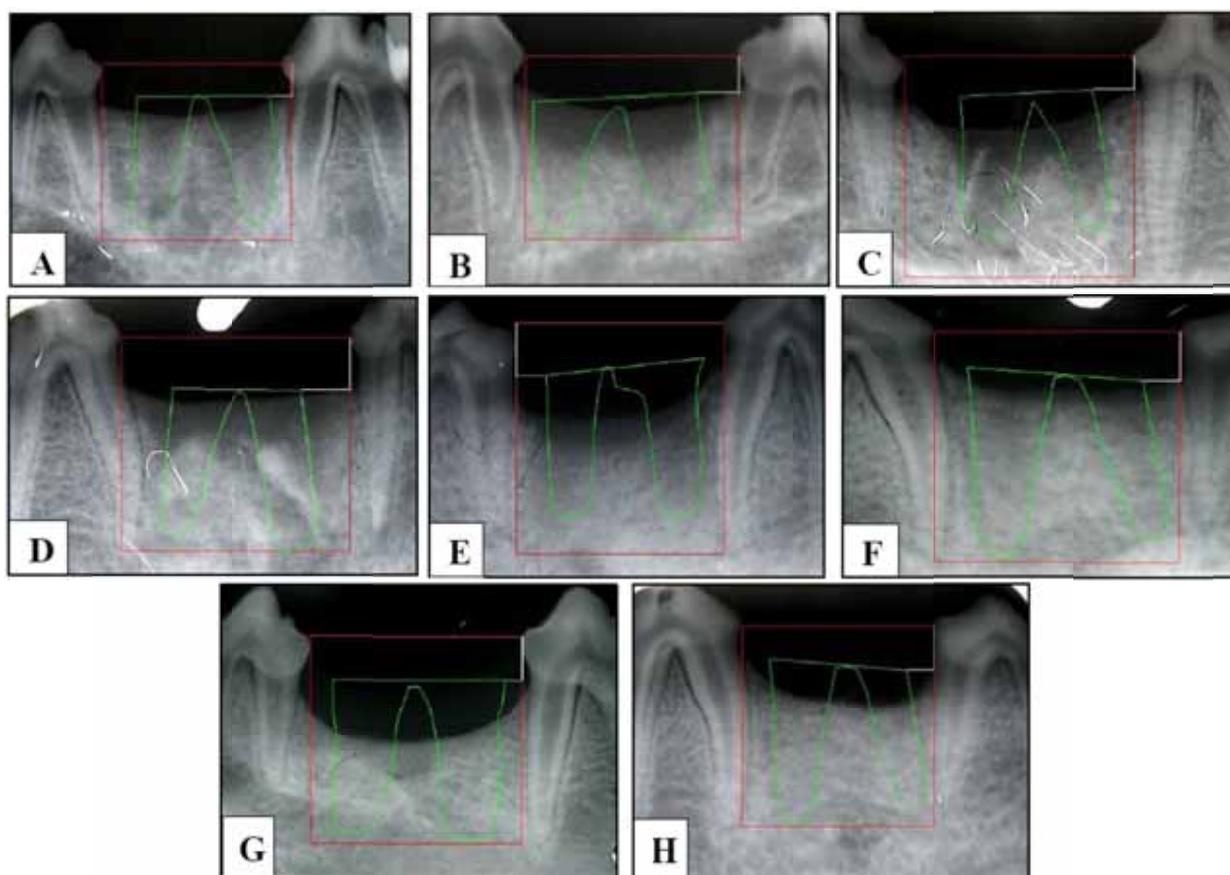


Plate 2: Differences in Extraction Sockets Densities: (A, C, E, G)= Lower left third premolar socket after 2, 4, 8, and 12 weeks respectively. (B, D, F, H)= Lower right third premolar socket after 2, 4, 8, and 12 weeks respectively.

Results of bone resorption % in extraction sockets (Table 3, Figure 3, Plate 3): Regarding the left side, significant difference was apparent between groups I and II in relation to groups III and IV. But no significant difference was noticed between group I, II, and between groups III, IV. Concerning the right side, no significant

difference between the study groups was noticed. Comparison between the right and left sides showed a significant difference only in group IV (12 weeks period).

Results associated with bone formation % in extraction sockets (Table 4, Figure 4, Plate 3): About the left side, significant differences were obvious between groups I and

II on one side, and groups III and IV on the other. However, no significant difference was noticed between groups I, II, and between group III, IV. However, no significant difference presented among the study groups in the right side. Significant difference was noticed only in group IV between the right and left sides.

Results related to the density of the newly formed bone (Table 5, Figure 5): No significant difference was observed between study groups in the left side, also no significant difference noticed between groups I, II, III and between groups III and IV in the right side. Conversely, highly significant differences were documented between groups I and II in one side and group IV on the other. In respect to comparison between right and left sides, significant differences were detected in groups III and IV.

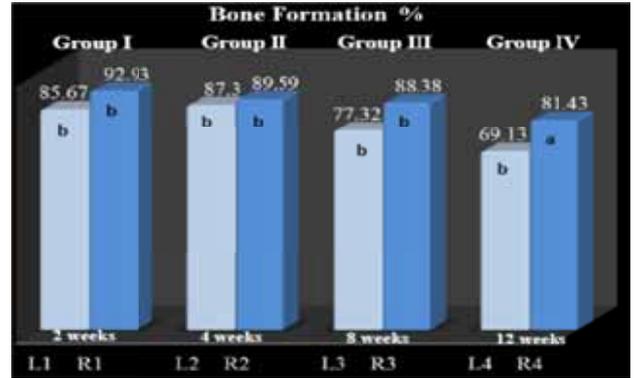


Figure 4: Means of bone formation percentage. (L= Left side R= Right side).

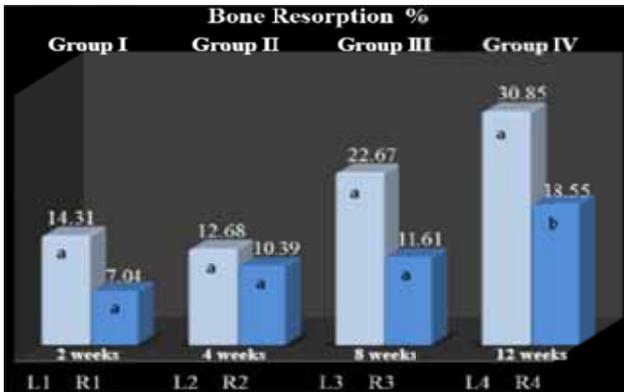


Figure 3: Means of bone resorption percentage. (L= Left side R= Right side).

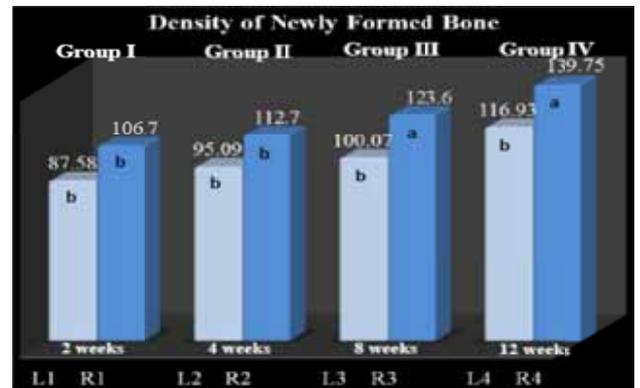


Figure 5: Means of density of newly formed bone. (L= Left side R= Right side).

Table 3: Radiographic analysis (by Image-J Program) related to bone resorption percentage in extraction sockets (Mean ± SD)

Tissue Response	Group Number and Period			
	Group I (2 weeks)	Group II (4 weeks)	Group III (8 weeks)	Group IV (12 Weeks)
Bone Resorption Percentage				
Left side	14.31±2.45 Ba	12.68±4.95 Ba	22.67±12.75 ABa	30.85±10.33 Aa
Right side	7.04±7.71 Aa	10.39±6.03 Aa	11.61±5.66 Aa	18.55±13.27 Ab

Capital letters mean significant difference between periods (horizontally). Small letters mean significant difference between left and right sides (Vertically). Level of significance horizontally and vertically: ** = P<0.01.

Table 4: Radiographic analysis (by Image-J Program) related to bone formation percentage in extraction sockets (Mean ± SD)

Tissue Response	Group Number and Period			
	Group I (2 weeks)	Group II (4 weeks)	Group III (8 weeks)	Group IV (12 Weeks)
Bone Formation Percentage				
Left side	85.67±2.44 Bb	87.31±4.96 Bb	77.32±12.75 ABb	69.14±10.33 Ab
Right side	92.93±7.71 Ab	89.59±6.03 Ab	88.38±5.66 Ab	81.43±13.27 Aa

Capital letters mean significant difference between periods (horizontally). Small letters mean significant difference between left and right sides (Vertically). Level of significance horizontally and vertically: ** = P<0.01.

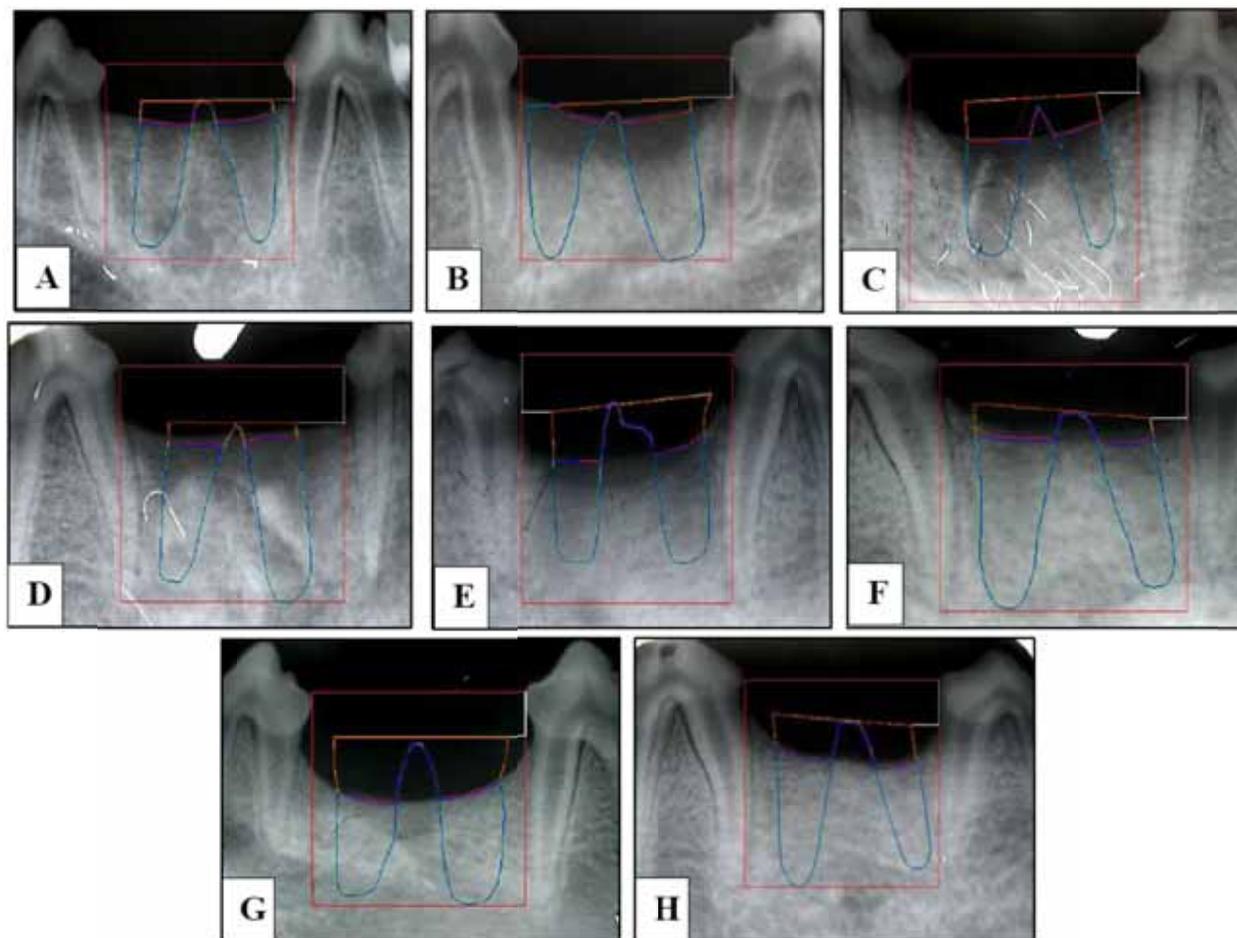


Plate 3: Bone resorption and bone formation in extraction sockets: (A, C, E, G)= Bone resorption (red color) and bone formation (blue color) outlined in the lower left third premolar socket after 2, 4, 8, and 12 weeks respectively. (B, D, F, H)= Bone resorption (red color) and bone formation (blue color) outlined in the lower right third premolar socket after 2, 4, 8, and 12 weeks respectively.

Table 5: Radiographic analysis (by Image-J Program) related to density of newly formed bone in extraction sockets (Mean \pm SD)

Tissue Response Density of Newly Formed Bone	Group Number and Period			
	Group I (2 weeks)	Group II (4 weeks)	Group III (8 weeks)	Group IV (12 Weeks)
Left side	87.58 \pm 15.66 Ab	95.09 \pm 16.45 Ab	100.07 \pm 11.38 Ab	116.93 \pm 24.97 Ab
Right side	106.78 \pm 15.18 Bb	112.72 \pm 11.26 Bb	123.66 \pm 13.66 ABa	139.75 \pm 11.52 Aa

Capital letters mean significant difference between periods (horizontally). Small letters mean significant difference between left and right sides (Vertically). Level of significance horizontally and vertically: *** = P<0.001.

Discussion

The dog is one of the more frequently used large animal species for musculoskeletal and dental research. Unlike

other animal species, there is a considerable amount of literature comparing canine and human bones with regard to the usefulness of the dog as a model for human orthopedic conditions. Most of the literature reported that

the dog is more suitable as a model for human bone from a biological standpoint. In terms of mineral composition, humans and dogs do not show significant differences (28-30). In the present study, the extraction sockets in dog mandible were the regions of interest (ROI) for demonstrating the response of bone tissue to an implant material. Moreover, dog was chosen due to the anatomical and histological resemblances to those of humans. Ease of access, simplicity of procedure, less traumatic placement, the healing properties and size similarities with humans are additional factors making this model appropriate for such study as proof of efficacy or safety of the material prior to registration or human clinical trials (16,31).

ImageJ program was used in the present study for radiologic assessment since it is an essential tool that fulfills most of routine image processing and analysis requirements. Another strength is the large number of automated image segmentation algorithms, again allowing the user to choose the most appropriate one, which is considered a significant advantage (32). ImageJ calculates area as a number of pixels, also it measures density in pixels depending on the gray scale difference in pixel value statistics for user-defined selections (33).

The early resorption of calcium sulphate as indicated by many previous researches (7,34-37) leaves calcium phosphate lattice in the area; the presence of high concentrations of calcium ions gives more radio-opacity compared to the control side, and it encourages the subsequent ingress of osteoprogenitor cells leading to early new bone formation; that's why a highly significant differences were observed between the right (experimental) and left (control) sides in relation to differences in extraction sockets densities (Table 2, Figure 2). This was in line with other previous confirmations (38-40). Many dental and orthopedic literatures concluded that resorption of calcium sulphate is rapid and complete when compared with other implantable regenerative materials such as hydroxyapatite. It seems to be completely resorbed in 4-10 weeks depending on the vascularity of the grafted site, ingress of osteoprogenitor cells and life span of the model (6,38,40-43). It was found that the presence of calcium sulphate (and the subsequent release of high concentrations of calcium ions) in the implantation site is associated with increased concentrations of bone morpho-genetic protein (BMP)-2, BMP-7, transforming growth factor- β (TGF- β), and platelet-derived growth factor (PDGF), all of which play a role in bone regeneration. In the aggregate, these results suggest that this material does not act simply as a bio-inert filler, but it may play a more active role in osteogenesis (44).

Animal studies have shown that the dissolution of calcium sulphate hemihydrate was accompanied by formation of resorption pits due to the attachment of osteoclasts (bone resorbing cells) to calcium sulphate

hemihydrate as they possess a calcium sensing receptor (CaSR), which may regulate their activity based on local calcium concentration, and subsequently lead to resorption of the material but not the surrounding bone (4,45), and this gives us a reasonable explanation for the less bone resorption in the experimental side compared to the control one in this study (Table 3, Figure 3). Meanwhile this resorption is principal to the precipitation of a calcium phosphate (CAP) lattice around the resorbed particles, to which osteoblasts (bone forming cells) attach resulting in new bone formation. This could interpret the greater bone formation percentage (Table 4, Figure 4) and the highest density of newly formed bone in this study at the experimental side (Table 5, Figure 5). Moreover, calcium ions released during dissolution of calcium sulphate will lead to local increases in calcium ion concentration, which may stimulate osteoblast proliferation and differentiation from undifferentiated mesenchymal present in the area due to the release of growth factors resulting in modulation of osteoid synthesis by a process of creeping substitution (46,47). The present study concluded that the use of β -calcium sulphate hemihydrate (CSH) prepared from Iraqi gypsum rocks as a bone substitute significantly reduced bone resorption and increased the rate of new bone formation. In addition, the density of the newly formed bone in the experimental side was greater than that noticed in the control side.

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