The Histopathological Effect of Local Injection of Fucose on Bone Fracture :An Experimental Study on Rabbits

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ABSTRACT

Aim: To evaluate the effect of sulcular injection of 50µl of 150mM fucose on rabbits with bone trauma.

Background: α-L-Fucose is a methyl pentose sugar similar to L-galactose except for the loss of alcohol group on carbon number 6.It is found in human serum bound to proteins by covalent bonding and it is a member of a large group of compounds known as covalent bond membranes .

Materials and Methods: The present study was carried out on 65 male rabbits, bone trauma was introduced into the mid- labial areas of facial plate of the alveolar bone of lower right central incisor for all rabbits . The rabbits had been left for 7 days after induction of bone trauma, then 5 rabbits were sacrificed and considered as a base line traumatic group . The remaining 60 rabbits were divided randomly into two 2 main groups, first group consist of 30 rabbits and were injected locally with 50µl of 150mM fucose solution into the bottom of gingival sulcus at the mid-labial area of lower right central incisor and were considered as fucose injected group and were divided randomly into six subgroups(each subgroup consist of 5 rabbits) , then periodontal tissue biopsy was collected from injected group at time intervals of 1, 3 ,7 ,14, , 21 , and 28 days after fucose injection , while the second group consist of 30 rabbits with bone trauma only and were not received any fucose injection and considered as non injected group and were divided randomly into six subgroups (each subgroup consist of 5 rabbits), sacrificed at time intervals of 8,10,14,21,28 ,and 35 days after induction of bone fracture.

Results: The results showed that fucose injection enhanced bone regeneration and healing of traumatic bone in short duration of time, 1 day after sulucular injection of fucose (8 days after induction of bone fracture).

Conclusion: α-L- Fucose can be used as a bone stimulating agent which stimulate and accelerate new bone formation, so fucose can be used in the treatment of alveolar bone defect in periodontitis patient

KEY WORDS:

α-L-Fucose, periodontal biopsy , sulcular injection .

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التأثيرات النسيجية ألمرضية للحقن ألموضعي للفيوكوز على تكسر العظم
دراسة تجريبية على الأرانب

المستخلص

بعد تكسير العظم الخارجي العائد للسن الامامي الاول للفك السفلي من جهة اليمين لجميع الارانب البالغ عمرها ٣٠ يوما وتركها لمدة ٧ أيام، تم حقن جيب اللثة موضعيا لنفس المكان بمادة السكر الاحادي (الفيوكوز).

تم ذبح الارانب حسب الطريقة الاسلامية للأيام التالية: ١،٢، ٣، ٧، ١٤، ٢١ يوما بعد حقن الفيوكوز، ثم أخذت الانسجة العائدة للفك السفلي من جهة اليمين وبعد فحصها مجهريا وجدنا ان مادة الفيوكوز تساعد على التئام تكسر العظام ويساعد على سهولة تكون العظم الجديد في مكان تهشم العظام في فترة قصيرة جدا تبدأ من ٨ أيام بعد عملية تكسير العظم (يوم واحد بعد حقن الفيوكوز) وتستمر في نمو العظم الجديد الى ان يكتمل نمو العظم كاملا بعد ٢١ يوما مما يثبت ان هذه المادة لها تأثير على تحفيز الخلايا المكونة للعظم.

نوصي بإمكانية استخدام مادة الفيوكوز في اماكن التشوهات العظمية في امراض الانسجة ما حول الاسنان المتقدمة (Periodontitis). يحتاج هذا البحث الى بحوث اخرى لدعمه وتأكيد النتائج التي توصل اليها الباحثون في هذه الدراسة لبحث امكانية تطبيقها على البشر.

INTRODUCTION

α-L-Fucose is a methyl pentose sugar similar to L-galactose except for the loss of alcohol group on carbon number six (C6), with a general formula $C_6H_{12}O_5$ and a molecular weight of 164.16 g⁄mol[1]. Fucose is a common component of many N-and O-linked glycans of glycoproteins and glycolipids produced by mammalian cells[2,3] . Two structural features distinguish fucose from other six-carbon sugars present in mammals, these include the lack of a hydroxyl group on the carbon at the 6-position and exist in L-configuration in contrast to the rest of sugars[4,5]. Fucose is naturally found in D- and L-forms.The L-form is the only common form of the sugar , while the D-form is a synthetic galactose analogue[6] .The L-form is found in mammalian tissues and fluids, while D- form is identified in plants. Purified L-Fucose is white powder and it melts at 153-155 ċ° , and L-fucose exist in two different

forms α-L-fucose (29.5%)and ß-L-fucose (70.5%) [7]. Fucose is a powerful immune modulator .It is distributed in macrophages, playing a vital role in immune function. Studies showed the importance of serum, saliva and gingival fluid fucose and its related parameters in the detection of oral disease, such as; gingivitis, periodontitis and oral cancers [8,9,10,11,12]. Researchers have shown that fucose has an important effect on inhibiting and reversing disease processes like inflammation, immunity, leukemia ,and breast cancer, including the suppression of tumors[13,14,15]. Researchers revealed that α-L-Fucose could be used as therapeutic agent for many diseases, throughout oral administration or intravenous injection(16.A study showed that fucose local injection into rabbit tongue muscle caused a reduction in the inflammatory process,168 hours after injection, accompanied by regeneration in both oral mucosa and muscle layer[17]. Another study reported that sulcular injection of α-L-Fucose had no harmful effect on the injected tissue and resulted in reduction of inflammatory reaction gradually, fibrous tissue regeneration, ,and healing of damaged gingival tissue in short duration of time in 3 days after injection [18] .

Methods

This study was conducted from 18 Th June 2012 to 10th June 2013. in Hawler Medical University, College of Dentistry, Department of Basic Science, and Clinical Biochemistry Laboratory, Baghdad University, College of Dentistry, Department of oral Diagnosis and Histology Laboratory. The study was carried out on (65) male rabbits of the same species and nearly the same age (10-12 months), with a weight range of (1-1.5 kg). These rabbits were allowed to acclimate at least 7 days prior to the experiment in well arrayed room to ensure the same type of food to be taken by these rabbits and the same condition of temperature (25-30 ˚c). All rabbits had bone trauma in the mid labial area of alveolar bone of the lower right central incisor and this was obtained by, firstly all the rabbits were weight , anesthetized with subcutaneous injection of xylazin (4 mg / kg) and ketamine (40mg/ kg) cited by[19,20], secondly a reamer (size 45)was inserted from beneath gingival margin into mid- buccal area of alveolar bone of lower right central incisor, then by making pushing and pulling action movement for 15 seconds to induce bone trauma , then K-File (size 45) was inserted to the same location and the movement action is the same as that was performed by a reamer and for the same period of times (15 seconds) until a alveolar bone fracture was achieved . All the rabbits had been left for 7 days , then 5 rabbits were sacrificed at 7 days after induction of bone fracture and considered as a base line traumatic group, and the remaining 60 rabbits were divided randomly into two main groups, first group was called fucose injected group which consisted of 30 rabbits and had been subdivided randomly into 6 subgroups (A1,A2,A3,A4 ,A5,and A6) ,each subgroup included five rabbits that received sulcular injection of a single dose of 50µl / kg of 150mM fucose into the mid –labial of the gingival sulcus of the lower right central incisor. Then periodontal tissue samples were collected after a specific time intervals of 1,3,7,14 ,21 and 28 days after fucose injection which mean at time intervals of 8,10,14,21,28,and 35 after induction of bone fracture. The second group called non injected group, consisted of 30 rabbits with bone trauma only and were not received any fucose injection , this group also subdivided randomly into 6 subgroups (B1,B2,B3,B4,B5,and B6) each subgroup consist of 5 rabbits, and periodontal tissue samples were taken after a specific time intervals of 8,10,14 ,21 ,28 and 35days after induction of bone fracture.

1.Fucose solution preparation.

- 1.2312 g of α- L-fucose was dissolved in normal saline (0.9% Na Cl), and completed into 50ml by normal saline (150mM fucose).
- 1.2312 g of of α- L-fucose was obtained from the following equation:
- weight of fucose(g)= mollarity (mol/L) × molecular weight×volume1(ml)/l000
- = 0.15(mol/L) ×164-.16 ×50/1000

2.Sulcular injection technique and administration of L-fucose.

In this technique the needle was inserted from the gingival margin approximately 5mm into the sulcular tissue at the bottom of the gingival sulcus of the lower right central incisor(the needle was painted at the level of 5mm distance from the tip and then stoper was placed over the painted area) 18.

3. preparation of histological specimens (Periodontal tissue sampling).

The head was separated from the rest of body , then the mandible was separated from the skull , muscles and the soft tissue covering the mandible were removed using a surgical blade ,then the anterior region of the lower jaws was excised and cut off with saw and prepared for histopathological examination[18,20] .

The samples were placed in recipients containing 10% formalin for 5 days, decalcified in 10% formic acid for 7-10 days, washed in running

water and dehydrated in a series of alcohol solution for ascending concentration of 40% to 100%,clearing with xylene , embedded in paraffin wax in a position that permit saggittal sections, from each specimen serial saggittal sections were cutted with microtome device at 5 microns thickness and then mounted on microscopical slides. 4 sections were selected from each sample and separated from one another by at least 15 microns. For staining the longitudinal paraffin sections are de-waxed with xylene from 20-30 minutes and dehydrated in descending grades of alcohol concentration, stained with Harris hematoxyline for 7-10 minutes, washed in tap water, stained with eosin for 1-2 minutes, dehydrated in absolute alcohol for 2-3min.and clear with xylene, then cover slips are fixed on the stained sections using the Canada balsam.

4. Histomorphometry, including counting of osteoblast cells at three different areas (coronal / crestal , middle, and apical/ cervical) of alveolar bone at 8,10,14,21,28, and 35 days after induction of bone fracture for non injected subgroups , and at 1,3,7,14,21, and 28 days after fucose injection for fucose injected subgroups, and at 7 days after induction of bone fracture for base line traumatic group. Counting was done by using a digital light microscope (MOTIC) and its software program under windows operating system, the counting was done by using a grid containing 30 squares, then the mean values of three location were calculated. The width of alveolar bone also had been measured at three location (crestal , middle, and cervical) of alveolar bone under (X100) median power field, then the mean value of three location were calculated.

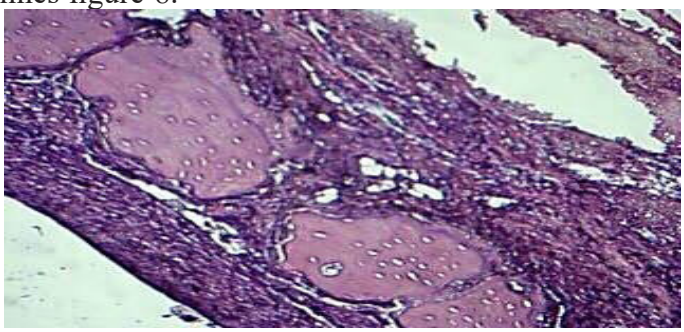
Statistical analysis

All data were expressed using descriptive statistic as mean ± standard deviation SD , and inferential statistics which include Paired T -test , statistical analysis were carried out by using statistical software (SPSS version 22),the results were considered significant if P value ≤0.05.

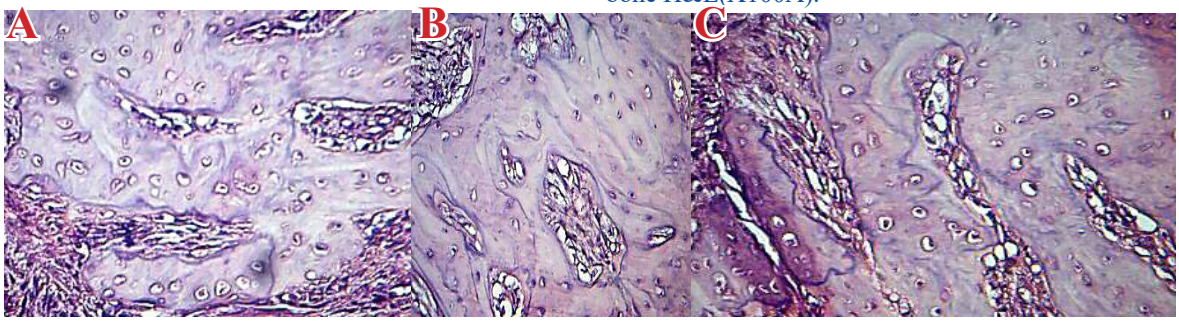
RESULTS

Histopathological results

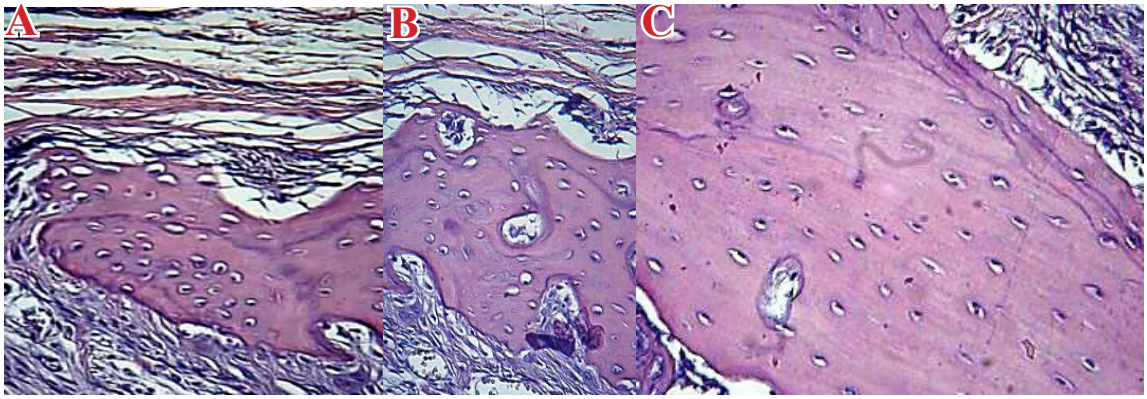
Figure 1 showed obvious bone destruction which appear as bone fragments in rabbits, 7 days after inducing of bone fracture. Little bone healing and new bone formation (bone regeneration) appeared, 1 day after fucose solution injection in fucose injected subgroup A1 which represented by presence of reversal lines and osteoblast cells at the periphery of bone (figure2) , then after 3,7,14 days of fucose solution injection more bone formation can be detected (more bone regeneration) which can be detected by the presence of more osteoblast cells at the periphery of bone, more osteocyte cells within their lacunae and presence of more reversal line (figure 3,4,5), and after 21 days of fucose injection the formation of new bone continued especially in the upper most layers toward coronal part of the tooth which filled the defect area that was previously induced by the experiment with presence of more osteoblast cells , reversal lines and more new blood vessels within the bone (figure6),and after 28 days of fucose injection more bone formation could be seen in the crestal, middle and apical part of alveolar bone with presence of more osteoblast cells ,more osteocytes, very prominent reversal lines, and with presence of fibrous tissue surrounded the area (figure 7). While in non-injected subgroups ; B1,B2,B3,B4, and B5 , the bone trauma remained without healing and new bone formation at 8, 10,14, 21,28 days after induction of bone fracture, until little bone healing and new bone formation appeared in B6 subgroup at 35 days after induction of bone fracture which characterized by presence of more reversal lines figure 8.



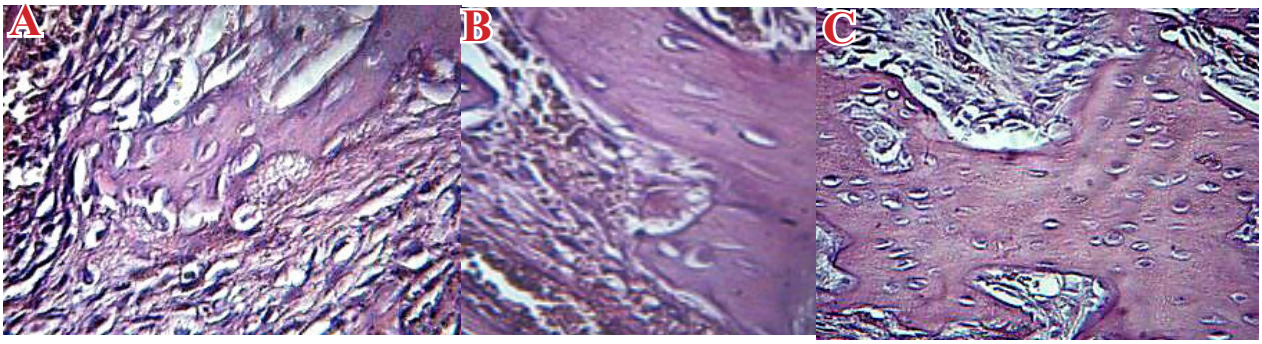
Figure(1). Prominent bone fractures , 7 days after induction of bone H&E(X100A).



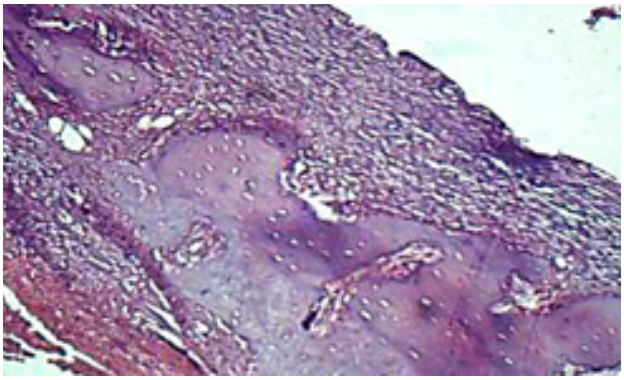
Figure(2): Histological features of rabbit bone , 1day after sulcular injection of fucose which show little bone healing in A- crestal portion, B-middle portion, and C-Apical potion of alveolar bone H& E (X200 A, X100B, X10 0C).



Figure(3): Histological features of rabbit bone ,3days fucose solution injection which show more bone healing (remodeling bone to restore the shape of crest of alveolar bone) and more new bone formation in A-crestal portion, B- middle portion, and C-apical portion of alveolar boneH&E (X200A,X200B,andX200B).



Figure(4): Histological features of rabbit bone,7days after sulcular injection of fucose solution which show A-new bone formation(osteoid tissue) B-presence of osteoclast cell C- presence of more osteoblasts cells at the periphery of the bone H&E(X200A,X200 B , and X200C).



Figure(5): Histological features of rabbit bone , 14 days after fucose solution injection which show a well alveolar bone formation surrounded by fibrous tissue(collagen fibers and fibroblast cells) H&E(X200).

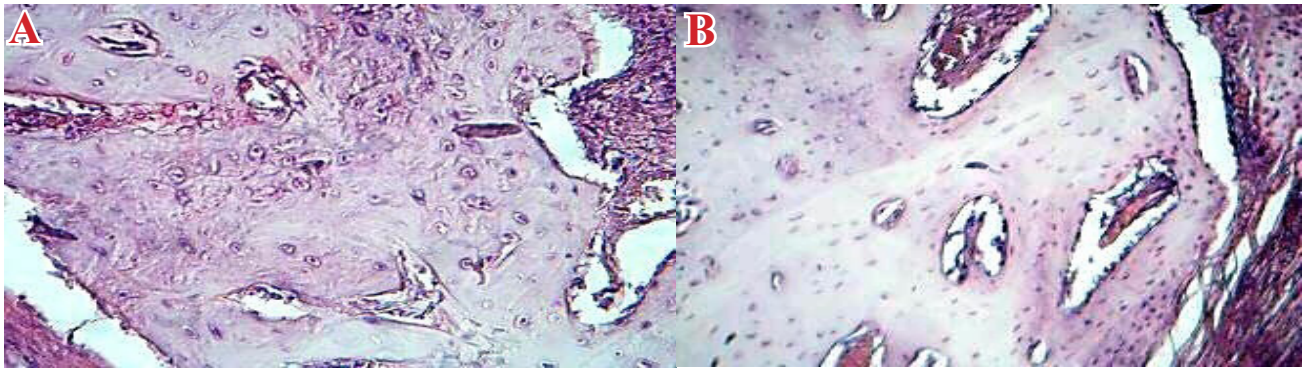
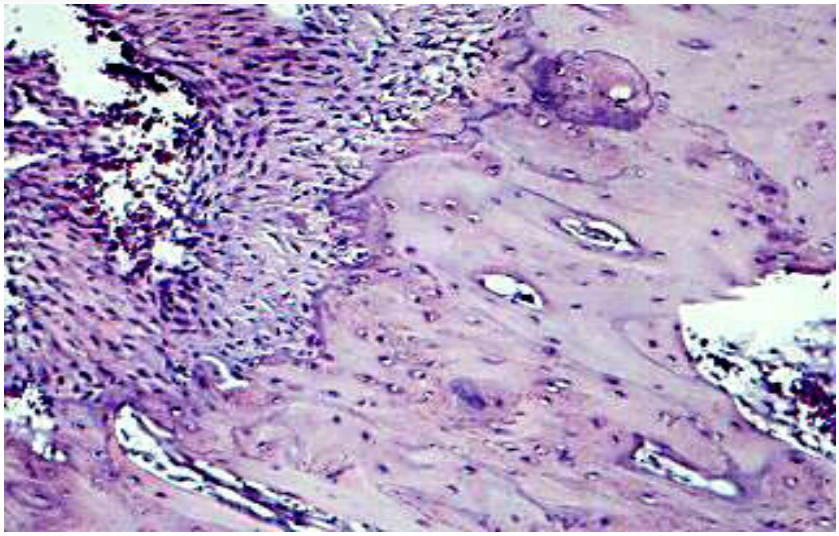
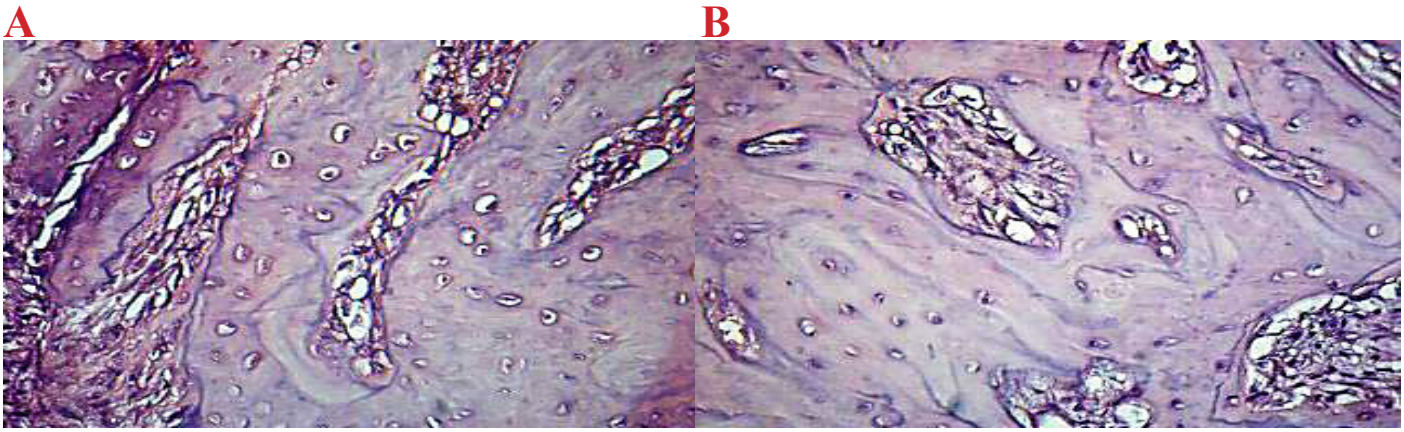


Figure (6): Histological features of rabbit bone ,21days after fucose solution injection which show A- formation of more bone (more osteoblast cells at the periphery of the bone , osteocytes within lacunae, and blood vessels within bone) B-more blood vessels within the bone H&E (X200 A and X100 B).



Figure(7): Histological features of rabbit bone , 28 days after sulcular injection fucose solution which show more bone regeneration with fibrous tissue surround bone H&E (X100).



Figure(8). Histological features of rabbit bone, 35 days after induction of bone fracture which show little bone healing in non injected sub group (B6) at A-crestal , B- middle, and apical third of alveolar bone H&E(X200A and X 100 B).

2-Histomorphometrical results.

Table 1 , shows that before fucose injection in base line traumatic group, and after 7 days of induction of bone fracture, the mean value of osteoblast cells at three different areas of alveolar bone was (6.2 ± 3.1) , then the mean values of osteoblasts 1 day after fucose injection increased (18.8±5.6), and this increase continued after 3 days (41.4±8.7) ,7days (72.6±5.5), 14 days (93.6±5.5), 21days (158.8 ± 19.9), reaching a peak in 28days (182.8±28.1),the differences between base line traumatic group and fucose injected subgroups A1, A2, A3, A4, A5,and A6 were statistically significant (P≤0.05) .Table 2 shows that there was no significant difference in the mean value of osteoblast cells at three different areas of alveolar bone between base line traumatic group and non injected sub groups B1, B2 ,B3, B4, B5 and B6 at 8, 10, 14, 21 , 28, and 35 days after induction of bone fracture(P>0.05).

Table 3, shows that the mean value of thickness of alveolar bone in base line traumatic group before fucose injection at three different areas was (181.406±30.854). This value increased 1day after fucose injection (308.365±9.396), and this increase continued after 3days (535.52±27.089) ,7days (665.69±34.99),14days (864.974±5.341), 21days (916.783 ± 20.148), reaching a peak in 28 days after fucose injection (948.177±9.479), the differences between base line traumatic group and fucose injected subgroups A1, A2, A3, A4, A5, and A6 were statistically significant (P≤0.05). Table 4 shows that there was no significant differences in the mean value of thickness of alveolar bone between base line traumatic group and non injected sub groups B1, B2, B3, B4, and B5(P>0.05) , then significant differences had been found between base line traumatic group and non injected subgroup B6 after 35 days of induction of bone fracture (P≤0.05).

Table1.shows the mean ± SD of osteoblast cells at three locations of alveolar bone in base line traumatic group and in fucose injected subgroups at 1,3,7,14,21, and 28 days after fucose injection (P≤0.05= Significant) .

Rabbit groups		N.off rabbits	Time intervals /days	mean ± SD	P-value
Base line		5		6.2 ± 3.11	
Fucose injected	A1	5	1 day	8.8±5.6	.000
	A2	5	3 day	41.4±8.7	.000
	A3	5	7 day	72.6±5.5	.000
	A4	5	14 day	93.6±5.5	.000
	A5	5	21 day	158.8 ± 19.9	.000
	A6	5	28 day	182.8±28.1	.000

Table2.shows the mean ± SD of osteoblast cells at three locations of alveolar bone in base line traumatic group and in non injected subgroups at 8,10, ,14,21,28 and 35 days after induction of bone fracture (P≤0.05= Significant) .

Rabbit groups		N.of rabbits	Time-intervals /days	mean ± SD	P-value
Base line		5		6.2 ± 3.1	
Non-injected	B1	5	8 day	6.2 ±3.0	1.000
	B2	5	10 day	6.2 ±2.9	1.000
	B3	5	14 day	6.2 ±3.1	.147
	B4	5	21 day	6.4 ±3.1	.110
	B5	5	2 8day	6.4 ±3.1	.847
	B6	5	35 day	27.4 ±4.3	.475

Table3.shows the mean± SD of thickness of alveolar bone at three locations of alveolar bone in base line traumatic group and in fucose injected subgroups at 1,3,7,14,21, and 28days after fucose injection (P≤0.05= Significant). .

Rabbit group		N.of rabbits	Time intervals / days	mean ± SD	P-value
Base line		5		181.4 ± 30.8	
Fucose injected	A1	5	1 day	308.3±9.3	.002
	A2	5	3 day	535.5±27. 0	.000
	A3	5	7 day	665.6±34.9	.000
	A4	5	14 day	665.6± 34. 9	.000
	A5	5	21 day	916.7±20.1	.000
	A6	5	28 day	948.1±5.4	.000

Table4.shows the mean ± SD of thickness of alveolar bone at three locations of alveolar bone in base line traumatic group and in non-injected subgroups at 8,10,14,,21, 28 and 35days after induction of bone fracture(P≤0.05= Significant).

Rabbit groups		N.of rabbits	Time intervals /days	mean ± SD	P-value
Base line		5		181.4 ± 30.8	
Non-injected	B1	5	8day	180.4 ±26.8	178
	B2	5	10day	183.6 ±4.3	.878
	B3	5	14 day	181.5 ± 15. 9	.989
	B4	5	21 day	181.4 ±24.4	.995
	B5	5	28 day	182.4 ±19.1	.877
	B6	5	35 day	291.3 ±7.4	.001

DISCUSSION

To the best of our Knowledge no previous study was done concerning the effect of sulcular injection of fucose solution on alveolar bone fracture. The effect of local injection of fucose solution on bone fracture resulted in marked bone formation (active

regeneration process) which begin in short duration of time, 1 days after fucose injection (8 days after induction of bone fracture) and continued until 30 days after fucose injection, this new bone formation represented by presence of more reversal lines on the surface of bone , increasing numbers of osteoblast cells at the periphery of bone. while in non injected subgroups, more time are required for healing and beginning of new bone formation reached about 35 days after induction of bone fracture. The enhancement of bone healing by fucose injection may occurred throughout early differentiation of osteoprogenitor cells into osteoblast cells for rapid bone formation, therefore the hyperactivity of osteoblast cells for rapid bone formation could be induced by stimulatory effect of fucose injection, more studies will be needed to confirm this results. Also the enhancement of bone formation may be due to the inhibitory effect of fucose injection on the production of pro-inflammatory mediators IL-1-beta and TNF-alpha production [18].

CONCLUSION

The present study concluded that fucose stimulate and accelerate new bone formation in short duration of time ,1 day after fucose injection , and this bone regeneration continued until its return into normal physiological appearance, so fucose can be used as a bone stimulating agent that stimulate new bone formation in rabbits with alveolar bone defect. Further studies are necessary to determine whether the histological finding in this study using L-fucose in rabbits will be translate to clinical benefits in the human especially in treatment of horizontal or angular bone defect in patient with periodontitis. .

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