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Histological and Histomorphometric Evaluation of 1% Bisoprolol Gel on Bone Healing in Male Rabbits

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Abstract: Bisoprolol fumarate a selective β1 – adrenergic receptor antagonist, bone remodeling is under β – adrenergic control via the sympathetic nervous system, in some human studies β- blockers were reported to increase bone mineral density or decrease fracture rate, the aim of study to investigate the local effect of 1% bisoprolol fumarate gel in healing femoral bone defect.

Materials and Methods: Thirty New Zealand white albino adult male rabbits weighting (2- 2.5) Kg were randomized to one of two, treated and control groups. An intra bony defect 3*5 mm diameter and depth was created in the femoral bone and filled with 1%bisoprolol gel (treated group) or gel base only (control group). The animals were sacrificed after 2, 4, 6 weeks after surgery and specimens were analyzed histologically and histomorphometrically,

Results: histological examination showed at the end of 4 weeks an obvious enhancement of new bone formation and new vascularization significantly more than control and treated group at end of 2,6 weeks, no inflammation seen in all treated groups ,the gel was degrading and biocompatible.

Conclusion: Result of the present study revealed that 1%bisoprolol fumarate gel promotes healing of bony defect and has good osteogensis.

Keywords: Bone healing, Bisoprolol fumarate, Rabbit, Histomorphometry.

Introduction

Bone regeneration is desirable objective in dental practice, the use of animals models to study bone healing is particularly useful to answer questions related to the most effective way to treat human beings, bone healing process involves cellular components, including osteoblast, stem cell ,growth factor and bone morphogenetic protein -2[(1),]The ideal material used in bone repairing should be able to seal the defect and should have an excellent biocompatibility further more induction of osteogensis, ease of handling and low product price are advantageous[(2)]two recent epidemiological studies showed that the use of β-blocker was associated with 30% decrease in fracture risk[(3,4)] . The involvement of sympathetic nervous system in the regulation of bone mass has demonstrated both pharmacologically and genetically by increase in osteoblast number and activity and subsequent increase in bone mass in mice characterized by low sympathetic tone[(5)] on the other hand mice or rats treated with β-agoinst isoproterenol or clenbuterol displayed a marked decrease in osteoblast number, activity, trabecular bone microarchitecture parameter and biomechanical properties[(6,7)] , Beta blockers are currently considered as potential drug under investigation for osteoporosis and fracture healing [(8)], Bisoprolol fumarate is selective β- adrenergic receptor antagonist has been clinically used to reduce the incidence of cardiovascular disease [(9)] the aim of this study was to evaluate histolological and histomorophometrical effect of 1%bisoprolol fumarate gel on bone healing.

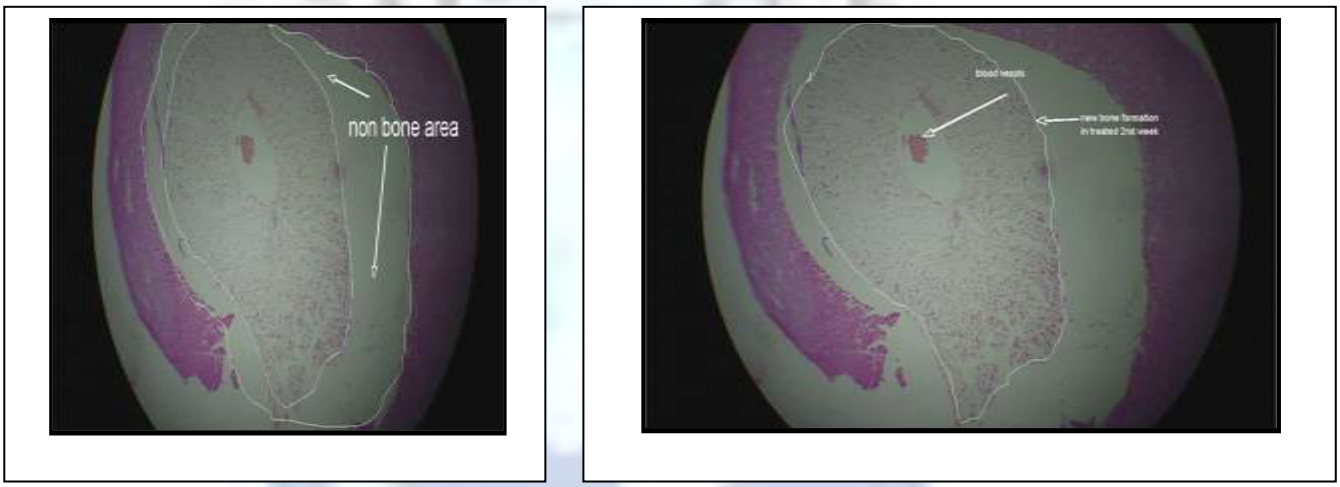
Materials and Methods

Preparation of Bisoprolol Fumarate Gel: The pure Bisoprolol fumarate powder was purchased (Bisoprolol fumarate, united company**,** Jorden). Bisoprolol gel was freshly prepared by mixing 0.01GM of bisoprolol powder in 1gm of gel base that contain (carboxy-methyl cellulose and propylene glycol) to give a final concentration (1%w/w) with continuous mixing using vortex device to prepare homogenous gel, which were kept in sterile plastic containers and stored at room temperature**.**

Experimental Animals: Thirty New Zealand white adult male rabbits of 6-8 months old, clinically healthy, Their average weight 2250 grams, Were used, the animal study protocol was approved by the ethics committee for animal experiments in the faculty of dentistry, Mosul University.

The animals were housed in wire mesh cages with standered condition food and water were provided at libitum, Rabbits were anesthetized with injection of 50mg/kg ketamine hydrochloride (Ketamine, Hameln, Germany)and xylazine 5 mg /kg (Xyla,Interchemie, Holland) respectively, and atropine sulfate injection (50) microgram / kg intra muscularly , femur was routinely asepsis , an incision was performed on left femoral bone , ,one cortical perforation of (3)mm in diameter and (5)mm depth with low speed hand piece of 2000rpm and profuse irrigation of distal water , the created defect was filled with 1% bisoprolol gel for treated groups, and filled with gel only for control groups ,the animals received antibiotic ceftrioxone at dose (40mg/kg) .I.M once daily for three days , and also received analgesic (40mg /Kg) diclofenamic acid I.M once daily to control pain .Five animals from each group were sacrificed at 2,4,and 6 weeks after the surgery. Sectioned femurs were fixed in 10% formalin in phosphate buffer saline (PBS) for 2 weeks, the bone tissue dehydrated with graded alcohol and embedded in paraffin, then sectioned at (5) µm with a steel Knife, the histological specimens were prepared in usual fashion haematoxyline and eosin staining, histololgical evaluation was performed at different magnifications.
Histomorphometric measurement of the samples was performed by using image J software version 1.42 (developed by the National Institutes of Health, Bethesda, MD, USA). First,the total area was determined by identifying the external and internal surfaces of the original femur, and then the area of the cavity and soft tissue (nonbone area) was circled and defined. The following histomorphometric parameters were determined (10)

Area new bone %= ((1 – (non-bone area / total area)] × 100 (%)



The Results

Group Two Weeks Control: At the area of injury, there was substitution with low density granulation tissue, large vacuoles between collagen bundles give the spongy appearance to tissue shape **(as Figure 1),** moderately diffuse fibroblast and osteoblasts with mild diffusion of Austen around osteoblasts as nidus and covering collagen bundles to be extended as fine spicules, the process of, ossification amount of maturation and mineralization is much more progressed at peripherally than in center of newly formed callus. as the (**Figure 2)** few remnants of fibrin cast still apparent, no sign of inflammatory reaction, few newly formed capillaries can be noted, few multinucleated osteoclasts were observed .as the**(Figure 3)**.

Figure (1): Spongy Appearance at Site of Bone Defect

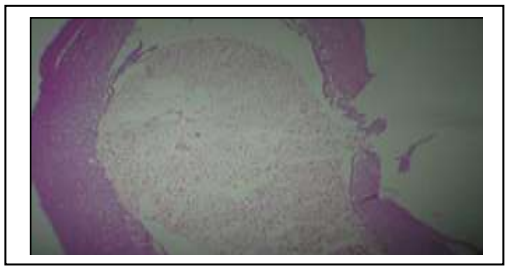


Fig. 2

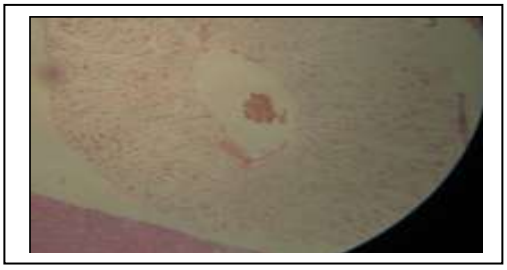


Fig. 3

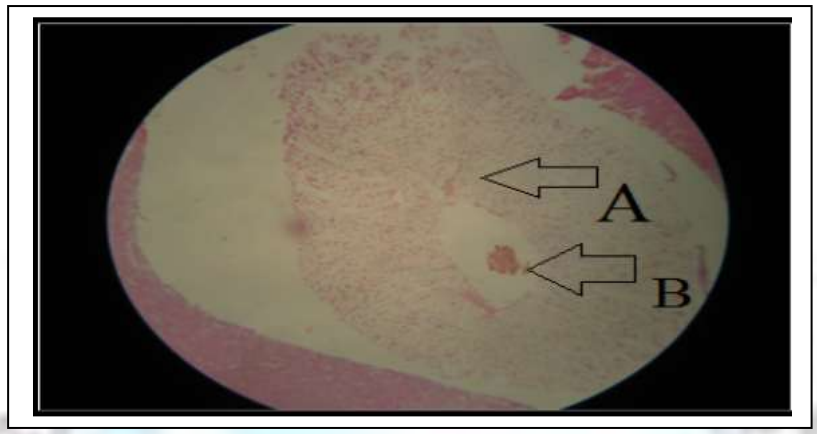


Figure (4): At the end of 2 weeks in control group , induced defect showed the newly formed soft callus composed of areolar tissue components and osteoid with in eosinophilic reaction coloration and many gaps giving the spongy appearance (A), Blood cast remnants still exist with a gap in the center of callus (B). Magnification 24 X, Staining H&E.

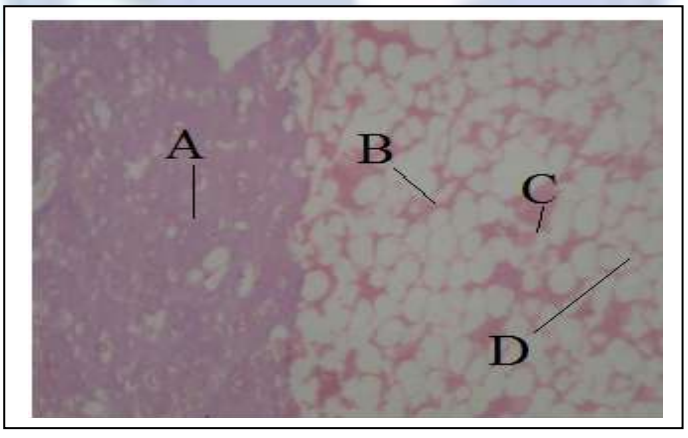


Figure (5): At the end of 2 weeks control group the induced defect showed the original mature bone (A) and the soft callus tissue formed on site of defect composed of mesh like collagen bundles (B), a nidus or foci of ousted precipitations (C) and newly formed capillaries (D). Magnification 165 X, Staining H&E.

Group Two Weeks Treatment

The area of injury showing substitution of same tissue described above, fibroblast and osteoblast diffusion , amount of osteoid precipitated and capillaries observing increased comparing with group two control.

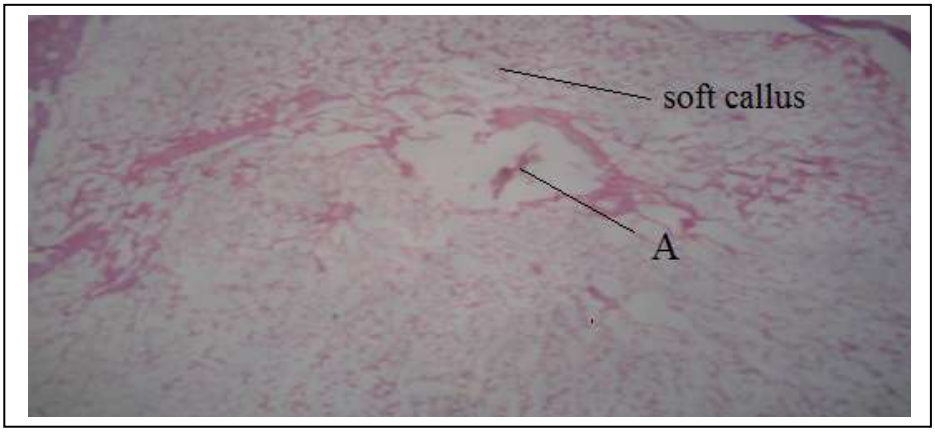


Figure (6): At the end of 2 weeks treated group the induced defect showed the soft callus tissue formed with better progression of ossification in the form of spicules and lamellae (A) comparing with control group sections. Magnification 56 X, Staining H&E.

Group Four Weeks Control

There is visual, significant increase to cellular component of the newly formed soft callus at the site of defect comparing with two week sections appeared as clusters of osteoblasts surrounded by variable amount of eosinophilic pink instead partially mineralized to appear slightly grayish pink at some foci, generally osteoid precipitation is more diffused comparing with two weeks sections (control) the areolar tissue bridges between those partially ossified segments still recognizable, vascularization and capillaries are more prominent.

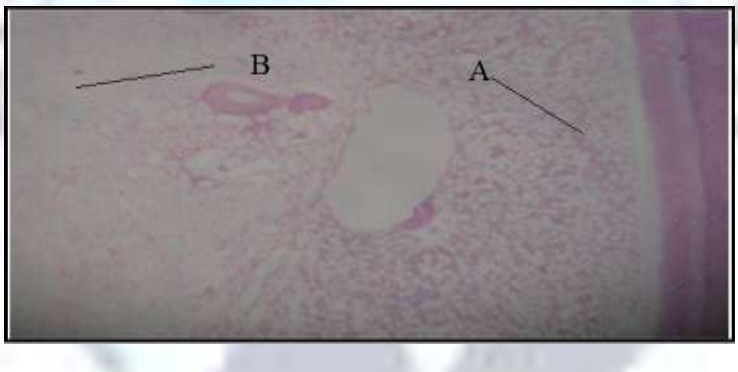


Figure (7): A t the end of 4 weeks control group ,induced defect showed, the soft callus tissue formed with better ossification and mineralization (A) on the periphery than in the center which is still occupied by a fibrous areolar tissue (B).Maturation of callus is better than 2 weeks sections Magnification 68 X, Staining H&E.

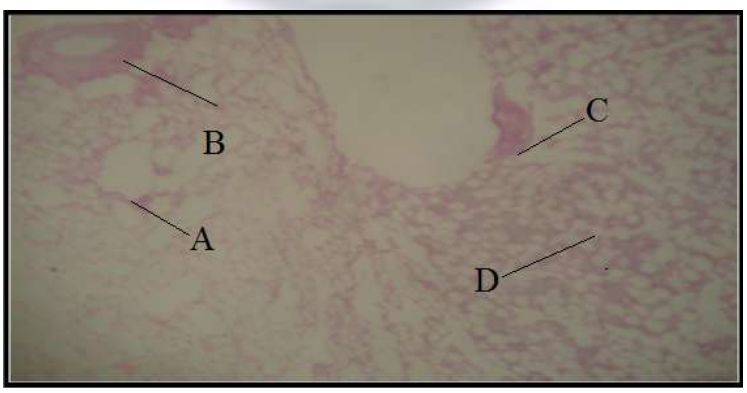
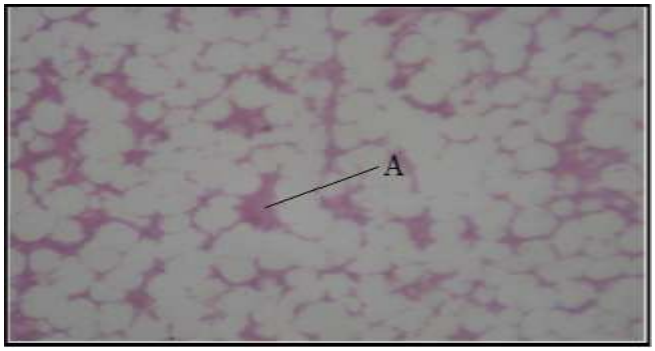


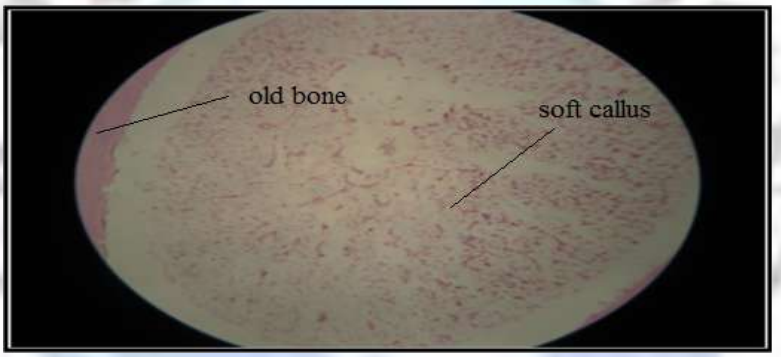
Figure (8): at the end of 4 weeks control group at site of induced defect showed a magnification of upper image in which a well formed capillaries (A), A grayish pink mineralized osteon matrix (B) , newly formed bone canaliculi (C), Mesenchymal cells and areolar tissue (D) . Magnification 115 X, Staining H&E.



Group Four Weeks Treatment

Figure (9): At the end of 4 weeks treated group at site of induced defect showed the soft callus tissue formed at site of defect with progression of process of healing and osteiod precipitation comparing with 2 weeks groups sections. Magnification 24 X, Staining H&E.

Osteoblast diffusion and multiplication as osteoid presentation visually progressed comparing with two week treatment group also those parameters appeared to be more prominent comparing with four weeks control group vascularity , capillaries maturation and soft callus mininralization is better performed than control, few multinucleated osteoclasts noticed in some sections.



Group Figure (10) At the end of 4 weeks treated group at site of induced defect showed the process of ossification of soft callus through distribution of osteoid matrix covering collagen and forming nidus and spicules (A) and appeared partially mineralized. Magnification 165 X, Staining H&E.

Six Weeks Control

After six weeks of healing the area of defect still shows the form of callus but with more advanced mineralization around aereolar component comparing with four weeks control group, an islets of blackish to dark bluish calcium salts deposits can be clearly noticified , few gaps containing blood casts still exist at some sections, better maturation of the new capillaries can be seen, increase density of osteoblast and fibroblast diffusion comparing with four weeks control, advanced accumulation of osteoid around mesenchymal cells taking the form of bone tissue spicules and lamella as **fig 11**

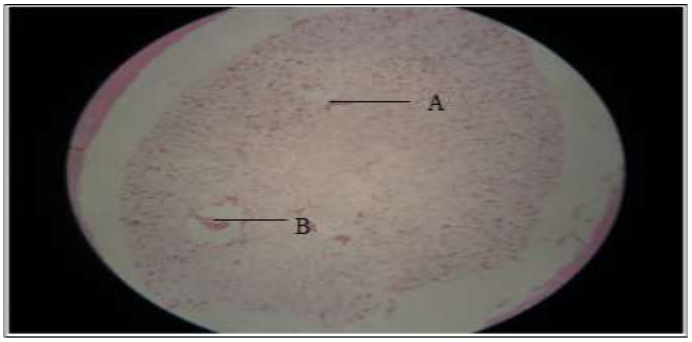


Figure (11) At the end of 6 weeks control group at site of induced defect showed progression of ossification of the soft callus (A) some gaps with blood casts still exist within callus at some sections (B) . Magnification 24 X , Staining H&E

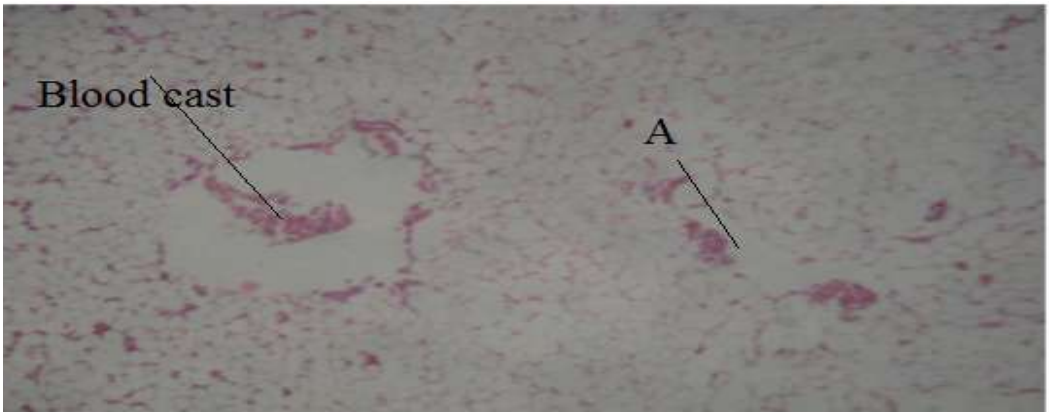


Figure (12) : At the end of 6 weeks control group at site of induced defect showed the center of the soft callus with incomplete ossification (A) and few gaps containing blood casts. Magnification 165 X , Staining H&E.

Group six weeks treated

The same progression in maturation of callus at site of defect mentioned at group sex weeks control is noted here m better localization of osteiod, heavier distribution of mesenchymal cell, observation of few mature osteocyts at periphery of site of defect surrounded with spicules of osteiod as primary stage of forming mature osteoid unit, blood vessels and capillaries completely developed with good blood perfusion. As figure 13,

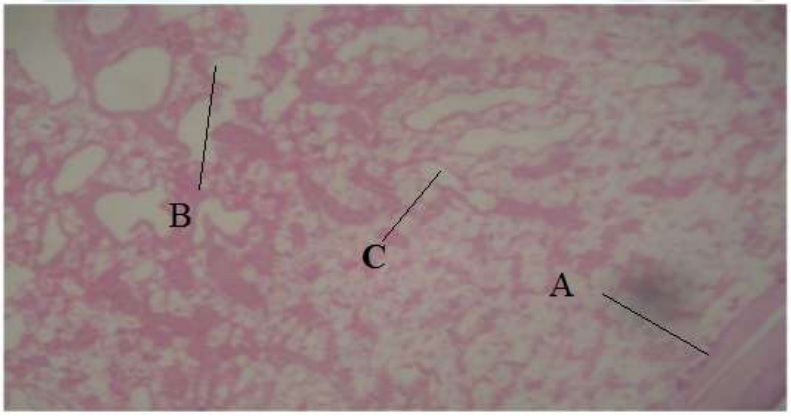


Figure (13) At the end of six week treatmente at site of induced defect showed progression in maturation in the callus and architectural details of osseous tissue manifested by formation of osteoid lamellae (A) osteoid ducts (B) osteoid spicules (C). Magnification 145 X , Staining H&E .

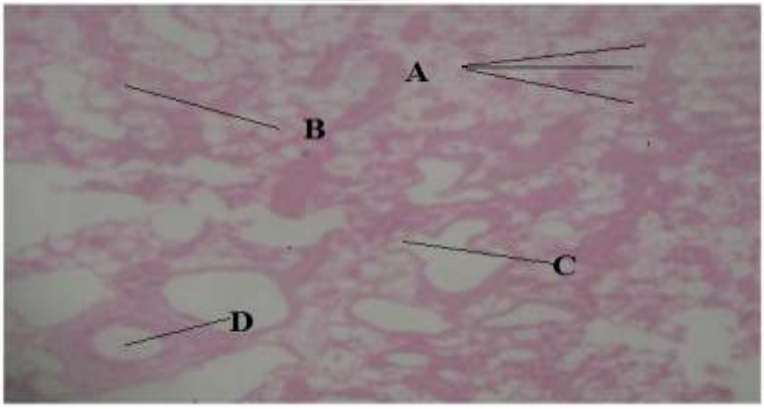


Figure (14) :-at the end of six weeks treated group at site of induced defect showed osteoid lamellae (A) newly formed osteon units with osteocytes (B) osteoid ducts (C) multiple foci of osteoblasts (D). Magnification 165 X, Staining H&E .

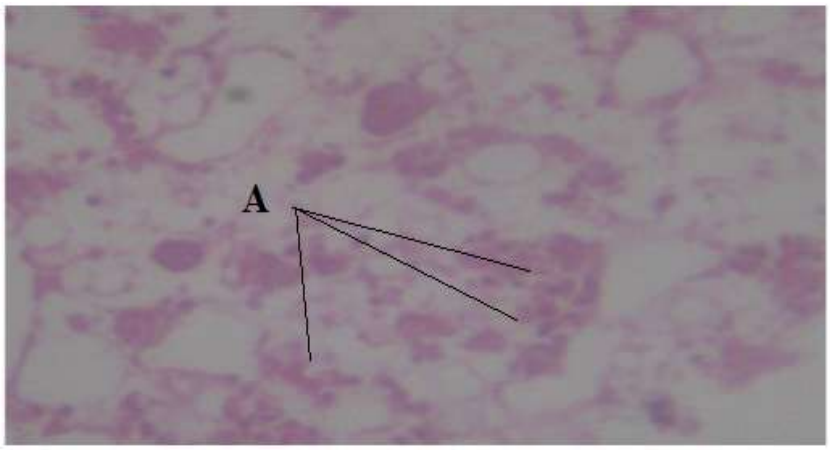


Figure (15): At the end of six weeks treated group at site of induced defect showed areolar tissue at the center of the callus demonstrating mature capillaries and foci of proliferating osteoblasts (A)

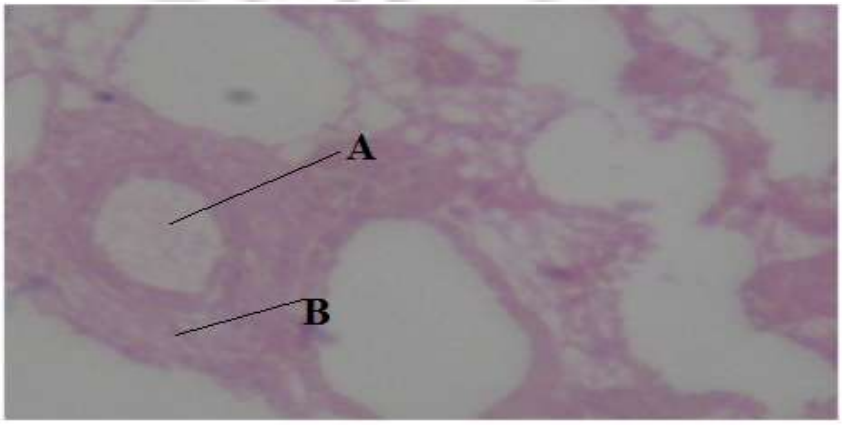


Figure (16) : At the end of six weeks treated group at site of induced showed formation of normal osseous architecture in the callus , Blood vessel (A) Surrounded with lamellae forming osteoid ducts (B). Magnification 280 X, Staining H&E

Histomorphometric Evaluation

Table (1) Values for the new bone formation as percentages within surgically created defects in rabbit femur (n= 5).

Time / Groups	Mean of New Bone Formation% after		
	2nd week (No= 5)	4th week (No= 5)	6th week (No= 5)
Control	62.735%	66.14%	71.0%
Treatment	78.036%	96.23%	74.7%

Discussion

Several regenerative procedures have been proposed for evaluation of bone formation in bone defects In the present study, we have analyzed the effect of Bisoprolol fumarate gel 1% in the healing of bone defects, the histological and histomorphometric results showed a significant increase in bone formation as compared to vehicle treated –control.
Recent clinical studies indicated that beta blockers and antihypertension drugs would reduce the risk of bone fracture in an elderly population[(11)] .In the present study local treatment with Bisoprolol fumarate gel1% resulted in an increased callus formation and osteoblast after 2,4 weeks this was most probably due to an increase of cell proliferation and

mineralization of callus. The effects of beta blockers on Bone mineral density have been formerly investigated in several studies. Inactivation of the sympathetic nervous system impairs osteoclastic bone resorption, and thus increases bone formation in animal models[12)] The data from human studies about the effects of beta blockers on osteoporosis is almost the same. Although in some studies improvements on Bone mineral density with beta blocker treatment have been proven [13] in other studies no effects of these drugs on bone metabolism have been reported.[14,15)]Similarly, the effects of beta blockers on fracture risk have also been studied, and they were found to decrease fracture risk in some studies.[13] . the improved histological healing of bone is further supported by the results of biochemical analysis of serum for bone specific alkaline phosphatase (BALP) which showed a significant increase after 2week of local treatment with 1%bisoprolol gel [16] increase of BALP correlates with increased bone formation and mineralization and reflects the metabolic status of osteoblasts,in the present investigation both osteoblast diffusion and mineralization were enhanced after 4 weeks treatment with bisoprol gel indicates osteoinductive ability of bisoprolol gel.

Some studies have demonstrated that low dose of propranolol (beta blocker) suppress bone resorption by inhibiting RANKL mediated osteoclastogenesis as well as inflammatory markers without affecting haemodynamic parameters [17]Other results showed that beta blocker propranolol stimulate OPG on its own osteoblast cells [18]While beta blocker propranolol showed increased bone formation in compact bone and tibia and inhibited bone resorption in cancellous bone of femur of ovariectomized and non ovariectomized[19] In contrast (Reid et al showed in recent study, mice were I/P treated for 3 days after ovariectomy using propranolol showed an increase BMD loss in mice [20] While Li-jie yu showed increase in circadian rhythm of serum Nitric oxide and endothelial nitric oxide synthase in rats treated with bisoprolol for 4 weeks [21]Nitric oxide (NO) a type of short lived signaling molecule plays important role in many biological processes including bone cell function [22)].Some studies reported that NO is an important regulator of bone metabolism [23] The results of these studies are controversial the studyConducted on the effect of NO on bone cell functions showed that bone cells produce NO in response to various stimuli includingEstrogens, pro-inflammatory cytokines, and mechanical stress [24]NO release in osteoblastic cells increases the cyclic guanosine monophosphate (cGMP) formation and cGMP signal regulates osteoblastic proliferation and differentiation [25]NO inhibits the osteoclasts, thus greatly increasing bone deposition[26] The histological evaluation of bisoprolol gel was assessed in this study at different intervals ,it reveals good osteoinductive and biocompatible with no sign of foreign body reaction and or sever inflammation[27]

Conclusion

In the light of the available information, we hypothesized that the local use of bisoprolol gel was beneficial in bone healing. However, clinical studies and investigations are required to confirm it .

Declarations

Conflict of interest
The Author(s) declare(s) that they have no conflicts of interest to disclose.

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