

Histological Evaluation of Local Estrogen Administration on the Healing of Traumatic Alveolar Bone Defect in Rabbits (Experimental Study)

Asmaa S Aldouri
BDS, MSc, PhD (Asst. Prof.)

Abdalsatar S Almaadhidi
BDS, MSc (Asst. Lec.)

Israa A Abdalhameed
BDS, MSc (Asst. Lec.)

Dept of Oral and Maxillofacial Surgery
College of Dentistry, University of Mosul

الخلاصة

الأهداف: لتقييم تأثير هرمون الأستروجين المحقون موضعياً بعد خلطه بليف الكولاجين المستخلص من العظم البقري؛ في التئام كسر العظم السنخي في الأرانب. **المواد وطرائق العمل:** ثمانية أرانب استعملت لهذه الدراسة، تم تحضير شقا عمودياً في العظم السنخي للأرانب. المجموعة القياسية تم ملء الشق بليف الكولاجين الطبيعي المستخلص من العظم البقري بينما خلط ليف الكولاجين بـ هرمون الأستروجين في المجموعة التجريبية. تم دراسة النتائج نسيجياً ثم تم تحليلها احصائياً. **النتائج:** أظهرت النتائج أن استعمال هرمون الأستروجين المحقون موضعياً مع ليف الكولاجين، حسنت بشكل ملحوظ تشكيل عظم جديد وأوعية دموية جديدة أثناء التئام العظم السنخي إذا ما قورن بالمجموعة القياسية التي استخدم فيها ليف الكولاجين فقط. **الاستنتاجات:** أثبتت هذه الدراسة أن حقن هرمون الأستروجين موضعياً في كسر العظم السنخي يكون فعالاً في تنشيط التئام العظم. كما وأن التحليلات الاحصائية أثبتت وجود علاقة قوية بين سرعة التئام العظم ووجود هرمون الأستروجين. الكلمات المفتاحية: استروجين، العظم السنخي، التئام.

ABSTRACT

Aims: This study carried out for histological assessment of the effect of local administration of estrogen hormone after mixing with collagen fiber of bovine bone on healing of traumatic alveolar bone defect in the rabbits. **Materials and Methods:** Eight Rabbits were used, the vertical fissure was done in the mandibular alveolar bone of the control group was filled with natural collagen fiber of bovine bone, while in the experimental group, this fissure was filled with estrogen hormone mixed with natural collagen fiber of bovine bone. Histological studies and statistical analysis were done. **Results:** The results showed that local administrations of estrogen hormone enhance new bone formation and new vascularization which is statistically significant when compare with control group. **Conclusions:** This study illustrate that the estrogen has an osteoinductive action to enhance bone healing process. Biostatistical analysis was shown significant relation; so bone healing enhanced by this material.

Key words: Estrogen, Alveolar Bone, Healing.

Aldouri AS, Almaadhidi AS, Abdalhameed IA. Histological evaluation of Local Estrogen Administration on the Healing of Traumatic Alveolar Bone Defect in Rabbits (Experimental Study). *Al-Rafidain Dent J.* 2013; 13(1): 72-80.

Received: 19/6/2011

Sent to Referees: 19/6/2011

Accepted for Publication: 13/9/2011

INTRODUCTION

Bone is a specialized vascular connective tissue consisting of cells and calcified intracellular materials. Although bone is one of the hardest tissues of body, it is dynamic tissue that constantly change its shape in relation to the stress placed on it. This tissue unlike other connective tissue, its matrix consist of physiologically mineralized, tiny crystallites of a bicarbonate-containing calcium phosphate called hydroxyapatite distributed in an organized collagen structure.⁽¹⁾ Repair of this tissue is a complex process involving a number of

cellular functions directed towards the formation of a scaffold and mineralization of the defect followed by an eventual remodeling of the defect site to attain the original structure.⁽²⁾

According to the function, alveolar process adapted itself; it is divided in to two parts, alveolar bone proper and supporting alveolar bone. The supporting alveolar bone surrounds alveolar bone proper and gives support to the socket, it consists of two parts, cortical plate and spongy bone. Histologically the cortical plate contains longitudinal lamellae and haver-

sian system.⁽³⁾ Spongy bone is cancellous bone which supports the bone proper, heavy trabeculae with bone marrow space are present in this bone. The bone marrow space contains blood forming elements and osteogenic cells.⁽⁴⁾ There are three types of cells constituting the bone osteoblast, osteocyte and osteoclast.⁽⁵⁾ Osteoblast are mononucleate bone-forming cells that descend from osteoprogenitor cells, they make a protein mixture known as osteoid, which mineralizes to become bone.⁽⁴⁾

Osteocyte originate from osteoblasts that have migrated in to and become trapped and surrounded by bone matrix that they themselves produce it, their functions include varying degrees, formation of bone, matrix maintenance and calcium homeostasis. Osteoclast cells are the cells responsible for bone resorption (remodeling of bone to reduce its volume), are large multi nucleated cells located on bone surface in Howships lacunae.⁽⁴⁾ Osteoblasts can be stimulated to increase bone mass through increased secretion of osteoid and by inhibiting the ability of osteoclasts to break down osseous tissue.⁽⁶⁾ Bone building through increased secretion of osteoid which is stimulated by the secretion of growth hormone by the pituitary gland, thyroid hormone and the sex hormones (estrogen and androgens). These hormone also promote increased secretion of osteoprotegerine.⁽⁷⁾ Estrogen is a group of compounds named for their importance in estrous cycle of humans and other animals, and functioning as the primary female sex hormones but also present in male as testosterone hormone. Natural estrogen are steroid hormones, while some synthetic ones are non steroidal. Their names come from the Greek words estrus. Estrogen is synthesized in all vertebrates.⁽⁸⁾ Like all steroid hormones estrogen readily diffuse across the cell membrane. Once inside the cell, they bind to and activate estrogen-receptors which in turn modulate the expression of many genes.⁽⁹⁾ Oestrogens are produced by developing follicles in the ovaries, liver, adrenal glands, breasts and fat cells,⁽¹⁰⁾ it accelerate metabolism, reduce bone resorption, increase bone formation, increase platelet adhesiveness, decrease fat deposition.^(11,12) The estrogen

seems to be an important factor in all stages of fracture healing. The application of estrogen enhances fracture healing of long bone at least in mice; larger chondrocytes and callus mineralization were increased. Estrogen deficiency is known to increase osteoclast bone resorption, whereas estrogen replacement can reverse this effect.⁽¹³⁾

MATERIALS AND METHODS

Eight rabbits weight 2 kg \pm 100mg. male were used in this study. The rabbits were divided into two groups; four rabbits were sacrificed for each of two healing periods, one week and three weeks respectively. The animal anesthetized generally (Ketamine hydrochloride 10% (Holden Batch NO.1640-INDIA) at a dose of 50mg/kg mixed with Xylazine 2% (Interchemie Batch NO. 351861 HOLLAND) at a dose of 5mg/kg) and the site of the operation anesthetized locally with Xylocaine and adrenaline to reduce blood field. The mouth opened by using elastic mouth gage and oral cavity washed by 2% chlorohixidine concentration (Biofresh-SYRIA). After anesthetizing the animals by general anesthesia the vestibule of the right side of mandible three centimeters away from crown of central incisor incised intraorally with horizontal incision of about one centimeter. In the alveolar bone of mandible, vertical fissure was done by round bur of micro handpiece with irrigation to avoid heat generation (0.5 mm. height, and 0.5mm. depth). In the control group this fissures filled with natural collagen fiber of bovine bone (Lyostypt, BRAUN, Malaysia. C€0123) as a haemostasis. In the experimental group the fissures is filled with natural collagen fibers of bovine bone mixed with estrogen hormone in two derivatives (1mg. Oestradiol benzoate and 4mg. Oestradiol fenilpropionat) in 0.1 benzyl alcohol (Organon, Santa Farma, Holland P.K.262, 34361).

The incision was sutured with black silk suture (size 3/0 Navo Batch NO. 7634507 CHINA) and the rabbits were treated with procaine penicillin (Guangdong Medicine Batch NO. 070571 CHINA) at a dose of 10000 Iu/kg. for three days post operatively to control infections and with diclofinac sodi-

um(Alshrak Batch NO. 75 SYRIA) at a dose of 5 mg /kg for one day post operatively to control pain.

Histological study: The rabbits killed under general anesthesia (four rabbits at the end of first week and four rabbits at the end of four weeks) and the anterior segment of mandible resected and the all soft tissue removed. The specimen prepared as decalcified section and stained with hematoxylin and eosin. The microscopical finding includes evaluation of cell forming bone as well as bone lamellae. The cell counting and measuring of lamellar thickness was illustrated by using special graduated microscopical lens at power of magnification of 200X and 400X. Four randomly selected location of each section was examined. Each location divided by graduation of graduated lens into four quarters. The measurements were applied to each quarter separately and take the mean for these four measurements of the same location. The mean of two locations of control

groups and two locations of experimental groups taken to be considered later in biostatistical analysis.

Statistical analysis: Biostatistically used compared means Duncan T-test to determine the groups differences (significance) between two groups after each healing period in the number of osteoblast and thickness of new bone trabeculae separately using SPSS program under WIN-DOSE operating system in Pentium IV computer. (P value ≤ 0.05 , significant).

RESULTS

Histological analysis:

Control group at the end of 1st week post-operatively: The histological finding showed the fissures that filled with collagen fibers of bovine bone accompanied by blood clot and mild proliferation of granulation tissue and blood vessels. Figure (1 and 2).

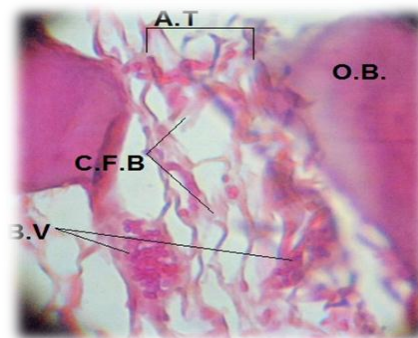


Figure (1): Digital micrograph of control group at the end 1st. week. A.T. area of trauma of mandible filled with C.F.B.: collagen fiber of bovine bone. O.B. original bone. B.V. blood vessels... (400X power of magnification

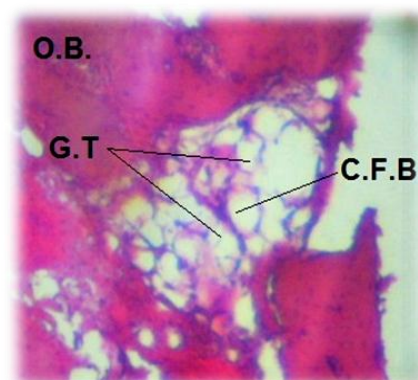


Figure (2): Digital micrograph of control group at the end 1st. week at traumatic side of mandible filled with C.F.B.: collagen fiber of bovine bone. O.B. original bone G.T. granulation tissue... (200X power of magnification)

Experimental group at the end of 1st week postoperatively: At the bone lining of the fissure there is marked migration of the osteoblasts toward the socket and aggre-

gated around the collagen fibers of bovine bone with mild formation of very thin bone trabiculea. Figures (3 and 4).

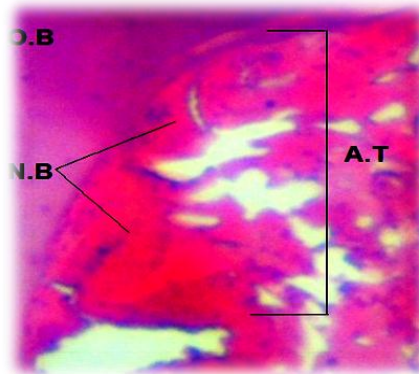


Figure (3): Digital micrograph of control group at the end 3rd week. A.T. area of trauma of mandible. O.B. original bone. N.B. new bone..(400X power of magnification).

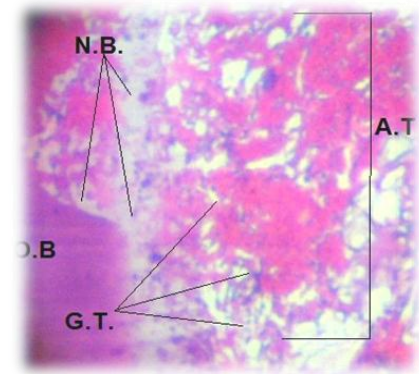


Figure (4): Digital micrograph of control group at the end 3rd week. A.T. area of trauma of mandible filled with. G.T. :granulation tissue. O.B.: original bone. N.B. new bone..(200X power of magnification).

Biostatistical analysis at the end of 1st week post operatively: Biostatistical analysis was show significant relation in both osteoblast numbers and thickness of new

bone trabeculae in comparison between both experimental and control groups. Table (1) Diagram (1).

Table (1):T.TEST. Comparison between control and experimental groups in the number of osteoblasts and thickness of new bone trabeculea formation at the end of 1st. and 3rd. week intervals.

	T. Tests	Control groups	Experimental groups	Significance P. value
Number. of osteoblast.	Means of first week	8.000	27.333	0.014*
	Means of third week	18.66	78.00	0.049*
Thickness of new bone trabeculea	Means of first week	4.5	18.33	0.04*
	Means of third week	18.66	88.33	0.036*

p. value ≤ 0.05 is significant.* : *p* value is significant.

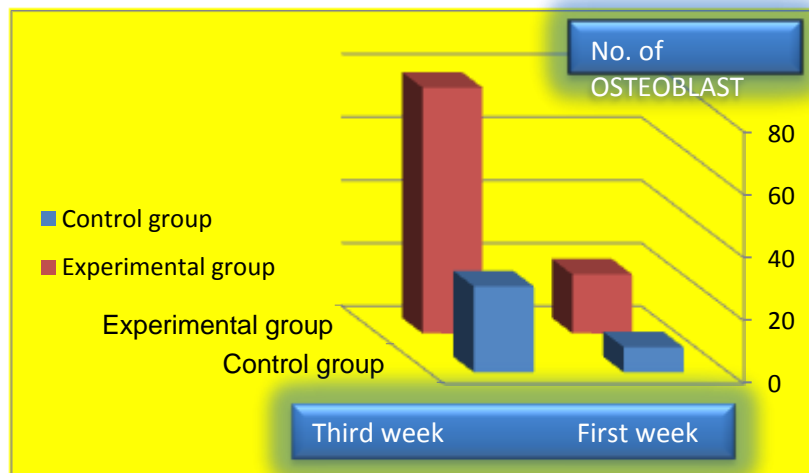


Diagram (1): Comparisons between control and experimental groups in the number of osteoblasts at the end of 1st. and 3rd week intervals.

Control group at the end of 3rd week postoperatively: The histological finding showed dense granulation tissue within the traumatic area with proliferation of osteo-

blast and thin bone trabeculae formation start from borders of the original bone. Figures (5 and 6).

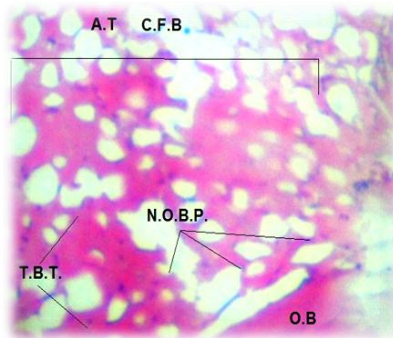


Figure (5): Digital micrograph of experimental group at 1st. week..A.T.: area of trauma of mandible filled with C.F.B.: collagen fiber of bovine bone with estrogen hormone. O.B.: original bone. N.O.B.P. new osteoblasts proliferation. B.T. new bone trabeculae....(400X power of magnification).

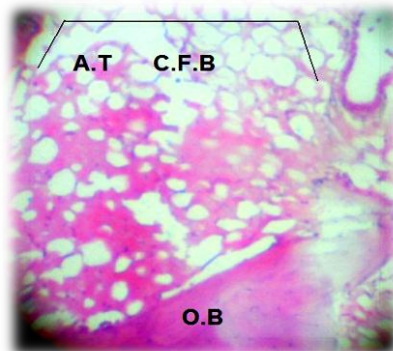


Figure (6): Digital micrograph of experimental group at 1st. week..A.T.: area of trauma of mandible filled with C.F.B.: collagen fiber of bovine bone with estrogen hormone. O.B.: original bone. ... (200X power of magnification).

Experimental group at the end of 3rd week postoperatively: Histologically, the fissure filled with natural collagen fibers of bovine bone, showed increase the number of the osteoblast migrated toward the

traumatic area and aggregated around the collagen fibers and increase thickness of newly formed bone trabeculae. (Figures 7 and 8).

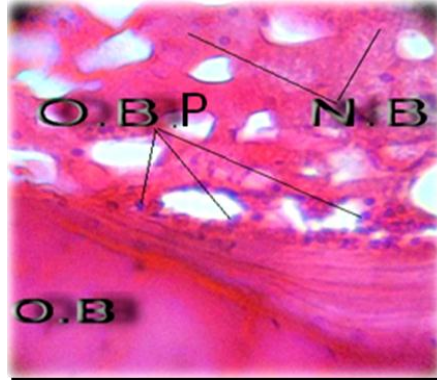


Figure (7): Digital micrograph of experimental group at 3rd week. . O.B.: original bone. O.B.P: osteoblasts proliferation .N.B.new bone ... (400X. power of magnification).

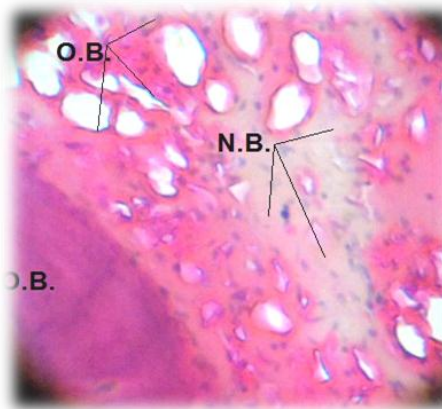


Figure (8): Digital micrograph of experimental group at 3rd week. O.B.: original bone. N.B. new bone. O.B.P: osteoblast proliferation ... (200X. power of magnification).

The histological finding showed osteoid formation and calcification of granulation tissue within the fissure with proliferation of osteoblast and increase thickness of bone trabeculae away from the border of original bone. The increasing in the number of the osteoblast migrated toward the socket is reduced as many of this cells convert to osteocytes and trapped within lacunae also the bone trabeculae became more prominent and the number of osteocyte appeared within lacunae of newly

formed bone increased.

Biostatistical analysis at the end of 3rd week post operatively:

During data collection there was wide range of variables between two groups in this stage.

Biostatistical analysis was show significant relation in both osteoblasts number and thickness of new bone trabeculae in comparison between control and experimental group. Table (1) Diagram (2).

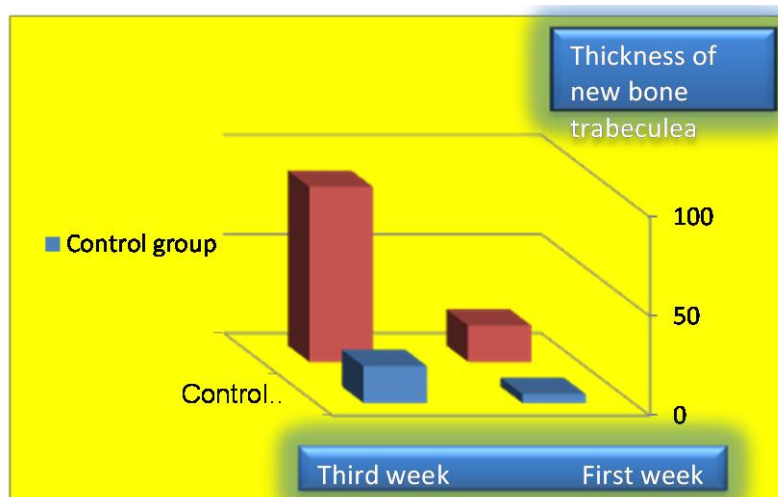


Diagram (2): Comparisons between control and experimental groups in the thickness of new bone trabeculae at the end of 1st. and 3rd week intervals.

DISCUSSION

Although the variation in size of rabbits produces a potential limitation in the experiment carried out for this study, this animal model was chosen for the ease of handling, and because rabbits reach skeletal maturity at around six months of age. In comparison to other species, such as primates and other rodents, the rabbit has a faster bone turnover.⁽¹⁴⁾

Biologically during the whole period of data collection postoperatively there were no any sign of severe inflammation or rejection to the materials. Using histological examination of the alveolar bone, all treated defects with estrogen showed greater bone formation than the control group. Histological evaluation of experimental group at first week postoperatively revealed that estrogen has important role in skeletal remodeling and healing, because osteoblasts migrate toward the defect area and aggregated around the collagen fibers of bovin bone with mild formation of very thin new bone trabeculae and may be that osteoblast estrogen receptors cause remodeling. It is reasonable to assume that the effect of estrogen on skeletal remodeling could be caused, at least partly by a direct effect on bone cells, osteoblast and osteoclast.⁽¹⁵⁾

In the present experimental study, at the end of the first week post operatively,

there was a significant reduction in both osteoblast number and thickness of new bone trabeculae in comparison the experimental and control group. So estrogen plays an important role in bone defect healing process. Estrogen has profound effects on bone metabolism; estrogen has potential direct action via its own receptors on bone cells.⁽¹⁶⁾

At the end of third week postoperatively, the histological examination has revealed dense granulation tissue and very thin bone trabeculae formation in the control group while in estrogen treated group, there was osteod formation and calcification of granulation tissue, increase bone trabeculae thickness. Also there was increase in number of osteocyte appeared within lacunae of newly formed bone increased, there was better healing. This result in agreement with Xiaohong et al. when they found better wound healing around the extracted sockets in rats when treated with estrogen when examined histologically by fluorescence observations.⁽¹⁷⁾

The rate and quality of wound healing depend on reproductive hormone levels (estrogen), because a marked repair of acute incisional wounds in ovariectomized rodent.⁽¹⁸⁾ Also at end of third week, there was significant increase in number of osteoblast and bone trabeculae in experimental group that indicate that estrogen

could affect bone healing and bone density causing more regular osseous tissue with a large number of osteocyte and neoformed bone and this in agreement with Durate P. et al. when they found that estrogen deficiency affect bone healing in rats.⁽¹⁹⁾

Estrogen may induce proliferation of undifferentiated mesenchymal cells (increase of the population of healing cells), angiogenesis (endothelial mitosis into functioning capillaries), and enhances bone regeneration and healing by acceleration vascular repair to the defect area.⁽²⁰⁾ Research has led to the general agreement among physicians and researchers that the progression of bone loss can be halted by estrogen replacement therapy.^(21, 22)

As a result, the estrogen would be a good candidate for use in combination with natural collagen fiber of bovine bone for use to repair bone defects.

CONCLUSIONS

This study demonstrated that local administration of estrogen has important specific role for promoting and accelerating bone healing.

REFERENCES

1. Gartnr LP, Hiatt JL. Color textbook of histology. Bone, 2nd ed. Saunders company. China. 2001; Pp; 134-54.
2. Oda T, kinshita and Ueda M., Effects of cortical bone perforation on periosteal destruction. *J maxillofacial surg.* 2009; 67(7): 1478-1485.
3. Nidheechandra, Mithilesh, Satish C, Shaleen C. Textbook of dental and oral histology and embryology. Jaypee-brothers medical publishers. Daryagani, New Delhi. 1st ed. 2004; Pp; 237.
4. Nancy A, Tencats oral histology, Development, structure, and function. Bone. 6th ed. Mosby company. USA. 2003; Pp: 111-44.
5. Boyle WJ, Simonet WS and lacey DL., Osteoclast differentiation and activation. *J. Nature.* 2003; 423: 337-342.
6. Bryan D, Gerard J, Tortora. Principles of anatomy and physiology. John wiley and sons. New York 5th ed. 2008; Pp: 512-524.
7. Boulpaep, Emile L, Boron and Walter F. Medical physiology a cellular and molecular approach. Philadelphia, Saunders. 1st ed. 2005; Pp: 1089-1091.
8. Ryan KJ Augist. Biochemistry of aromatase: significance to female reproductive physiology. *Cancer Res.* 1982; 42: 3342-3344- PMID.
9. Whitehead SA, Nusseys. Endocrinology. An integrated approach. Oxford: BIOS: Taylor and francis. ISBN. 2001.
10. Nelson LR, B ulunsE, Estrogen production and action. *J Am Acad Dermatol.* (2001), 45(Suppl.) 5116-24.
11. Warnock JK, Swanson SG, Borel RW. Combined esterified estrogen and methyl testosterone versus, sterified estrogen alone in the treatment of loss of sexual interest in surgical menopausal women. *Menopauses J.* 2005; 12(4): 359-60.
12. Massaro D, Massoro GD., Estrogen regulates pulmonary alveolar formation, loss and regeneration in mice. *Am J physiol Lung cell Mol Physiol.* 2004; 287(6): L1154-9.
13. Beil F, Barvenick F, Gebauer M, Seitz M. Effects of Estrogen on fracture healing in mice. *Journal of Trauma- Injury infection and critical care.* 2010; 69(5): 1259- 1262.
14. Pearce AI., Richards RG, Milz S. Animal models for implant biomaterial research in bone. A review. *Eur cell Mater.* 2010; 13: 1-10.
15. Joae PM, Rodrigo T, Amaro Sd. Regulation of the bone healing process by-hormones, *International J morphology.* 2010; 197(6): 154-9
16. Boden SD, Joyce ME, Hehdemunn A, Bolander E. Estrogen receptors Mrna expression in callus during fracture healing in the rat. *Calcified tissue international.* 1989; 45(5): 324-325.
17. Xiaohong LI, Xiaoshu ZH, Xianyan ZH. The effect of estrogen on bone wound healing of extracted sockets in osteoporotic rats: fluorensence labeling observation. *Chinese J osteoporosis.* 2007; 187: 649-657.
18. Gillon S, Aschrof t, Joanne D, Egonvan B. Estrogen accelerates cutaneous wound healing associated with an increase in TGF.B, level *sNature medicine J.* 1997; 3: 209-1215.
19. Durate PM, Cesar NJB, Goncalres PF,

- Sallum EA, NocitJF. Estrogen deficiency effects bone healing around titanium implant histometric study in rats. *Implant dentistry J.* 2003; 12(4): 340-6.
20. Kerstin S, Nikos E, Jan B, Andreas L. , Estrogen increase bone marrow derived endothelial progenitor cell production and diminishes neointima a formation. *Circulation J.* 2003; 107: 3059.
21. ReckerRR., Effect of estrogen and calcium carbonate on bone lesion in postmenopausal women. *Ann Intern. Med., J.* 1977; 87: 649-655.
22. Claus C, Merete SC, NielsEL. Pathophysiological mechanism of estrogen effect on bone metabolism. Dose-response relationship in early postmenopausal women. *J Clinic Endocrinol Metab.* 1982; 55(6): 1124, Copyright, 2011 by the endocrine society.