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Effect of Sodium Fluoride on Cancellous Bone Defect Healing in Rats

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Abstract: Problem statement: The purpose of this study was to histopathology and biomechanics evaluate the effect of sodium fluoride on cancellous bone defect healing in rat model. **Approach:** The experiment was conducted on 40 male adult SD rats which were divided into four groups of control and experiments. After induction of general anesthesia, a hole in size of 2×3 mm in diameter and depth was made using a dental bit in the inner aspect of the between condyles of right femor. In all groups, defect was left untreated. Control group was given distilled water and the other three groups were exposed to fluoridated water at different concentrations (8, 30 and 60 mg F^-/L). After 45 days all rats were sacrificed and the histopathological and biomechanical penetration tests of the distal femoral bone were performed. **Results:** In control group, defect seemed to be filled with adipous tissue and sparse hematogenic cells and in spite of a poor osteogenic activity and some osteoblasts could already be seen. In experiment groups, many osteoblasts groupings and young bone trabeculas increased in number and bone trabeculas more organized. Histopathologically findings show that 30 mg F^-/L sodium fluoride in drinking water has been better effect than other groups. The mean load for fracturing in control group was 173.01±23.05 and in experiment groups 8, 30 and 60mg were 177.31±33.71, 181.90±36.81 and 168.51±54.35. Differences observed in biomechanically findings were significant between 30mg group and other groups ($p<0.05$). **Conclusion:** The results of this study show that fluoride level at 30 mg $F^- L^{-1}$ in drinking water increases compressive strength of cancellous bone defect in healing process and could stimulate osteogenesis in femoral cancellous bone defect in rats.

Key words: Sodium fluoride, cancellous bone, healing process, adult male rats, osteoblasts groupings, general anesthesia, biomechanics evaluate, osteoporotic patients, laboratory animal

INTRODUCTION

Sodium fluoride in clinical use for treatment of osteoporosis and vertebral fractures (Demos *et al.*, 2001; Apostolova and Brenne, 2010). Clinical studies have been shown that sodium fluoride is effective in bone formation and increase in cancellous bone volume in the ilium and axial skeleton (Demos *et al.*, 2001; Gibson-Moore, 2009; Roschger *et al.*, 1997) and it is an effective agent for increasing spinal bone density in osteoporotic patients (Demos *et al.*, 2001). Current studies show that the osteogenic actions of fluoride are mediated through a fluoride-dependent increase in the number of osteoblasts (Ohta *et al.*, 1995; Bohatyrewicz *et al.*, 2001; Monjo *et al.*, 2008; Qu and We, 2006). But the mechanism of action of fluoride on the skeleton is not known. The purpose of the present study was to investigate the healing effect of sodium fluoride on cancellous bone defect in rats.

MATERIALS AND METHODS

Animals: Investigations using experimental animals were conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in the United States guidelines (United States National Institutes for Health publication no. 85-23, revised in 1985) and our ethical committee on animal

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care approved the protocol. Forty male adult Sprague-Dawley rats, 250-300 gram were used. Rats were obtained from the central animal laboratory of Islamic Azad University-Tabriz Branch and were housed in colony rooms with 12/12 h light/dark cycle at 21±2°C for 2 weeks before initiation of the study, fed with laboratory pellet chow and drinking water was given ad libitum. Rats divided into four groups of 10 animals each, according to the procedure performed. Groups were differentiated by the varying concentrations of fluoride in the drinking water after surgery for 45 days.

Surgical procedure: Animals were anesthetized with Ketamine hydrochloride (Ketamine 10%, Alfasan, Woerden-Holland, 50 mg kg^{-1}) and Xylazine (Xylazin 2%, Alfasan, Worden-Holland, 5mg kg^{-1}) intraperitoneally. The right hind limb was shaved and prepared aseptically with povidone iodine. A 2-cm skin incision was made on the medial aspect of the distal femoral condyles. The muscle and articular capsule were dissected bluntly to expose the lateral and medial condyles. A confined cancellous defect was drilled in between of lateral and medial condyles using a low-speed dental bit, saline-cooled in a stepwise fashion. A hole in size of 2×3 mm in diameter and depth was made and left untreated. Tissue was closed in layers. Animals were monitored postoperatively and then returned to their cages. Animals received intramuscular injections of Penicillin G, 60000 Iu kg^{-1} immediately after surgery and 24 h later and orally Celecoxib 6 mg kg^{-1} for 3 days after surgery. The concentrations of fluoride used were 0 (control), 8, 30 and 60 mg $F^{-} L^{-1}$ (Bohatyrewicz *et al.*, 2001).

Animals were sacrificialed after 45 days postoperatively under general anesthesia, with an injection of over dosage of Thiopental sodium (60 mg kg^{-1}). The distal of right femurs include osseous defect were harvested, stripped of soft tissues and prepared for histopathologic and biomechanic analyses.

Histopathology analyses: Five femurs of each group sunk in 10% formalin buffer during seven days, for fixation; then, it was decalcified in a water solution of 10% nitric acid during additional 5 days. Each of those segments were included in paraffin blocks, all specimens were serially sectioned longitudinally at 5-µm intervals and stained with hematoxylin-eosin (H and E) method and used for light microscopic examination under a Nikon microscope (ECLIPSE E200, Japan) (Kaveh *et al*., 2010).

Biomechanical analysis: Five femurs of each group were removed, wrapped in saline-soaked gauze, frozen and stored. Specimens were thawed at room temperature in a saline bath prior to mechanical testing. All mechanical testing were performed in biomechanics laboratory of Polymer Engineering Department of Sahand University of Technology, using a Biomechanics measurement device (Zwick/Roell Z010, TI-PR010TH.A50 model) with a crosshead speed of 0.01 mm sec^{-1}. A load-distance curve was recorded to determine mechanical properties. The central load support was applied to the lateral and medial condyles of femur. Maximum stress and maximum strain were calculated based on standard engineering equations for three-point bending (Peck, 1985).

RESULTS

Histopathology results: Histopathological evaluation revealed that the defect in control group was filled with immature granulation tissue with angiogenesis and some degrees of inflammatory cells. Fibrous connective tissue seemed in deeper portion of the defect. No new bone formation was observed in the central part of the defect area (Fig 1). Histopathological evaluation in group of sodium fluoride treated at a dose of 8 mg L^{-1}, revealed that the newly formed lamellar bone middle portion of the defect is united with old bone tissue at lateral aspects of the repaired construct (Fig 2). Histopathological evaluation in group of sodium fluoride treated at a dose of 30 mg L^{-1}, revealed that quantity of newly formed lamellar bone in healing site is more than other groups. Bone neoformation was well organized and advanced stage of remodelling is seen (Fig 3). Histopathological evaluation of the healing site in fluorine treated group at a dose of 60 mg L^{-1}, reveals the apical portion of defect to be filled with immature granulation tissue (Fig. 4).

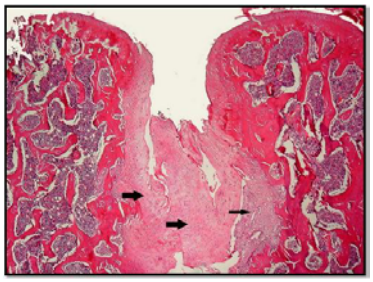

Fig 1: Histologic section from the healing site, in a control rat. The repaired construct reveals Chondrogenesis in fibrose tissue give evidence of substitution of granulation tissue with hyaline cartilage (thick arrows). Imperfect organic bone matrix, the osteoid, deposition and calcification has lead to smaller improvement in bone formation. A thick layer of mature granulation tissue (thin arrow) has remained at lateral portion of the defect with abundant capillary buds. Only a few inflammatory cell infiltrate is present in healing sites (H-E, Orig. Mag. ×40)

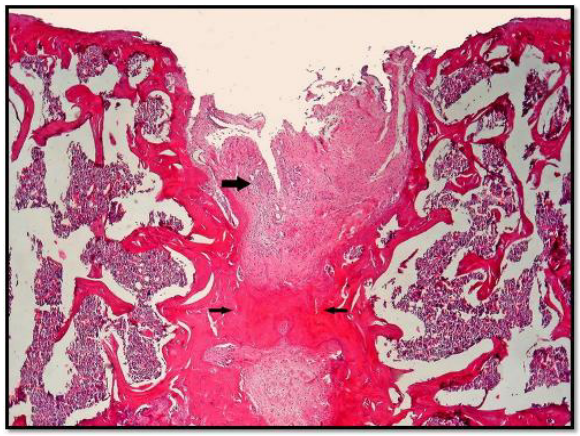

Fig 2: Histologic section from the healing site, in a fluoride treated rat at a dose of 8 mg/lit. Well matured granulation tissue is present in apical portion of the defect (thick arrow). Newly formed lamellar bone in middle portion of the defect is united with old bone tissue at lateral aspects of the repaired construct (thin arrows). This newly formed bone because of vital deposition and calcification of osteoid is more acidophilic. Quantity of newly formed lamellar bone in healing site is less than those of 30 mg/lit fluoride treated rats. However, defect site is bridged with thin layer of trabecular bone buds (H-E, Orig. Mag. ×40)

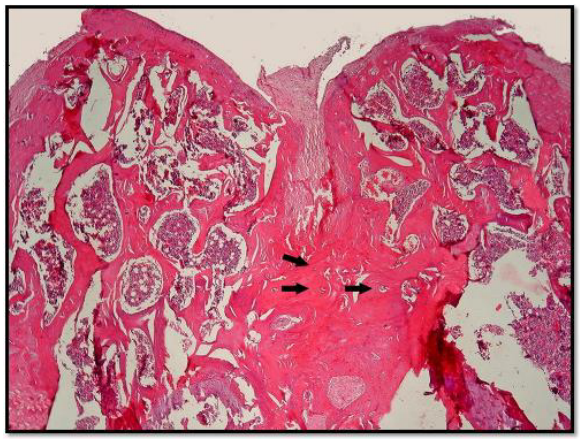

Fig 3: Histologic section from the healing site, in a fluoride treated rat (30 mg L^{-1}). Advanced stage of remodeling and consolidation developing haversian systems (arrows) is seen in repaired construct and almost the defect is filled with newly formed compact trabecuar bone (H-E, Orig. Mag. ×40)

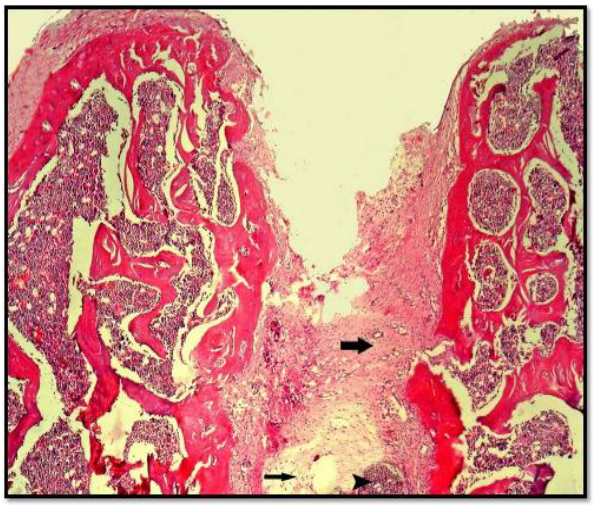

Fig 4: Microscopic section of the healing site in a fluoride treated rat at a dose of 60 mg L^{-1}, reveals the apical portion of defect to be filled with immature granulation tissue (thick arrow) showing active angiogenesis and some degrees of inflammatory cells. Deeper portion of the defect is occupied with loose areolar connective tissue (thin arrow) and bone marrow (arrow head) (H-E, Orig. Mag. ×40)

Table 1: Mean±STD of maximum stress and maximum strain

Max Stress	Control	8 mg L^{-1} Group	30 mg L^{-1} Group	60 mg L^{-1} Group
(Force N)	173.01±23.05	177.31 ±33.71	181.90±36.81	168.51±54.35
Max Strain (mm)	0.83±1.26	0.83 ±1.31	0.76±0.08	0.67±0.19

Biomechanical results: The mean±STD of mechanical test results for each group are provided in Table 1 and Fig 5-8 show biomechanical results for each group. The mean load for fracturing in control group was 173.01±23.05 and in experiment groups 8, 30 and 60mg were 177.31±33.71, 181.90±36.81 and 168.51±54.35.

During the strain energy for all mechanical assessment parameters, the highest mechanical test results were obtained in the group of sodium fluoride treated at a dose of 30 mg L^{-1} and lowest mechanical test results were obtained in the group of sodium fluoride treated at a dose of 60 mg L^{-1}. Differences observed in biomechanically findings were significant between 30mg group and other groups ($p<0.05$).

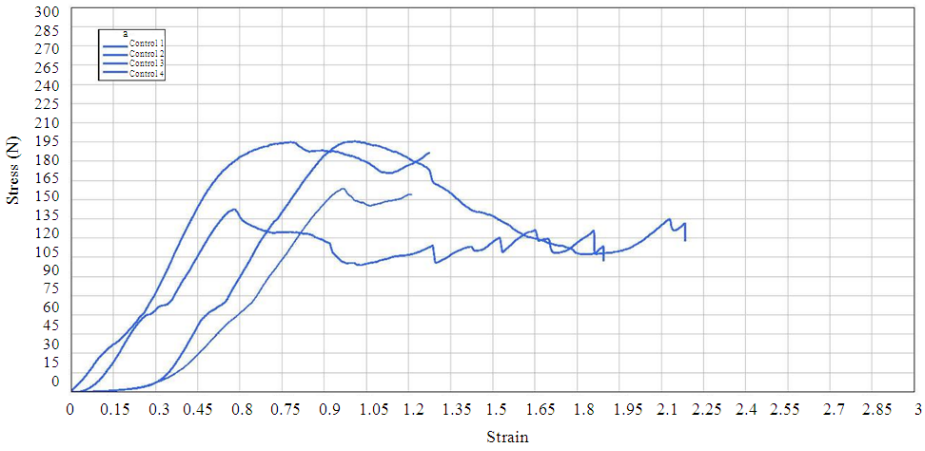

Fig 5: Bone mechanics in the control group

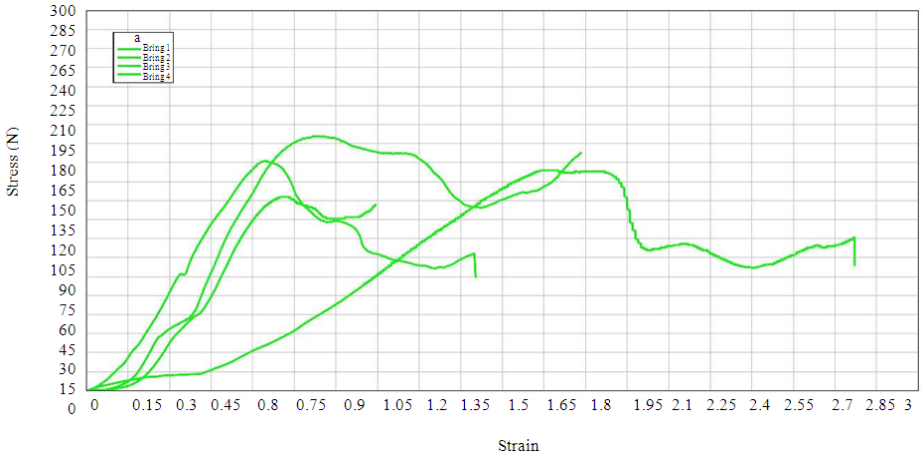

Fig 6: Effects of sodium fluoride on bone mechanics in fluoride treated group at a dose of 8 mg L^{-1}

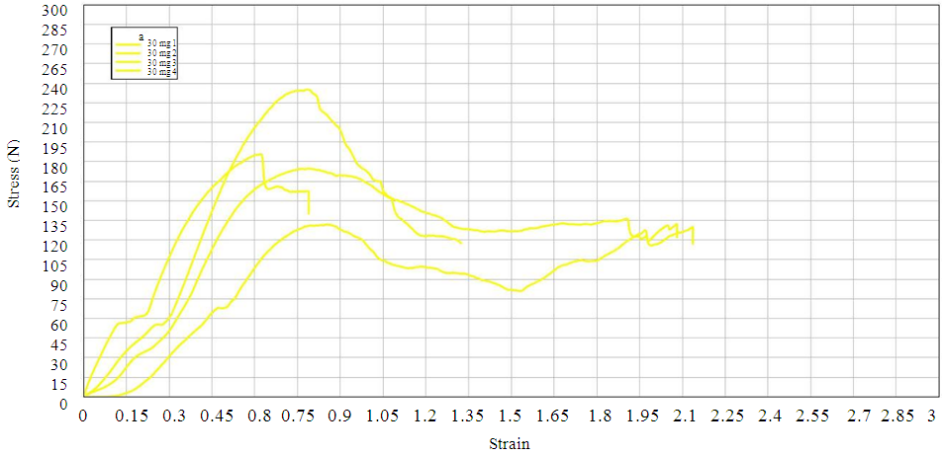

Fig 7: Effects of sodium fluoride on bone mechanics in fluoride treated group at a dose of 30mg L^{-1}

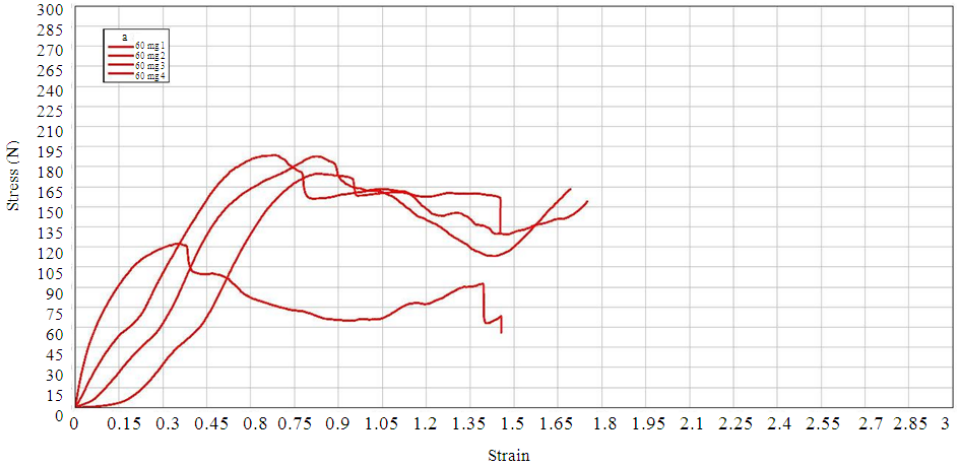

Fig 8: Effects of sodium fluoride on bone mechanics in fluoride treated group at a dose of 60 mg L^{-1}

DISCUSSION

Nowadays, many researchers are trying to find materials or drugs that can improve bone healing (Mousavi *et al.*, 2010; Sharifi *et al.*, 2009; Sadi *et al.*, 2010). This study has shown that exposure to fluoride in drinking water stimulated bone formation in defect area and increase canclous bone volume at a concentration 30 mg F^-/L. Significantly increases the compressive strength of cancellous bone in defect area only at a concentration 30 mg F^-/L. Higher concentration (60 mg F^-/L) decrease the compressive strength of cancellous bone in defect area than other experiment groups and control group. The higher dose of fluoride did not alter bone formation in defect area. The fluoride demonstrated of increased bone mineral mass and significant increase in BMD after treatment with fluoride salts (Bohatyrewicz *et al.*, 2001). Bohatyrewicz has reported the anabolic action of fluoride demonstrated in the form of increased bone mineral mass of whole femoral bones (Bohatyrewicz *et al.*, 2001). The mechanism of the osteogenic actions of the fluoride has not been established. However, Lau *et al*. (1989) have shown a mitogenic action of fluoride on osteoblasts (Lau *et al.*, 1989). Ohta *et al*. (1995) reported fluoride can act as a mitogen enhancer in osteoblast (Ohta *et al.*, 1995). Other studies have shown fluoride inhibits fluoridesensitive phosphotyrosyl protein phosphatase activity (Suzukia *et al.*, 1995; Lau and Baytink, 1993). In this regard, fluoride prevents dephosphorylation protein phosphatase activity in osteoblast cells and fluoride prevents dephosphorylation of phosphotyrosyl proteins believed to be key in mediating proliferation and differentiation of cells and it causes increasing the mitogenic signals generated by endogenous growth factors (Lau *et al.*, 1989; Wergedal and Lau, 1992; Ohta *et al.*, 1995). Similar studies conducted with this study show that findings of these studies are similar with our results.

CONCLUSION

We have recently obtained that fluoride increased cancellous bone formation at defect area in the rat at 30 mg F-/L and reduced cancellous bone formation at defect area in the higher dose.

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