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Histological and Histometrical Studies on the Effects of Fluoride on the Femur in Rats

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Abstract

Background: Fluoride (F^-) is a trace element that is incorporated into bone mineral during bone formation. This study assessed the effect of increasing Fluoride doses on the bone formation and microarchitecture on the Femur of rats by histological, and histometrical methods.

Materials and Methods: A total of 16 rats was divided into one group of control and three groups of animals that received 0.2, 0.4, 0.8 mg/kg of Fluoride daily for 3 weeks by gavage. Rats which were exposed to inorganic Fluoride in drinking water produced significantly more levels of bone lesions than the controls.

Results: Numerous osteocyte lacunae buried at various depths were evident, and the lacunar walls were irregular with mineralized segments running in all directions. The trabeculae of cancellous bone in these animals contained large amounts of osteoid.

Conclusion: The results of the present study indicated that the ingestion of Fluoride affected morphological changes in the Femur of rats.

Keywords: Fluoride; Femur; Osteoid; Rat

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Introduction

Fluoride is a highly electronegative trace element which is the 13th most abundant element in the earth's crust (1, 2). It is highly reactive and it occurs ubiquitously as Fluorides in nature. Extensive research has been carried out on the chemistry and the biology of Fluoride, and on its impact on the human health. Fluoride is toxic when consumed in excess, and has lead to a condition known as fluorosis. Many vital organs and tissue in the body, such as liver (3), kidney (4), cerebrum and cerebellum (5-7) the skeleton (8) and teeth (9) may be damaged by excessive accumulation of Fluoride. Fluoride can show up in surprising places, in our daily needs from chewing gum to drug and cosmetics. Tiny Fluoride can float in the air in the form of dusts/fumes and inhale by workers in the industry, Fluoride also can be consumed from drinking water and tea (10, 11). Water is the major concern. Fluoride is present in the water of any type, but underground water is more contaminated. Fluoride plays a role of a sword with a double edge. Its lower limit (0.6mg/l) and higher limit (1.2mg/l) in human are both detrimental and can cause skeletal and tooth problems. The most common skeletal abnormalities associated with Fluoride toxicity are hyperostosis, osteopetrosis, osteoporosis (12, 13) osteoosclerosis, osteonecrosis and bone deformations (14, 15). These conditions are far more common than many doctors imagine and they are usually misdiagnosed with arthritis or other conditions such as ankylosing spondylitis (16).

The lactating cattle are in the higher risk of developing skeletal Fluorosis than other animals, followed by sheep, and horses (17, 8). Although information regarding fluorosis in human, cattle and other species is extensive, but literature for Fluorosis in horses is scarce. Typical Fluoride teeth and skeletal lesions including hyperostosis and enostosis developed in horses after consuming artificially Fluorinated drinking waterfor an extended period of time (17).

Fluorides are present everywhere and can have a

significant imprdingact in human physiology and health, so, the present study aimed to assess the effects of increasing doses of fluoride on bone microstructure and bone formation in the rat femur using a histologically approach.

Materials and Methods

Sixteen animals of the Wistar rat albino species weighing 180 ± 20g were used in this experiment. Rats were then divided into four groups, settled in polycarbonate cages bedded with paddy husk; commercial pellet diet and water were provided *ad libitum*. Each group consisted of four rats. The animals were maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to food and water in the animal house. All experimental procedures and animal maintenance were conducted in accordance with the accepted standards of animal care. Sodium fluoride (Merck, Germany) was dissolved in distilled water, and administrated in animals in each group. The animals in Group I, were given orally 0.2mg/kg of Sodium Flouride, group II received 0.4mg/kg, and Group III, received 0.8m g/kg oral dose of Sodium Fluoride daily. Group IV (negative control group) received no amount of sodium fluoride. This experiment lasted for 21 days. On the last day of the experiment, all rats were euthanized and a complete necropsy was performed and the femur was dissected and fixed in 10% formalin (Sigma, USA) for 48 hours. After fixation the tissues were dehydrated with descending degrees of ethanol, cleared with xylene (Sigma, USA) and embedded in paraffin wax (18). The sections were cut at 5 µm thick (Leica, Germany), mounted on glass slides and routine stain procedure was performed. The fixed femur bones were decalcified in formic acid (Sigma, USA) for 3 weeks. The specimens were embedded in paraffin blocks in a routine manner, sectioned longitudinally into 4 m thickness and stained with Hematoxylin, Mayer's and Eosin, Y (Sigma, USA). The stained sections were observed under light microscopy (Nikon, Japan) and analyzed using the amount of new bone, mineralized cartilage and the cartilage/bone ratio were measured at the metaphysis beside chondro-osseous junction of femur and expressed in percentage. Alkaline phosphatase activity was determined by Andersch and Szecypinski method (19).

Statistical analysis

The quantitative variables were described by themean and standard deviation. These variables were compared among the groups by the test of variance analysis (ANOVA) followed by the Tukey test to conduct multiple comparisons. The thicknesses of bone trabecules were measured in ten different places using a graticule eyepiece lens and the average of these areas were taken as the thickness of the ostoid. Ten different areas in the sections were measured and mean values were taken into account. Differences were considered significant at $P<0.05$.

Results

Significant increases were observed in the levels of Fluoride, alkaline phosphatase and acid phosphatase in serum in the experimental groups (Figure 1, 2).

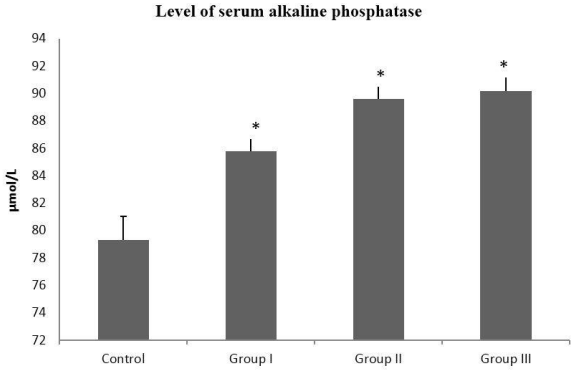

Figure 1. Level of serum alkaline phosphatase in control and experimental group.
* Values are statistically significant at $p<0.05$. Groups I, II, III compared with Group Control.

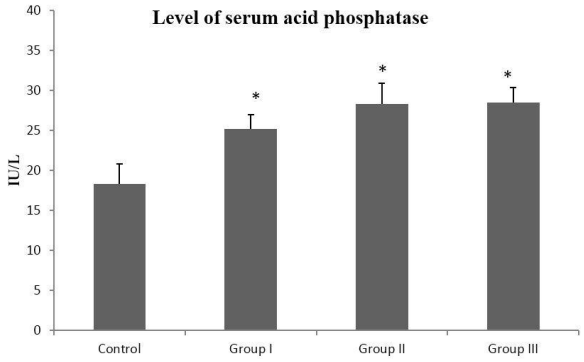

Figure 2. Level of serum Acid phosphatase in control and experimental group
* Values are statistically significant at $p<0.05$. Groups I, II, III compared with Group Control.

The light microscopic studies revealed extensive alterations in the bone cells of Fluoride treated rats (Figure3, 4). The bone cells of control rats showed a normal architecture (Figure3). Significant increase in bone lesion observed in rats treated with fluorosis drinking water (Figure3). The amount of mineralized cartilage was higher in Fluoride-supplemented group than the control group. Clinical growth and mineral consumption of the animals were monitored daily

during the entire 3-week experimental period. Most of the experimental animals died during the first two weeks. Fluoride treatment had a significant effect on bone microarchitecture. All the three treatments increased osteoid formation at the largest Fluoride dose. So, Fluoride treatment demonstrated a significant increase in all osteoid values when compared with the placebo control group.

Discussion

The effect of Fluoride on bone and tooth mineralization (12) has been well recognized. Fluoride is the only one of the few known agents that can stimulate osteoblast proliferation (18) and can increase new mineral deposition in cancellous bone. Fluoride incorporation into the bone increases the size and thus decreases the solubility of bone apatite crystals (1). It has been used clinically for the prevention and treatment of osteoporosis, although controversies still exist. Therapeutic medication for sodium Fluoride in osteoporosis lies with early administration and low-dose regimens in which toxic levels are avoided and mineralization is not impaired (14). Enzyme activities show that a particular tissue is in good health. When a specific enzyme in a particular tissue becomes abnormal, it shows that the structure and function of that organ most probably disturbed. Osteoblasts and osteoclasts secret some of these enzymes and proteins in skeletal response to injury and is a valuable noninvasive tool in determining the extent of bone injury, such as alkaline phosphatase, serum osteocalcin and serum procollagen type I C- and N-terminal propeptides during bone formation (20).

Bone resorption enzymes results from the breakdown of type I collagen and specific enzymes expressed in bone-resorbing osteoclasts, namely tartrate-resistant acid phosphatase, serum carboxyterminal telopeptide of type I collagen and urinary collagen type I cross-linked C-and N- telopeptide (21).

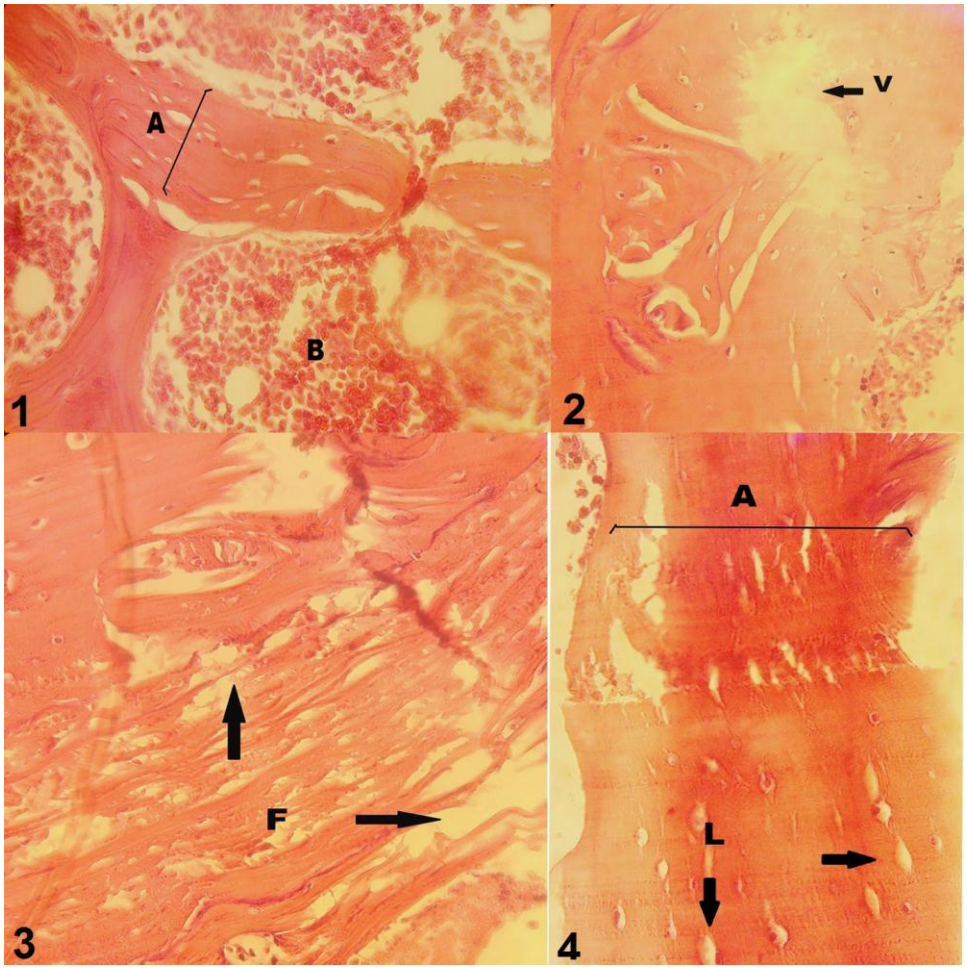

Figure 3. 1- Shows a medium power view of spongy bone of the femural head. The normal size of bone trabeculae is evident (A). In the spaces between the spicules, the myeloid elements of bone marrow can be seen as bluish patches (B). 2- Cracks and vacuoles in the thickened osteoid (V). 3-Widening of the trabeculae with intramembrenous ossification with empty spaces among the unmineralized collagen fibers (F). 4- Thickening of the bone trabeculae in the femural head (A). The lacunae are widened and devoid of osteocytes (L) H&E×40

Alkaline phosphatase and Acid phosphatase are the specific enzyme for bone injury and has been reported in many experiments as the sign of Fluoride toxicosis in bone tissue (22, 23). The results showed that the alkaline phosphatase and Acid phosphatase were higher in the treated group (group II and group III) than control. This assay is readily available and is very efficient in determining the bone lesions in live animals. In treated animals the thickness of the osteoid strikingly increased compared to that of the control. This increase in osteoid parameters was observed in our study already at Fluoride concentrations above 0.4 mg/kg of Fluoride. In this study, histomorphometric analysis of endochondral ossification showed that Fluoride supplement increased the amount of mineralized cartilage. During endochondral ossification, hypertrophic chondrocytes alter the matrix they produce by adding type X collagen and fibronectin to enable it to become mineralized by calcium carbonate. It has also been recognized that resorption of hard tissue is mandatory for bone growth and remodeling (1).

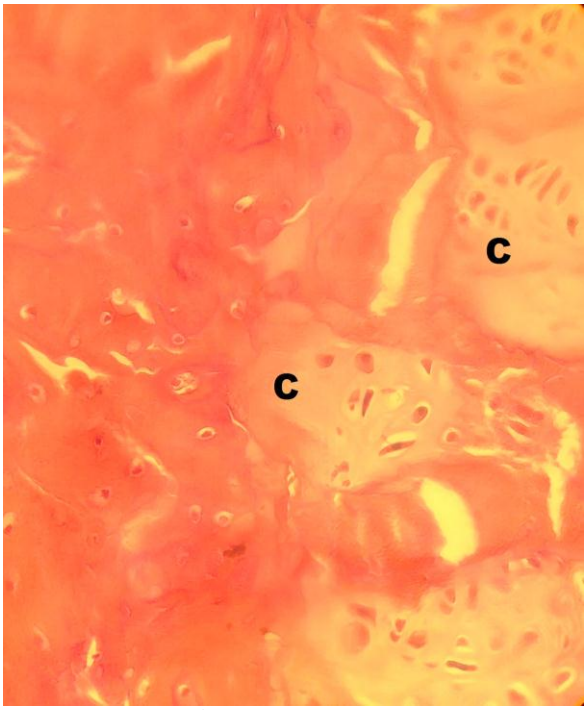

Figure 4. Remnants of the purple cartilage matrix are surrounded by bone (C) staining pink. The bone marrow is not visible in the field of view. H&E×400

Fluoride may stimulate bone formation but it also prolongs the time period of mineralization (12). This was also reported in one study wherein the high Fluoride intake, caused osteomalacia and diminished bone strength in mice and rats (24). The evidence also indicates that Fluoride produces bone mineral with intrinsically weaker material properties than normal mineral. This could be related to one study wherein Fluoride may have some effects on the organic matrix like in the post-translational assembly of the glycosaminoglycan chains which may be an influential factor in the mineralization process (20).
It has been reported that treating both rats and rabbits with Fluoride decreased bone strength despite increases in bone mass and bone volume fraction (25). Based on hisopathological findings, it is concluded that the fluoride supplement to rats with the concentration of 0.8mg/kg significantly affected the shape of the trabecules in femoral bone, the bone spicules thickened and filled the medullary cavity, increasing the bone density.

Conflict of Interest

The authors declare that they have no conflict of interest in this work.

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