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Osteoporotic Features In Rat's Bone after Exposure to Mixed Field of Electromagnetic Field

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Abstract

The aim of the present study is to investigate the biological effect of exposure to extremely low frequency magnetic field (0.5 Gauss), fast neutrons FN (10µSV/h) and a combined field of both for a period of 4 weeks at a rate of 8 h/day, 5 days /week on some of the rat's bone properties. For this purpose 80 adult male albino rats were divided into four equal groups: group A, (control group), group B (exposed to magnetic field), group C (exposed to FN), group D (exposed to combined field of magnetic field and fast neutron).After each experiment bone samples were subjected to the following study: Evaluation of Cu ,Zn ,Mg ions concentrations using atomic absorption technique, measuring of collagen cross linking and degree of crystallinity using FTIR technique and surface scan of bone using scanning electron microscope .The results indicate a highly significance decrease in collagen-cross linking.

Keywords: Extremely low frequency electromagnetic field; Fast neutrons; Collagen cross-linking atomic absorption; FTIR technique and SEM technique

Introduction

Several studies have investigated the biological effect of extremely low electromagnetic field on bone and on some studies increased risk of brain tumors, leukemia and breast cancer have been reported to be associated with exposure from overhead power lines and various consumer devices [1,2,3] recent researches reported deterioration of the mechanical properties of rats bone accompanied by an increase of calcium ions concentration of blood which in turns associated with its decrease in bone after exposure to 50 Hz [4]. However other researchers found that exposure to 50 Hz can induce the uptake of calcium ions in osteoblast [5]. Collagen is the protein that provides bone flexibility and contributes to its tensile strength. A proper cross-linking of collagen is an important determinant of bone mechanical performance, as demonstrated in pathological conditions such as lathyrism, where the cross-linking pathway is blocked. Lysyl oxidase, an enzyme needed in crosslink formation, is activated in the presence of copper ions and pyridoxine (vitamin B 6). Thus, copper or pyridoxine deficiency may weaken the collagen in bone because of the reduced concentration of mature cross-links [6]. Bone mineral content and mineral composition as well as changes in crystal size and perfection, mineral distribution, and/or mineral composition are affected by the health status of the organism. For example, Paget's disease of bone in which the crystal size is decreased, and osteopetrosis where crystal sizes and mineral content are increased [7] Also, it is proved that radiation induces changes in bone [8] and increased crystal size in agreement with the increase seen in magnesium-deficient animals. Recently it was observed that abdominal irradiation as in the adjuvant treatment of gastric cancer results in decrease in BMD and osteoporosis. Insufficiency fracture risk in the radiation exposed vertebral bones is increased [9]. The permissible doses of ionizing radiation, recommended by the ICRP, considered only pure nuclear radiation which can be only possible from radioisotopes [10]. The question now; does these recommended safe limits of exposures can be valid for mixed radiations of non-ionizing and ionizing fields which can occur from nuclear accelerators and x-ay machines? Therefore, the aim of the present work is to study the effects of whole body exposure of rats to 50 Hz - 0.5mG magnetic field, fast neutrons within the permissible dose limits and mixed effects of both magnetic field and fast neutrons on the biophysical and biomechanical characteristics of bone as a step-forward to evaluate the risk associating exposures to mixed radiation fields.

Materials and Methods

One hundred male albino rats of about two months age and average weight of (~150 g ± 10%) were equally divided into four groups namely A, B, C and D. Animals of group A were used as control and did not receive any treatment, Group B animals were exposed to 50 Hz, 0.5 Gauss magnetic field at a rate of 8 hours/day, 5 days/week. Group B was equally divided to two subgroups, namely B1 and B2. Animals of the subgroup B1 and B2 were exposed to the magnetic field for periods of 3 weeks and 4 weeks respectively, Animals of group C were exposed to fission neutrons from ^{252}Cf point source at a rate of 8 hours/day, 5 days/week.at an average dose rate of 10 µSv/h. This group was equally divided to subgroups C1, C2, C3, C4; animals of these subgroups were exposed to neutrons for 1 week, 2 weeks, 3 weeks and 4 weeks respectively. Group D animals were exposed to mixed field of neutrons and 50 Hz, 0.5 G magnetic field for periods like those in the fore mentioned steps. This group was equally divided to subgroups D1, D2, D3, D4. Animals of these subgroups were exposed to Mixed field of neutrons and magnetic field for 1,2,3 and 4 weeks respectively. Left tibia bone was defatted in chloroform /methanol mixture, dried at 110 ^{o}C for 18 hr for water elimination. Then the bone was ashes at 560 ^{0}C for 72 hr and the ash residue of the tibia was digested with 1 ml of 60% HNO3 and diluted up to 10 ml with distilled water .in such preparations, the concentration of Zn, Cu, Mg were determined by atomic spectrophotometer method(AAS). The FTIR spectra of the samples from 4000-400 cm^{-1} were obtained for qualitative and quantitative chemical analysis of the groups exposed to magnetic field, fast neutron, and combined field of both magnetic and fast neutron. The analysis of the normal bone of the animal was served as a reference. Percent crystallinity was measured after peak splitting. The ratio of the areas was calculated above and below the double peak of the phosphate anti-symmetric bending frequency .at approximately 563 to 603 cm^{-1}. The double peak becomes increasingly separated, or split, as crystallinity increases [11]. The splitting factor (SF) or crystallinity index have become the dominant crystallinity measure in archaeology and among the infrared measurements [12] and hence it was used in this study. SF was calculated by summing the peaks heights at 563 cm$^{-1)}$ (A) and 603 cm^{-1}(B) and dividing the obtained value by the height of the line passing in between them (C); All heights were measured from a common baseline drawn from approximately 495 to 780 cm^{-1} under the two peaks 563 cm^{-1} and 603 cm^{-1}.

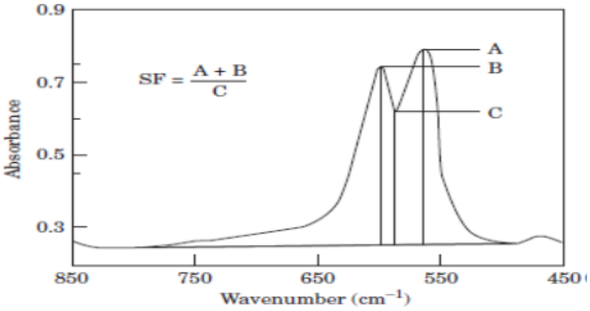

Figure 1: *Crystallinity measurements.*

Table 1: *The selected parameters analyzed for bone quality assessment.*

Parameter	IR peaks assignment	Comments
Crystallinity Index or Splitting Factor	Summing the heights of the 563 and 603 cm^{-1} peak and dividing this value by the height of the trough between them	Degree of HA crystallinity
Collagen Maturity	1660 cm-^{1}/1690 cm^{-1}sub-band area ratio or intensity ratio	Related to variations in collagen cross-linking.

Scanning Electron Microscopy (SEM) is a useful tool to assess microscopic features of materials after failure. The right tibia collected from both control and rats exposed to different radiation kinds are dried well at 60 ^{0}C for 5-6 hours to remove humidity and then coated by thin layer of gold to be examined under the scanning electron microscope.

Table 2: *shows the average value for* ***Mg, Cu and Zn ions*** *concentration for each group demonstrated.*

Group	Mg concentrations mg/g±SD	Cu concentrations mg/g ±SD *10^{-2}	Zn concentrations mg/g±SD
A	75.047±0.46	44.5±0.18	24.226±0.86
B1	59.3±0.92**	5.57±0.02**	13.55±0.32**
B2	28±1.06**	1.98±0.02**	11.25±0.18**
C1	69±1.8**	30.4±0.09**	19.2±0.69**
C2	66±1.07**	26±0.96**	13.18±0.43**
C3	63±2.5**	24±1.92**	7.06±0.09**
C4	52.2±0.83**	14.5±0.42**	6.5±0.43**
D1	71.23±0.87 *	43.382±0.97 n*	20.34±0.47*
D2	67.5±1.05**	40.98±0.01**	19.15±0.25**
D3	65.7±0.46**	36.5±1.06**	17.67±1.2**
D4	50.2±0.85**	12.5±0.42**	7.5±0.35**

It can be observed that the average Mg, Zn, and Cu concentration for each exposed sub-group suffered highly significant decreases relative to control (A), which can be indicated by the degree of significance.

Table 3: *shows the changes of collagen cross-linking for each group demonstrated.*

Group	P1/P2±SD
A	1.13±.06
1B	0.97±.001
2B	0.79±.007
1C	0.79±0.09
2C	0.73±0.05
3C	0.71±0.012
4C	0.626±0.007
1D	0.67±0.03
2D	1.06±.042
3D	0.89±.014
4D	0.79±.004

Where p1/p2 corresponds to 1660cm-1/1690cm-^{1} sub-band area ratio or intensity ratio and vice versa. It can be observed that the average value of collagen cross-linking for each exposed group shows highly significant decreases relative to control (A).

Table 4: *shows the changes in the degree of hydroxy apatite crystallinity for each group demonstrated.*

Group	p1	p2	p3	p1+p2/p3±SD
A	0.068	0.084	0.06	2.52±0.01
1B	0.076	0.093	0.056	3.02±0.013
2B	0.18	0.23	0.12	3.32±0.045
1C	0.35	0.075	0.048	2.78±0.053
2C	0.23	0.29	0.18	2.9±0.007
3C	0.075	0.094	0.049	3.3±0.07
4C	0.15	0.12	0.075	3.64±0.028
1D	0.109	0.137	0.091	2.76±0.042
2D	0.055	0.069	0.043	2.91±0.002
3D	0.12	0.144	0.085	3.09±0.007
4D	0.18	0.22	0.12	3.4±0.07

Where p1+p2 /p3 is the splitting factor and represent the peak area at 563 cm-1 + peak area at 603 cm-1 / peak area between them. It can be observed that the average value of hydroxy apatite crystallinity for each exposed group shows highly significant increases relative to control (A).

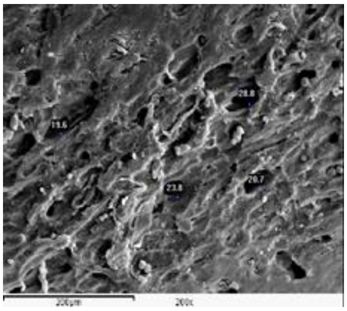

Figure 2: *SEM for control tibia bone. The surface of the cortical plate showed minimal porous architecture denoting compacted well oriented surface apatite crystals.*

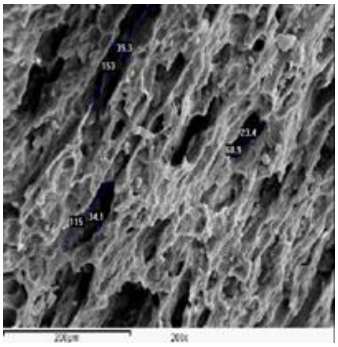

Figure 3: *Variation in bone exposed to magnetic field for 3 weeks (B1). A cleft-like destruction of the cortical plate surface was noted denoting destruction of surface apatite crystals. Around these defective areas an irregular corrugated wavy surface was also observed.*

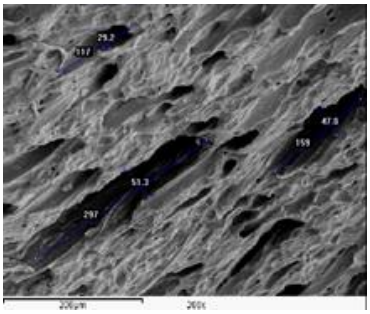

Figure 4: *Variation in tibia bone exposed to 4 weeks magnetic field (B2). Cleft-like defects were more pronounced. Wide loss of the surface crystalline architecture was also noted with exposure of sub-surface layer. The remaining trabeculae were short and sharp icicles-like and showed tapering and breakage.*

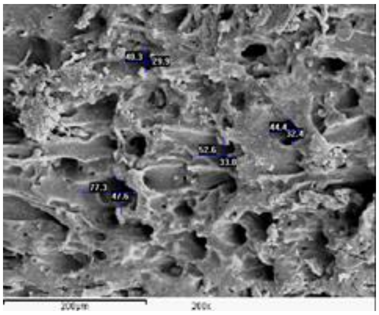

Figure 5: *Abnormalities in tibia bone exposed to one-week neutron. (C1). The normal crystalline structure was lost, and the remaining trabeculae were tapered and irregular. The surface defects were wide-spread with breakage and perforations of surface trabeculae.*

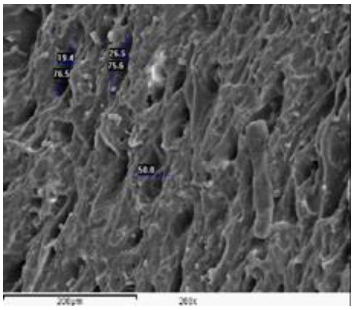

Figure 6: *Shows abnormalities in tibia exposed to two weeks neutron. (C2). The surface of the cortical plate was covered with amorphous irregular layer masking the normal crystalline architecture.*

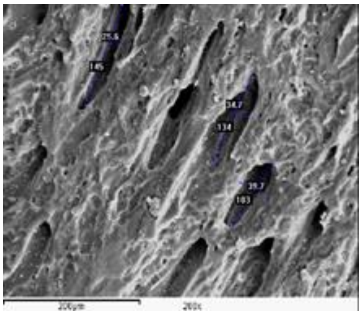

Figure 7*: Abnormalities in tibia exposed to 3 weeks neutron (C3). Cleft-like defects were evident. Wide loss of the surface crystalline architecture was also noted. The remaining surface showed micro porosity with precipitation of thread–like material leading to a sharp outline of these micro pores.*

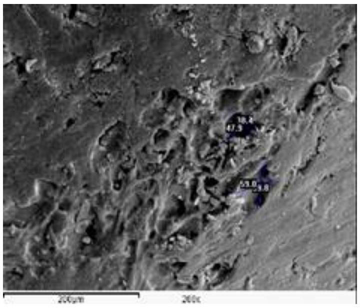

Figure 8: *Variations in tibia exposed to 4 weeks neutron (C4). Filling of the cleft-like defects was noted leading to formation of a homogeneous layer masking the normal crystalline architecture of the cortical plate. However, remnants of defective crystalline structure with surface destruction were observed.*

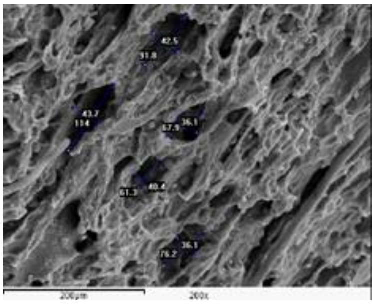

Figure 9*: Variations in tibia exposed to1 weeks mixed field (D1). The normal surface architecture was lost. A total loss of surface crystalline was noted with only remains of the cortical plate. Some of the matrix fibers were exposed.*

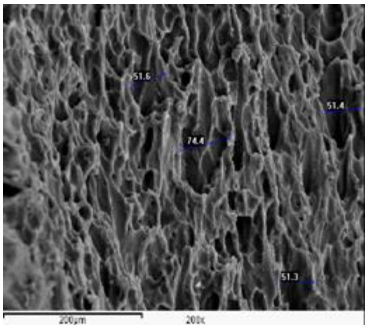

Figure 10*: Variation in tibia exposed to 2 weeks mixed field (D2) Cleft-like defects were more pronounced. Wide loss of the surface crystalline architecture was also noted with exposure of sub-surface layer and formation of osteoporotic-like defects. The remaining trabeculae were short and sharp, icicles-like and showed tapering and breakage.*

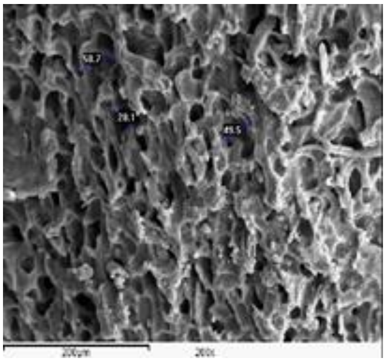

Figure 11: *Variations in tibia exposed to 3 weeks mixed field. (D3) Osteoporotic defects were observed with total loss of the surface crystallinity. The cortical plate was severely affected with total loss of the surface architecture. Exposure of some matrix fibers was also noted.*

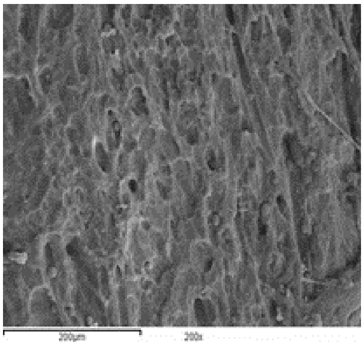

Figure 12: *Variation in tibia exposed to 4 weeks mixed field. (D4). Filling of the cleft-like defects was noted leading to formation of a homogeneous layer masking the normal crystalline architecture of the cortical plate. The remaining micro pores were sharply demarcated and most of them were partially filled with small crystalline material.*

Discussion

To analyze and understand the present finding it is important to discuss the mechanism of interaction of each type of radiation with biological system, especially with bone. The interaction of fast neutrons with biological systems which are all rich with hydrogen is the formation of nuclear recoils, mainly highly energetic protons, these energetic recoiled nuclei migrate in the network of the tissue to cause secondary recoils and destructive highly active species to the network. These highly active species may interact with the molecules forming the main structure of the tissue and the resultant is the formation of deficient regions. As the main structure of bone is hydroxyapatite crystals and collagen; the most probable interactions of fast neutron with bone constitutes is the breaking of the chemical bond between collagen fibrils leading to degradation of collagen fibrils and formation of highly active species of smaller molecules these active species may recombine again at random forming new molecular forms .The degradation of collagen fibers in bone samples after exposure to fast neutrons and /or 50 Hz magnetic field as indicated by the IR results table (3) to table (4) and the scanning electron microscopy images (SEMI) from figure (3)to figure (12) is supported by the fore mentioned analysis. The increase in the appearance of the hydroxyapatite crystals figure (7) occurred as a result of the degradation of the collagen fibers attaching the hydroxy-apatite crystals which permitted their movement away from the bone structure network .A proper cross-linking of collagen is an important determinant of bone mechanical performance ,so when the cross-linking pathway is blocked ,lysl-oxidase ,an enzyme needed in cross linking formation(which is activated by the presence of copper ions and vitamin B6),thus copper deficiency may weaken the collagen in bone because of the reduced concentration of mature cross-links. From table (1) and (2), it can be noted the deficiency of Cu^{++} and the decrease in the collagen cross-linking after exposure to fast neutrons and/or magnetic field. Osteoporotic Trabecular bone has been reported to have a decreased in magnesium content [13] and increased crystal size in agreement with the increase seen in deficient animals, which is the case of the present finding as seen in table (3) and (4).It may be concluded that exposures to mixed radiation fields of both fast neutrons and magnetic field causes loss of proper collagen cross-linking and increase the risk of damage to bone. From this research it was found that:

- Exposures to 50 Hz, 0.5 Causs magnetic field for a period of one month can cause damage to the structural properties of bone which leads to bone damage.
- Exposures to fast neutrons at a dose rate 10 micro-Sievert for a period of one month is biologically toxic to bone.
- Exposures to mixed radiation field of both fast neutron and magnetic field causes loss of collagen cross-linking and therefore bone mechanical properties and can highly increases the risk of osteoporosis.

All these findings are supported by our previous article that studied the effect of such exposures on bone mechanical properties, therefore, it seems necessary to revise the dose limits recommended by ICRP/103 to cover the exposures to different kinds of mixed radiation fields.

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