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RESEARCH ARTICLE

MONITORING OF RELATIVE CHANGES IN RATS' TIBIA BONE CHARACTERISTICS AFTER EXPOSURE TO 4KV/M-50HZ ELECTRIC FIELDS

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ABSTRACT

Recently considerable concerns are increased for the health effects of extremely low frequency electromagnetic fields ELF EMFs, especially power lines frequency of 50-60 Hz. The need for complementary work to assess the health consequences that may be attributed to the exposure to such fields is a must. The present work is aimed to study the effects of 4kV/m -50 Hz electric fields EF on biomechanical and biochemical properties of rats' tibia bones. Similarly to occupational workers the assigned experimental animals were exposed 8 h/day for different exposure regimes of 5,10,15,20 and 30 days.At the end of the exposure periods, thetibia bone samplesare carefully collected, dissected free and cleaned of soft tissues. Effects of exposure on collected tibia samples from each group were obtained and monitored relative to un-exposed ones. Characteristic stress-strain curves were established at rate of 0.5 mm/min and biomechanical parameters were determined for all assigned tibia samples. Furthermore, all tibia samples were put in furnace to obtain bone ash and concentration of calcium, phosphorus and collagen were measured. The results obtained by overall biomechanical and biochemical parameters were significantly decreased and relatively changed by remarkable ratios as compared to unexposed ones. On conclusion, the present findings supporting the hazardous effect of exposure to ELF EFs and its effects are highly correlated to the exposure duration. In addition, bone is substantially sensitive organ for exposure to ELF EFs and may be losing its fine structure and its material form as long as it exposed.

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INTRODUCTION

Electric and magnetic fields are appearing in the environment, existing in homes and all other locations powered by electricity. Electrical workers are thought to have special sources of exposure due to work in proximity to energized equipment, such as power lines, electrical appliances and electricity engineering equipment (Kunt *et al*., 2016). Still the question that can exposure to electric field and magnetic fields at extremely low frequency cause any health consequences and adverse health problems?. Recently, the public has explicit concerns about the possible biological effects of non-ionizing and non-thermal electromagnetic fields.

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Some researchers suggested that these fields negatively affect blood biochemistry, digestive and circulatory systems, and increase the risk for cancer. Moreover, exposure toELFEM radiation may have effects on the nervous, cardiovascular, liver, and hematology system of workers (Liu, 2013). Bone cells regeneration is a complicated process at which resorption of old bone by osteoclasts and the subsequent formation of new bone by osteoblasts are always performed in our lives. The former process is highly based on bioelectrical cell activity and therefore the possibility of any externally appliedelectric energy could modify the behavior of those cells is highly considered (Diniz, 2002). Worthy note that, the balance between construction and destruction in the bone metabolism of the electrical workers who were employed in high voltage electric transmission power lines is shifted towards destruction and led to a decrease in osteoprotegerin levels and thyroid metabolism and also increases oxidative stress index by increasing the total oxidant status and

decreasing the antioxidant status. Although this sounds to be true, the pathways of the effects of such fields are not clear. Regardless of that the effect of extremely low frequency (ELF) magnetic field (MF) (17.96 µT- 50 Hz) on spinal cord injury (SCI) induced osteoporosis in rats (Manjhi, 2013). Howeverexposure to electromagnetic fields of extremely low frequency (ELF, less than 100 Hz) and amplitude may have relevant effectson parameters such as body weight, blood glucose and fatty acid metabolism (Gabriele Gerardi *et al*., 2008). GöknurGüler *et al*. 2009 (Kaune, 1979), found that there is statistically increasing in hydroxyproline levels which may be caused by early formation of stable oxidized protein and changing the direction of the formation reaction of oxidized proteins then structural damage in protein synthesis due to ELF electric field. Sert C *et al*, 2002 (Frahm *et al*., 2006), showed that the low-frequency electromagnetic fields have some biological effects on the action of the cellpopulations of bone. It increases the maturation bone trabecula, bone volume, and boneformation. The present work is aimed to study the effects of 4 kV/m-50 Hz electric field on the biomechanical and biochemical characteristics of rats' tibia bones. Same as occupational workers animals were exposed 8h/day for different periods of 5,10,15,20 and 30 days/week.

MATERIALS AND METHODS

The exposure mean is carried out by using cage of Perspex chamber, with an exposure volume of dimension 100x30x35 cm^3 located between two parallel aluminum plates, which extended vertically along two parallel sides of the exposure cage. In order to prevent any animal shock from direct contacts with the electrodes, the aluminum plates were covered by front fixed Perspex plates of 0.01m thickness. It is worthy to mention that, the Perspex material has a negligible effect on the field homogeneity (Charles, 2001). The two Al-electrodes were connected to a step up transformer with an output voltage of 4kV/m and 50Hz when connected to the main supply. For more precautions an electric timer was used to adjust the exposure times specially when mains fall. The electric field inside the chamber was measured through the use of field meter (ETS-Lindgren HI-3638 ELF/VLF Electric Field Meter) and was found to be homogeneous and reads (4± 0.04) kV/m.
In the presence work 65 male albino rats, each of average weight 200± 10 gm. divided into five main groups, namely group A, B, C, D, E and F. Animals of group A (15 animals) are used as a unexposed group and didn't receive any treatment and housed at normal environmental conditions (the temperature inside the lab varied between 240 and 270 C, lighting condition are natural light from large windows during the day and complete darkness during the night). Animals of group B, C, D, E and F(10 animals per each) were exposed 8 h/day to an electric field of 4 kV/m-50 Hz for periods of 5, 10, 15, 20 and 30 days respectively. At the end of the exposure period the animals were immediately sacrificed and bone samples from each animal's tibiawere collected for experimental investigation.For further biomechanical and biochemical analysis each replicates of tibia bone samples was dissected free, cleaned of soft tissues, soaked into saline and then frozenat –20°C in an airtight plastic bag until testing.

The biomechanical analysis was carried out by usingconfigurable motorized tensile testing stand (ESM300-MARK-10) for tension and compression applications with force gauge up to 300 lb F (1.5 kN). It is worthy to mention here that before testing, bone specimens were thawed in saline at room temperature for at least 3 h (Turner, 1993). Further, each bone specimen was exposed to different loads and its corresponding displacement was recorded and then after load-displacement curve is performed.The structural and material characteristics were obtained from samples load-displacement and stress-strain curves.Maximum load, displacement, stiffness, energy absorption capacity (structural properties); ultimate stress, ultimate strain, elastic modulus and toughness (material properties) were calculated. On the other hand, bone specimens were extracted for calcium assays and put in furnace to obtain bone ash. Each specimen is weighted before analysis then prepared by dissolving in 10% nitric acid over a period of 24 hr. Calcium assays are preformed via atomic absorption spectrophotometer. Calcium ions form a violet complex with o-cresolophthaleincomplexone in alkaline solution. The intensity of violet color of this complex measured at 570-580 nm is proportional to the calcium concentration in the sample. Then after, the raw assay data were normalized to the initial specimen weight. Similarly, the phosphorus content was measured with a spectrophotometric method using a reagent kit (Analco Co., Poland) (Burr, 2002). Moreover, the bone collagen concentration was performed by calculating the total amount of hydroxyprolinein each sample, assuming300 hydroxyprolineresidues per collagen molecule and a molecularweight of collagen of 300,000 D, and was normalized toeither tissue dry weight or total protein weight (Gonzalez-Riola, 1997). The statistical analysis was performed using the student's *t*-test with a minimal confidence level of 0.05 for statistical significance. Each experiment was performed at least three times per each sample replicate and its average values were considered with its standard deviations.

RESULTS AND DISCUSSION

Recently, considerable concerns are increased to evaluate any hazardous health effects attributed to exposure of electromagnetic fields specially non-ionizing and non-thermal ones. All of interest was assigned to the exposure of ELF magnetic fields and its possible interaction modes with biological systems. On the other hand, little concerns were directed to the exposure of ELF electric fields which is not less importance than magnetic fields especially around systems where it draws low currents and high voltages. The importance of electric fields comes from its being generated without need the mains to be plugged in as it could be found elsewhere a static charges are found. These types of fields consist of low energy that not sufficient to cause neither ionization nor thermal effects. Several mechanisms by which weak electric or magnetic fields might produce biological non thermal-effects are being proposed. Even if strong intensity fields have effects based on physical principle as field-charge interactions, field induced dipole, electrical breakdown of cell membranes (electroporation), weak field effects are major concern and have been much debated in the literature.Mechanisms are related to chemical kinetic effects or physics phenomenons.
Several authors have speculated in the bioeffects literature that alternating magnetic fields may interact with biological systems by affecting chemical reactions involving free radical mechanisms. Many others have proposed mechanisms for biological effects of ELF fields based on resonance response in biological systems. Bone is considered as one of the most sensitive organs in the body to such fields and could be affected in different manners. Moreover, intrinsic bone bioelectricity may increase the ability of external fields to

Table 1. Biomechanical parameters for all collected tibia samples from each group (mean values ± S.D)

Groups	Displacement at Max. Load (mm)± S.D.	Maximum Load (N) ± S.D.	Stiffness (kN/mm)± S.D.	Energy to max. force (mJ)± S.D.	Elastic Modulus (MPa) ± S.D.	Ultimate stress (MPa) ± S.D.	Ultimate strain ± S.D.
A	0.1125 ± 0.0023	610.49 ± 11.8	7.06 ± 0.7	40.5 ± 0.9	950.1 ± 7.32	87 ±.6.8	0.075 ± 0.004
B	0.1154 ± 0.0034	550.69 ± 22.2	7.04 ± 0.4	37.6 ± 0.7	937.7 ± 8.03	79 ± 7.4	0.078 ± 0.003
C	0.1147 ± 0.0025	460.59 ± 17.9	7.15 ± 0.6	31.2 ± 1.8	931.2 ± 6.81	66 ± 9.4	0.076 ± 0.005
D	0.1267 ± 0.0031	390.48 ± 30.4	6.29 ± 0.7	29.3 ± 1.1	887.3 ± 9.33	56 ± 9.1	0.085 ± 0.002
E	0.1305 ± 0.0029	370.42 ± 24.4	6.58 ± 0.5	28.6 ± 2.1	842.7 ± 7.92	53 ± 8.2	0.087 ± 0.006
F	0.1317 ± 0.0019	350.41 ± 17.9	6.31 ± 0.9	27.3 ± 1.2	836.1 ± 8.16	50 ± 7.1	0.089 ± 0.004

Table 2. Calcium, Phosphorus and Collagen concentrations for all collected tibia samples from each group (mean values ± S.D)

Groups	Average calcium conc. (mg/dl). ± S.D.	Average phosphorus conc. (mg/dl). ± S.D.	Average collagen conc. (mg/100mg)% ± S.D.
A	319 ±9	188±5	4.3 ±0.1
B	292 ±9	174±8	3.6 ±0.4
C	276 ±9	153±6	3.1 ±0.1
D	257 ±9	155±6	2.7 ±0.3
E	234 ±8	140±6	2.4 ±0.2
F	229 ±8	119±4	2.2 ±0.2

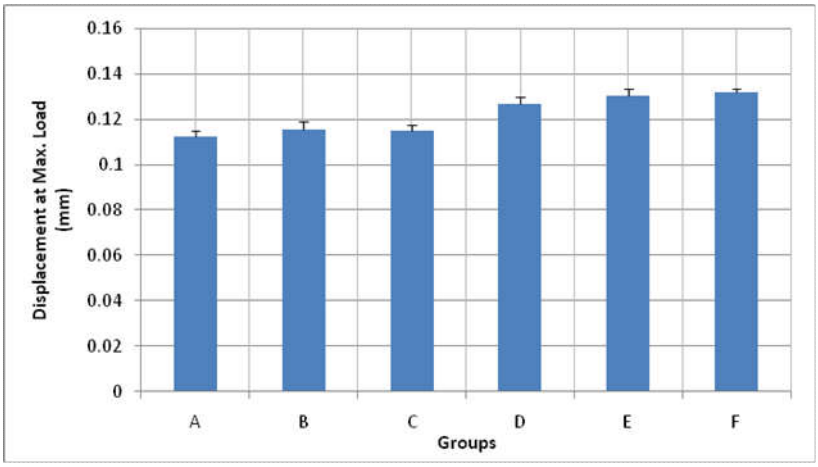

Figure 1. Average values of displacement at maximum force for tibia samples collected from each group

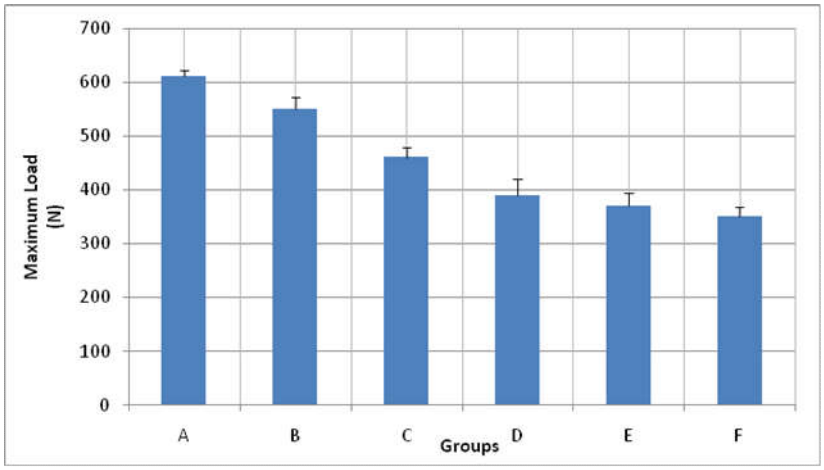

Figure 2. Average values of maximum load for tibia samples collected from each group

cause behavioral changes in bone cells (Gürgül, 2008). An ELF magnetic field can induce differentiation of cartilage cells and alter alkalinephosphatase activity in rat osteoblastic cells (Oksztulska-Kolanek, 2016). ELF-MF couldbe effective in epiphysial growth, bone build-up, and fracturerepair (Goodyear, 2012). The quality (biochemical and biomechanical properties) of rats bone are decreased by magnetic field exposure (Walleczek, 1992).

The present work is evoked to study the effects of 4kV/m-50 Hz electric field on the biomechanical and biochemical characteristics of rats' tibia bones. The chosen exposure level could be found elsewhere in the close area to power lines, some electric appliances and even much smaller as compared with electric fields close to x-ray transformer units. Similarly to occupational workers animals were exposed 8h/day for different periods of 5,10,15,20 and 30 days/week. Functionally, the most important mechanical properties of

bone are its strength and rigidity. These and other biometric features are best evaluated by analysis of their behavior under loads (Martin, 2013 and Shankar, 1998). Therefore, rats' tibia samples from all studied groups were exposed to tensile stresses of different loads at specific rate of 0.5 mm/sec, its corresponding displacements were measured and load-displacement curves were performed.

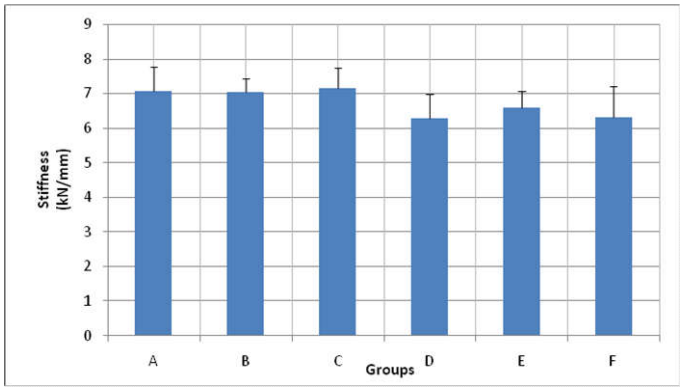

Figure 3. Average values of stiffness for tibia samples collected from each group

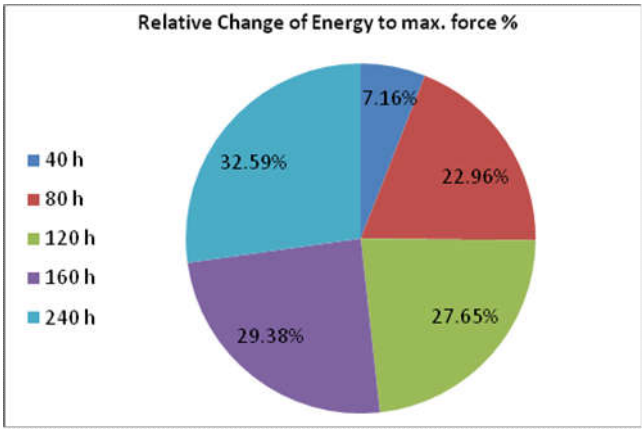

Figure 4. Relative change percentages in energy needed to break at maximum force for tibia samples from all groups

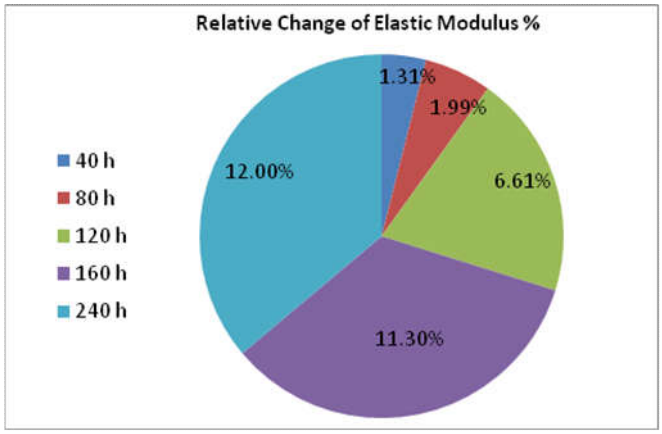

Figure 5.Relative change percentages in elastic moduli for tibia samples from all groups

The ultimate tensile strength, displacement, stiffness and energy absorption capacity (structural properties) were determined. Furthermore, the load-displacement recordings were normalized by cross sectional area and converted to stress-strain curves. Ultimate stress, ultimate strain, elastic modulus and toughness (material properties) were obtained. Nevertheless, the results of biomechanical testing may vary depending on the analyzed disease and should be compared to a control group of animals (Ali, 2007).

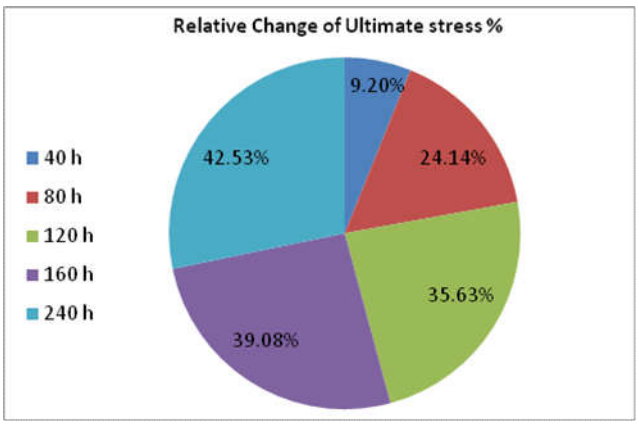

Figure 6. Relative change percentages in ultimate stress for tibia samples from all groups

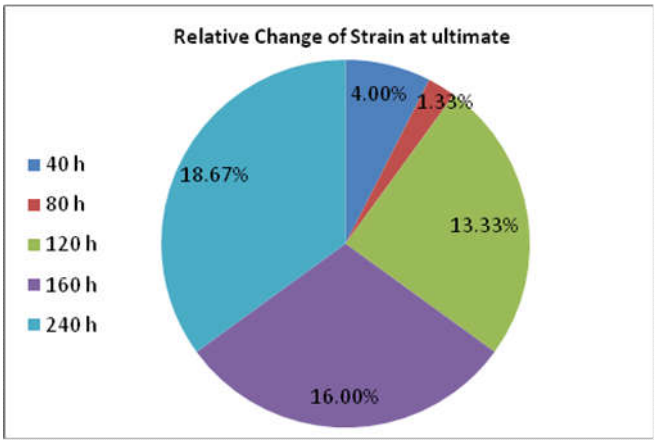

Figure 7. Relative change percentages in strain at ultimate values for tibia samples from all groups

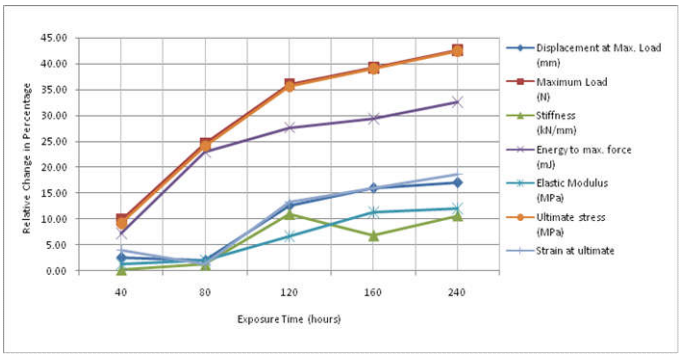

Figure 8. Relative change percentages in each biomechanical parameter at different exposure times

Significant differences in all assigned biochemical and biomechanical parameters were monitored relative to unexposed values and its relative change percentages were calculated. Table-1 lists the biomechanical parameters values for examined tibia rat's samples from each group. The displacement at maximum load data didn't show vast differences compared to unexposed group A but statistically it were significant ($p<0.05$) as depicted in Figure-1. Mean maximum loads were very highly significantly ($p<0.001$) decreased for groups D, E and F by ratios of 36%, 39% and

42.6% respectively and insignificantly ($p<0.09$) were decreased 9.7% and 24.5% respectively) for groups B and C as compared to group A. Figure-2 shows the prominent differences in mean maximum loads for exposed groups which may be attributed to the changes of the general integrity of bone structure as a result of exposure (Lilien, 2009; Roda-Murillo, 2005). Extrinsic stiffness of tibia samples showed enormous highly significant ($p<0.01$) reduction for groups D, E and F in comparison to group A, while the other tibia samples from groups B and C didn't show notable differences as revealed in Figure-3. The extrinsic stiffness is a structure property which is closely linked to the mineralization of bone (Lilien, 2009; Roda-Murillo, 2005 and Frahm, 2006). and so, it may be directly correlated to the calcium and phosphorus concentrations in tibia samples. Our results of mineral concentrations showed similar conformity on obvious reductions for groupsD, E and F.

The data presented in Table-2 indicated thatfor groups D, E and F; the calcium concentrations showed highly significant rebate ($p<0.01$) by ratios 19.4%, 26.6% and 28.2% and the phosphorus concentrations showed significant lowering ($p<0.05$) by ratios 17.6%, 25.5% and 36.7%. Despite of the tibia samples collected from groups D, E and Fbeing broken at farthest displacement but still the maximum load needed for the break is low and so, the work done for the breaking process is small compared to group A. Figure-4 indicated remarkable shift in the energy absorbed to maximum load into lower values as much as exposure time increased with its maximum shift for group F (32.6%) in comparison to group A. On the same regards the material properties of tibia samples depicted intrinsic remarkable changes as much as exposure time increased. Modulus of elasticity for groups D, E and F showed significant ($p<0.05$) decrease (6.6%, 11.3% and 12% respectively) as shown in Figure-5. Worthy note that modulus of elasticity is a value measure of intrinsic stiffness of bone material (Lilien, 2009 and Frahm, 2006), and it is associated to the collagen concentration in bone specimen. Moreover, ultimate stress and strain parameters contribute to bonecollagen integrity (Yavuz, 2008). The results of groupsD, E and F indicated highly significant ($p<0.01$) decrease in the ultimate stress (35.6%, 39% and 42.5% respectively) as depicted in Figures-6 and significant ($p<0.05$) changes in the ultimate strain (13.3%, 16% and 18.6% respectively) as shown in Figures-7. The results of collagen concentration in agreement to the former biomechanical results as presented in Table-2, collagen content in tibia samples from groups D, E and F is significantly ($p<0.05$) deteriorated by ratios 37%, 44% and 49% respectively.

Our results are in accordance with data of Gonzalez-Riola etal. (Adiguzel, 2008), as it showed that 30 days exposure to ELF-MF at aflux density of 3 mTcould reduce bone mineral density and bonemineral contentvalues of Oncis France rat femurs. Also similarly, SerkanGürgülst al. suggested that the exposure to ELF-MF (50 Hz, 1 mT) may reduce the bone quality by affecting mineralizationand collagen integrity (Kaya, 2009). The observed differences in bone mineral content may be turned to the decrease and damagingthe regulation of Ca2+ uptake into cells (Walleczek, 1992). Another review supported possibility of the activation of voltage-gated calcium channel which in turn leads to rapid elevation of intracellular Ca2+, nitric oxide and in some cases at least, peroxynitrite (Martin L. Pall, 2013). It may be presumed that the effect of such fields on a live biological system is the interference of these fields waves with bioelectric impulses generated during physiological processes. This interference may cause either inhibition or enhancement of the physiological mechanisms, depending on the mode of interaction. Shankar *et al*. (1998) suggested that ELF-MFranging from 0 to 1.8 mT could damage the signal processbetween Ca2+ receptor system and calcitonin in osteoblasts.They also indicated that ELF-MF might stimulate theresorptional activity of osteoclasts by inhibiting the effect ofcalcitonin. One other effect of the electric field on a biological process is the fact that when a charged particle is moving in an electric field, it suffers deviation from its pass-way in addition to its acceleration. These changes in the ionic motion under the influence of the electric field may be the main reason for the changes in elemental concentrations in the different organ. Exposure to external ELF electric and magnetic fields induces electric fields inside the body (Lilien *et al*., 2009). The cell membrane has been supposed to be a target for the EMF. This hypothesis pointed that an EMF can induce ligandreceptor interaction and stimulates ion transport channels (Roda-Murillo *et al*., 2005; Frahm *et al*., 2006). One possible explanation as of why the trace elements of enamel and dentin were affected by ELF treatment is that ion transports can be altered by the ELF magnetic field (Yavuz *et al*., 2008; Adiguzel *et al*., 2008; Kaya *et al*., 2009). The former explanations depicted clearly accordance of exposure on the bone quality in different way especially ionic transportation and cell membrane structure.

Moreover, the results showed relationship between exposure periods and its effects on bone. The results started at the beginning of exposure with steep differences and followed by shallow changes in comparison to unexposed group as reveled in Figure-8. It is noteworthy, the shallow changes in the bone parameters at the beginning of exposure is agreed to the undetected responses of the exposure to such fields in daily life as it need very long time of exposure to cause remarkable health consequences.

Conclusion

It is concluded from the present findings that exposure to such fields may cause significant physiological alterations on properties of bone. Also, one may conclude that exposure to 4-kV/m; 50-Hz electric field for occupational workers is risky and may cause health consequences. Further, the need for more concerns should be taken regarding such fields and new exposure limits have to be proposed in the future.

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