



## **HISTOLOGICAL AND ULTRASTRUCTURAL EFFECT OF ZINC SULPHATE SUPPLEMENTATION ON THE ALVEOLAR BONE OF CALCIUM DEFICIENT RATS**

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### **ABSTRACT**

**Objective:** The aim of the present study was to investigate the efficiency of zinc sulphate supplementation in treatment of the alveolar bone osteoporosis in rats induced by calcium deficient diet.

**Design:** Thirty adult male Wistar rats with an average weight of 200-250 grams were randomized into 3 groups. Group I (control group): received standard rat chow, Group II (osteoporotic group): received calcium deficient diet and Group III (zinc treated group): received calcium deficient diet plus oral supplementation of zinc sulphate. After 3 months, the rats were sacrificed. The mandibles were examined histologically, histomorphometrically, ultrastructurally and by energy dispersive X ray microanalysis (EDX).

**Results:** Histologically the impaired bone quality induced by calcium deficiency were significantly improved by zinc supplementation. The alveolar bone restored its structural organization in form of thick bone trabeculae, decrease number of osteoclasts and regularly distributed osteocytes. Histomorphometric analysis showed significant reduction in area percentage of alveolar bone trabeculae in osteoporotic group ( $p < 0.001$ ), while zinc treated group showed significant increase in the area percentage of bone compared to the osteoporotic group ( $p < 0.001$ ). Ultrastructurally, surface roughness and irregularity of the buccal cortical plate were observed in osteoporotic group. However, zinc treated group showed smooth and regular bone surface with minor irregularities. EDX microanalysis indicated a significant decrease in calcium content in osteoporotic group ( $p < 0.001$ ), while zinc treated group showed significant increase in the calcium percentage of bone compared to the osteoporotic group ( $p < 0.001$ ).

**Conclusions:** In a rat model zinc supplementation prevents the bone loss induced by calcium deficient diet.

**KEY WORDS:** Zinc, Alveolar bone, Osteoporosis, Calcium deficiency

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## INTRODUCTION

Osteoporosis has been identified as one of the major public health problems characterized by decrease in the bone mass and micro-architectural deterioration of bone tissue. The most dramatic presentation of the disease, especially in the elderly, is increased bone fragility and susceptibility to fracture<sup>(1)</sup>.

The development of osteoporosis usually occurs in postmenopausal women<sup>(2)</sup>, however in men it is thought to be primarily related to aging, glucocorticoid use, and hypogonadism. Some modifiable factors, including excess alcohol consumption, smoking, physical inactivity, and deficiency or excess of some dietary components, are also among the predisposing factors<sup>(3)</sup>.

It has been observed that the general aging population suffer from both malnutrition and under nutrition, with subsequent osteoporosis. This emphasizes the concept that bone homeostasis is regulated through various nutritional factors in maintaining bone health in long life<sup>(4)</sup>.

Calcium is a vital component of bone architecture and is essential for deposition of bone mineral throughout life. It is also necessary for neuromuscular activity, blood coagulation, and normal cardiac function<sup>(5)</sup>. Although the body stores more than 99% of its calcium in the bones and teeth, it is also found in the extracellular fluid (ECF) or plasma. If the plasma calcium level decreases, bone resorption increases to restore its normal level<sup>(6)</sup>.

In growing animals, calcium deficiency impairs growth, delays consolidation of the skeleton. In adult animals, it causes mobilization of bone and leads to osteoporosis<sup>(7)</sup>.

Nutritional and food factors supplementation may play a part in the prevention of bone loss with aging and have been to be worthy of notice in the prevention of osteoporosis<sup>(8)</sup>. Zinc, has been identified as one of the essential trace elements

that have a stimulatory effect on osteoblastic bone formation and an inhibitory effect on osteoclastic bone resorption, thereby increasing bone mass<sup>(9)</sup>.

Zinc enhances osteoblastic bone formation and mineralization by directly activating aminoacyl-tRNA synthetase, a rate-limiting enzyme at the translational process of protein synthesis, which stimulates cellular protein synthesis<sup>(10)</sup>. It is also present in the active site of alkaline phosphatase enzyme (ALP) which is involved in the production of inorganic phosphate and the calcification of the extracellular matrix (ECM). Depriving pre-osteoblasts of zinc reduces their differentiation capacity by decreasing ALP and osteopontin expression, as well as calcium deposition<sup>(11)</sup>.

Moreover, zinc has been shown to inhibit osteoclastic bone resorption through inhibition of osteoclast-like cell formation from bone marrow cells and stimulation of apoptotic cell death of mature osteoclasts. In addition, it has a suppressive effect on the receptor activator of nuclear factor kappa B ligand (RANKL)-induced osteoclastogenesis<sup>(10,12)</sup>.

The oral administration of zinc has been shown to prevent bone loss in ovariectomized rats<sup>(13)</sup>. Animal feeding with calcium deficient diet is another method for induction of osteoporosis in animal models which has a more negative effect on bone volume and microarchitecture than ovariectomy<sup>(14, 15)</sup>. So the aim of this study was to determine whether zinc supplementation has positive effects on alveolar bone structure deteriorated by calcium deficiency in rats.

## MATERIALS AND METHODS

### Experimental procedure

The study protocol was approved by the Ethical Committee of Faculty of Dentistry, Alexandria University. Based on PASS program version 12 (power analysis and sample size calculation) which is used to calculate the minimal required

sample size of study, a power calculation was performed to determine the sample size. The animal was considered the study unit. A sample size was desired that would provide 80% power to recognize a significant difference of 10% among groups with a 95% confidence interval ( $\alpha=0.05$ ), considering the change in alveolar bone surface area as the primary outcome variable. Therefore, a sample size of ten animals per group was required.

Thirty adult male Wistar albino rats with an average weight of 200-250 grams were used in this study. They were obtained and kept in the Institute of Medical Research, Alexandria University. The animals were housed four rats per one metal cage at  $25\pm 1^\circ\text{C}$ , 12 hour light/12 hour dark cycle and  $55\%\pm 10\%$  humidity. A standard diet was provided with water ad libitum. After 15 days of

acclimatization, rats were randomly divided into three groups, 10 rats each (Fig. 1).

**Group I (control group):** rats were fed the standard rat chow, which contains 1.2% calcium.

**Group II (osteoporotic group):** rats were fed calcium deficient diet containing 0.13% calcium.

**Group III (Zinc treated group):** rats were fed calcium deficient diet containing 0.13% calcium and additionally received daily zinc sulphate solution at a dose of 50 mg/kg using oral gavage syringe. The zinc sulphate solution was prepared by dissolving 10 grams of zinc sulphate in one liter of saline. Therefore, the rat weighing 200 grams were given one ml of this solution<sup>(16)</sup>.

Both group I and group II received daily 1 ml of saline orally. All oral solutions were given daily at 10 am by oral gavage syringe.

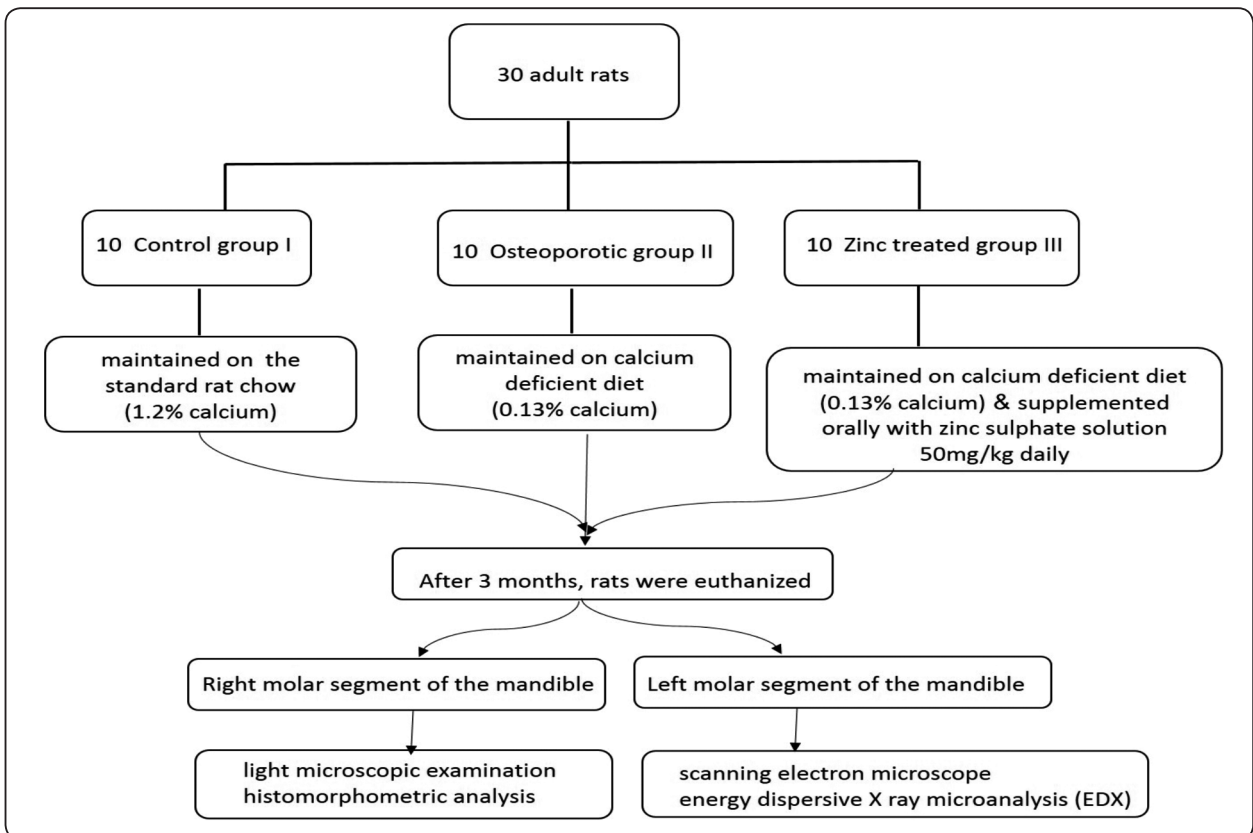


Fig. (1) Flow diagram showing allocation of animals into different experimental groups and different methods of samples' assessment.

Three months after the beginning of the study, all the animals were euthanized with a lethal dose (150 mg/kg body weight) of sodium thiopental.

The mandibles were excised. The molar region of the right side of the mandibles were processed for light microscopic examination and histomorphometrical analysis, while the molar region of the left side of the mandibles were processed for scanning electron microscope examination and energy dispersive X ray microanalysis (EDX).

### Light microscopic examination

The right halves of the mandibles were fixed in 10% neutral buffered formalin for 48 hours, and then rinsed in distilled water. Specimens were decalcified in 10% EDTA, dehydrated in ascending grades of alcohol, cleared in xylene and then embedded in paraffin wax. From each specimen, 20 mesiodistal serial sections of 5  $\mu\text{m}$  thickness were cut. The sections were stained with haematoxylin and eosin stain according to the conventional method<sup>(17)</sup>. Histological examination was performed by a blinded and certified histologist using light microscopy.

### Histomorphometrical analysis

Morphometric evaluation of the percentage of surface area of the alveolar bone was assessed for

each specimen using the (Image J 1.46) software. Five mesiodistal sections of tissues from each animal, at different standardized depths (taken every 100  $\mu\text{m}$ ) were used for quantification. One photograph was taken from each section using the same magnification power. A rectangle with a standard dimension was drawn. The total surface area of this rectangle was measured using the image J program by choosing the region of interest (ROI) manager and the measurement was recorded.

For determination of the area occupied exclusively by bone tissue, the bone marrow spaces were measured (Figure 2), summed and the result was subtracted from the total area of the standard rectangle. The results were expressed as percentage values (the proportion of area occupied by bone tissue in relation to the total area of the standard rectangle).

The mean of the values from the five sections of each specimen was obtained. The same procedure was repeated for each of the ten animals in each group. Histomorphometric analysis was performed by one calibrated examiner who was blinded to the experimental groups and treatments rendered. The terminology and units used are those recommended by the Histomorphometry Nomenclature Committee of the American Society for Bone and Mineral Research<sup>(18)</sup>.

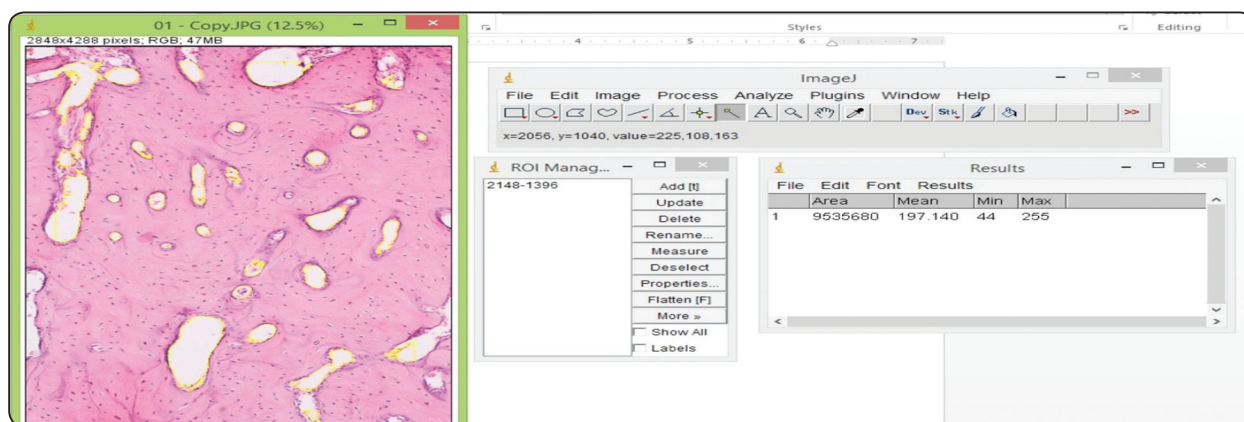


Fig. (2) Measurement of bone surface area using Image J program. The bone marrow spaces are traced using wand tracing tool.

### **Scanning electron microscopy and quantitative X-ray microanalysis**

The left molar segments were separated from the rest of the mandibles and then fixed in 2.5% glutaraldehyde in phosphate buffer. The specimens were then washed under running water, dehydrated, air-dried and exposed to X-ray analysis using EDX system attached to scanning electron microscope. This was performed to analyze the different percentages of calcium and phosphorus with the aid of a software system (LINK ISIS, Oxford, UK). Afterwards, the specimens were coated with a thin layer of gold using a sputter coater to be examined on JOEL 5300 JSM scanning electron microscope<sup>(19)</sup> by one calibrated examiner who was blinded to the experimental groups and treatments rendered.

### **Statistical analysis**

Data obtained from both histomorphometrical and elemental microanalysis were statistically described in terms of mean and standard deviation. Comparison of the mean between the groups was done using analysis of variance (ANOVA) test. A probability value (p value) less than 0.05 was considered statistically significant. Statistical analysis was done using Statistical Package for the Social Sciences (SPSS) version 20.0<sup>(20)</sup>.

## **RESULTS**

### **Light microscopic results**

#### ***Group I (control group)***

The histological examination of the control group revealed the normal architecture of the alveolar bone which includes smooth bone surface facing the periodontal ligament (PDL). Large interconnecting bony trabeculae were seen encircling medullary cavities which contain the bone marrow (Figure 3a).

#### ***Group II (Osteoporotic group)***

The structure of the alveolar bone was greatly affected by calcium deficiency. The bone lining the socket showed an irregular outline along most of the root length. Many osteoclast cells were detected in some areas along the endosteal surface of the bone (Figure 3b&c). The bony trabeculae appeared thinner and more widely separated (Figure 3d). Some osteocytes exhibited pyknotic nuclei and occupied relatively widened lacunae (Figure 3e).

#### ***Group III (zinc treated group)***

A generalized restoration of the normal structural features of the alveolar bone was the prevailing finding. The boundary of the alveolar bone appeared relatively smooth; however, some areas appeared slightly irregular (Figure 3f). The bony trabeculae appeared dense in comparison to the osteoporotic group (Figure 3g). The osteocytes showed regular distribution with large nuclei filling most of the lacunae (Figure 3h).

### ***Histomorphometric analysis***

The percentage of area occupied by alveolar bone trabeculae in the mandible in the different experimental groups are summarized by means and standard deviation in (Table 1).

There was a statistically significant decrease in the bone surface area in the osteoporotic group II in relation to the control group I ( $p_1 < 0.001$ ). In the zinc treated group III, the mean bone surface area increased to values close to the control group I ( $p_2 = 0.213$ ). Moreover, the difference in the mean bone surface area between both osteoporotic group II and zinc treated group III were statistically significant ( $p_3 < 0.001$ ).

### **Scanning electron microscopic results**

Examination of the buccal cortical plate of the mandible of control animals revealed the normal architecture of the alveolar bone with smooth

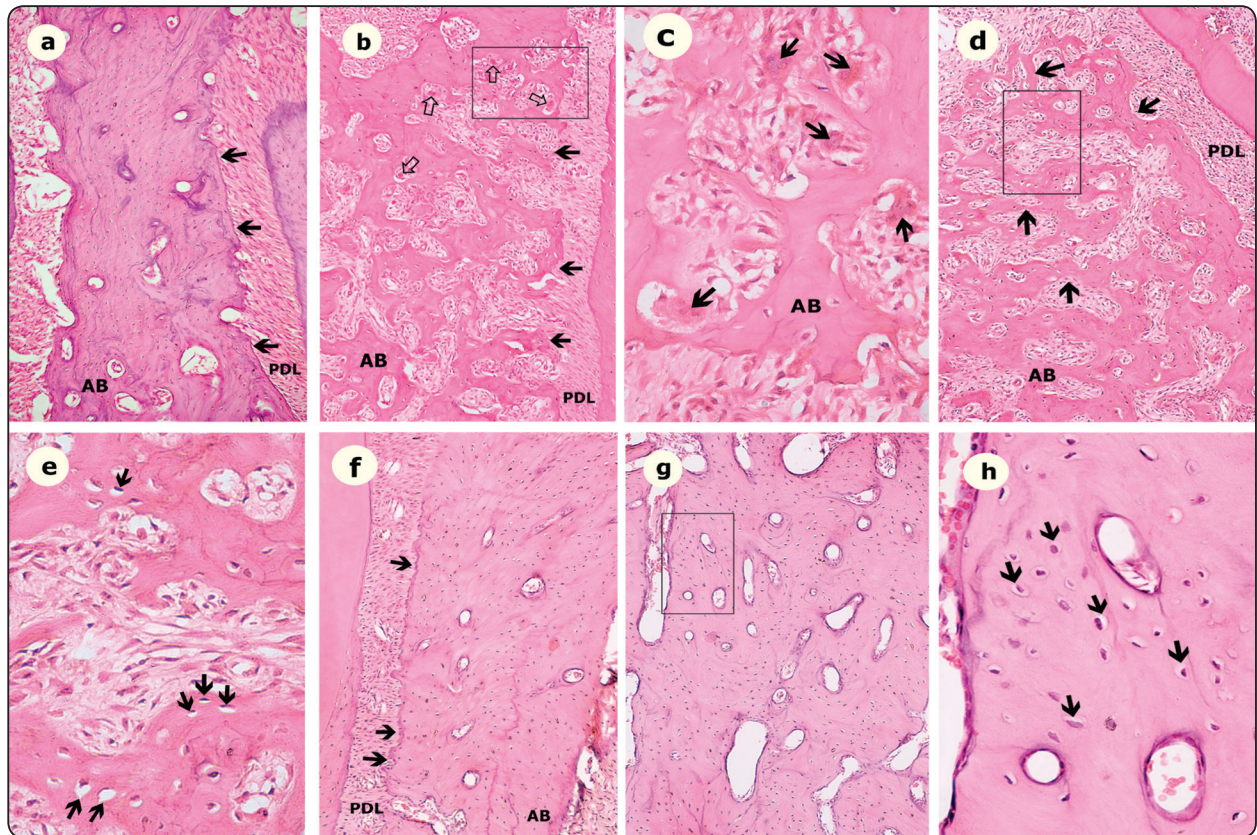


Fig. 3 (a-h): Light micrographs (LM) of the alveolar bone (AB) in different groups. **Control group I (a):** The alveolar bone shows an outstanding density and relatively smooth boundary (arrows) adjacent to PDL. **Osteoporotic Group II (b-e).** (b): The bone surface lining the socket appears irregular (arrows) with many osteoclasts (hollow arrows) lining the bone marrow spaces. (c): Higher magnification of the previous micrograph inset showing osteoclast cells (arrows) housing the Howship's lacunae. (d): The cancellous bone shows thinning of the bony trabeculae (arrows). (e): Higher magnification of previous micrograph inset showing osteocytes with pyknotic nuclei (arrows). **Zinc treated group III (f-h).** (f): The alveolar bone boundary appears relatively smooth with some few irregularities (arrows). (g): Cancellous bone shows regular trabecular organization surrounding narrow marrow spaces. (h): Higher magnification of the previous micrograph inset showing regular distribution of osteocytes' lacunae (arrows). PDL: periodontal ligament. (H&E a, b, d, f &g 100x; c, e & h 400x)

TABLE (1) Comparison between the studied groups regarding percentage of mean bone surface area.

	Group I (n=10)	Group II (n=10)	Group III (n=10)	P	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>
<b>Percentage of bone surface area</b>							
Mean	91.189	80.828	88.602	<0.001*	<0.001*	0.213	0.001*
SD.	2.235	6.942	2.893				

Normally quantitative data was expressed in (Mean. ± SD) and was compared using F: F test (ANOVA)

p<sub>1</sub>: p value for Post Hoc test (LSD) for comparing between group I and group II

p<sub>2</sub>: p value for Post Hoc test (LSD) for comparing between group I and group III

p<sub>3</sub>: p value for Post Hoc test (LSD) for comparing between group II and group III

\*: Statistically significant at p ≤ 0.05

surface containing numerous nutritive canals with regular borders (Figure 4a). On the other hand, in the osteoporotic group II, the bone surface showed massive abrasion and discontinuation of the cortical plates, where deep resorptive craters of different sizes and irregular outlines were seen (Figure 4b). In the zinc treated group III, there was a marked improvement in the surface topography. The bone surfaces exhibited a marked masking of the osteoporotic changes which were seen in association with group II. The bone surface appeared smooth and regular with minor irregularities observed at some areas (Figure 4c).

**Energy dispersive X ray microanalysis (EDX)**

The elemental composition of calcium and phosphorus in the molar region of the alveolar bone is summarized by means and standard deviation in (Table 2). Energy dispersive X ray analysis revealed a decrease in calcium and an increase in phosphorous content in osteoporotic group II compared to control group I ( $P_1 < 0.001$ ). In zinc treated group III, the percentage of calcium increased and that of the phosphorus decreased. However, the difference between both control and zinc treated groups was statistically significant ( $P_2 < 0.001$ ).

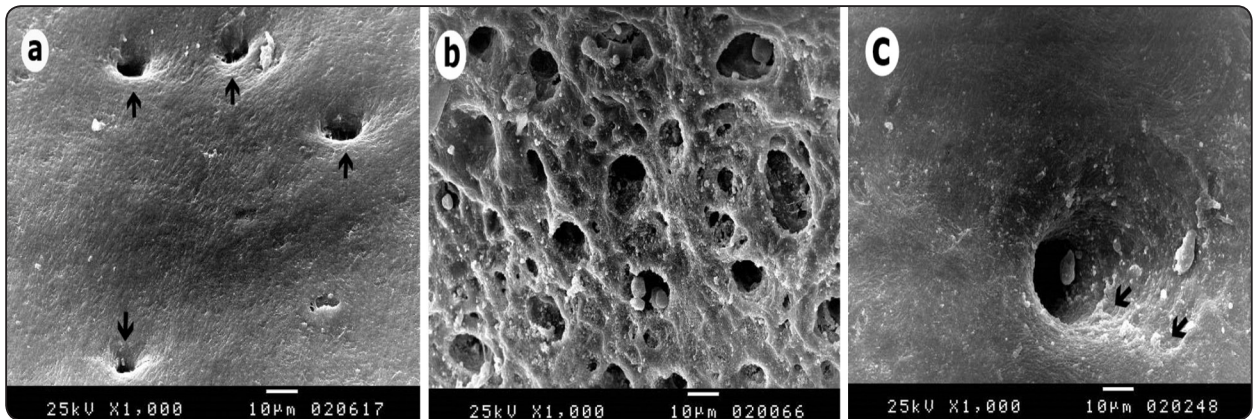


Fig. 4 (a-c): Scanning electron micrograph of the buccal cortical plate in different groups. **Control group I (a):** The bone surface shows a generalized smooth surface topography with nutritive canals (arrows) exhibiting regular borders. **Osteoporotic group II (b):** The surface shows severe resorption with multiple deep resorptive pits and craters. **Zinc treated group III (c):** The buccal cortical plate shows smooth surface topography with minor roughness (arrows). (1000x)

TABLE (2) Comparison between the studied groups according to the percentage of Calcium and Phosphorus.

	Group I (n=10)	Group II (n=10)	Group III (n=10)	P	P <sub>1</sub>	P <sub>2</sub>	P <sub>3</sub>
<b>Ca</b>							
Mean	72.70	59.05	68.41	<0.001*	<0.001*	<0.001*	<0.001*
SD.	1.52	1.20	0.85				
<b>Phosphorus</b>							
Mean	27.30	40.95	31.59	<0.001*	<0.001*	<0.001*	<0.001*
SD.	1.52	1.20	0.85				

Normally quantitative data was expressed in (Mean. ± SD) and was compared using F: F test (ANOVA)

p<sub>1</sub>: p value for Post Hoc test (LSD) for comparing between group I and group II

p<sub>2</sub>: p value for Post Hoc test (LSD) for comparing between group I and group III

p<sub>3</sub>: p value for Post Hoc test (LSD) for comparing between group II and group III

\*: Statistically significant at p ≤ 0.05

## DISCUSSION

Osteoporosis is among the most common bone diseases that affects millions throughout the world. The worldwide prevalence of osteoporosis contributes to about 9 million fractures each year. In addition to being a common disease, osteoporosis increases healthcare costs<sup>(21)</sup>.

There is an evidence that osteoporosis affects the oral structures. It has been found to be associated with alveolar bone loss and subsequent tooth loss. Moreover, it has been suggested that alveolar osteoporosis may affect the course of periodontal disease, so that if bacterial infection occurs, periodontitis will progress more rapidly than if the bone had normal density<sup>(22)</sup>.

Zinc, a nutritional trace element, is essential for the growth, development, and maintenance of healthy bones. Patients with senile osteoporosis have lower zinc ion levels in serum and bone tissue than normal patients (10). Furthermore, in postmenopausal women, zinc intakes are positively associated with bone mass<sup>(23)</sup>.

Since the prevalence of osteoporosis increases with the aging of the world population, it has been of particular interest to identify anabolic agents that would prevent bone loss. So the aim of the present study was to investigate the effect of dietary supplementation of zinc on calcium deficient rats to evaluate whether it may ameliorate the detrimental effect of calcium deficiency on alveolar bone structure.

Concerning our experimental model, the rat was chosen because it is one of the most frequently used laboratory animal for studying osteoporosis. Moreover, it has a relatively adequate lifespan, a well characterized skeleton and is widely available<sup>(24)</sup>. Calcium deficient diet is one of the approaches used to induce osteoporosis. Rearing rats with less than 0.3% calcium diet for two months was approved to be an effective rat model of osteoporosis<sup>(25)</sup>. The

percentage of calcium in the calcium deficient diet used in our study was 0.13%. This dose was given according to previous studies made by Teófilo et al<sup>(26)</sup> and Prado et al<sup>(27)</sup> who used the same calcium percentage to induce osteoporosis.

In the present study, calcium deficient diet showed a marked negative effect on the alveolar bone structure and led to the appearance of osteoporotic features. Also, this study revealed that dietary zinc supplementation can overcome and treat most of these features. This was demonstrated histologically, histomorphometrically, ultrastructurally and by Energy dispersive X ray microanalysis (EDX).

Histologically, examination of group II receiving low calcium diet showed thinning of bone trabeculae, wide lacunae of osteocytes with pyknotic nuclei, marked increase in osteoclasts along the bone surface. This can be explained relying on the fact that calcium deficiency leads to parathyroid hormone (PTH) - mediated bone breakdown in order to restore normal calcium level in blood. The body is clearly more concerned with maintaining the plasma calcium concentration than with preserving the integrity of the skeleton<sup>(7)</sup>. Parathyroid hormone stimulates osteoclast formation by increasing the production of RANKL and inhibiting the expression of osteoprotegerin (OPG)<sup>(28)</sup>. Moreover, in condition of severe calcium deficiency, osteocytes can also act directly to resorb bone around their lacunae through a process known as osteocytic remodeling<sup>(29)</sup>.

Our histological findings were confirmed by scanning electron microscopic observations of group II which showed pronounced surface roughness and multiple deep and wide resorptive craters. This is supported by Iwamoto et al<sup>(30)</sup> who found that calcium deficiency increased eroded surface and the number of osteoclasts with decreased rate of bone formation and mineralization.

Energy dispersive x-ray is considered an efficient method to measure different percentages of minerals in the bone. The other method available



for measurement of mineral composition in isolated bones is ash analysis, which is a destructive method and only provides information about total bone composition. The present method has an advantage over ash analysis in that it allows for region specific analysis of elemental composition<sup>(31)</sup>.

The results of the elemental microanalysis in the present study coincide with those of the histological and scanning electron microscope results. Decrease in the calcium concentration in relation to the phosphorous was noted in the calcium deficient diet group. This is in agreement with Kato et al<sup>(32)</sup> who observed that the calcium deficient diet reduced Bone Mass Density (BMD) in rats by approximately 12%. Moreover, Jiang et al<sup>(33)</sup> proved that dietary calcium deficiency caused significant decreases of BMDs of the mandible compared to control rats.

Bushinsky et al<sup>(34)</sup> stated that the increase in osteoclasts activity may play a role in changing the ratio of calcium to phosphorus in the osteoporotic bone. During the process of bone resorption, osteoclasts increase the release of calcium leading to a decrease in the ratio of surface ion concentration of bone.

In the ongoing study, histomorphometrical analysis showed a statistically significant decrease in mean bone surface area in the osteoporotic group II in relation to the groups I and III. These results are supported by Ohya et al<sup>(35)</sup> and Hong et al<sup>(36)</sup> who demonstrated that rats fed with calcium-deficient diet showed low bone mass, significantly decreased bone density and mineral content.

All of the previous histological, SEM and elemental microanalysis findings of calcium deficient diet group, showed remarkable differences from those of the zinc supplemented group. The observed histological findings of the present study assured the positive influence of zinc administration on the restoration of the structure of alveolar bone. In zinc treated group, the bony trabeculae appeared dense with marked decrease in

osteoclasts in comparison to the osteoporotic group. The osteocytes showed regular distribution with large nuclei filling most of the lacunae.

The anabolic effect of zinc on bone metabolism was investigated by Yamaguchi et al<sup>(37)</sup> who found that the dietary intake of zinc for 8 weeks in men or women caused a significant increase in serum bone specific alkaline phosphatase activity and c-carboxylated osteocalcin concentration and a significant decrease in serum bone tartrate resistant acid phosphatase (TRACP) activity and N-telopeptide of type I collagen. Moreover, Igarashi and Yamaguchi<sup>(38)</sup> revealed through their work that culture of osteoblasts from femoral-diaphyseal tissues with zinc resulted in increased production of insulin like growth factor I (IGF-I) and transforming growth factor beta 1 (TGF- $\beta$ 1) from bone tissues with fracture healing.

Zinc has been shown to exhibit mitogenic effect on osteoblasts<sup>(39)</sup>. Seo et al<sup>(40)</sup> noted that prolonged cultivation of osteoblasts with zinc produced a remarkable increase in alkaline phosphatase activity and protein concentration in osteoblastic cells. In addition, zinc has been shown to stimulate the expression of runt-related transcription factor 2 (Runx2) mRNA, which is related to the differentiation to pre-osteoblastic cells<sup>(41)</sup>.

The light and scanning electron microscopic results of zinc treated group revealed a pronounced decrease in signs of resorption associated with calcium deficient diet. The bone surface appeared relatively smooth with decrease in resorption craters. This is supported by Bortolin et al<sup>(42)</sup> who proved that zinc supplementation to diabetic rats resulted in improvement in bone quality deteriorated by induction of diabetes.

Zinc has been shown to have inhibitory effects on osteoclastogenesis and to cause osteoclastic cell death that is generated with differentiation of bone marrow cells. Yamaguchi and Kishi<sup>(43)</sup> found that culture of mature osteoclast-like cells (isolated from

rat femoral tissues) with zinc caused apoptotic cell death. In addition, zinc has been shown to have a suppressive effect on RANKL-induced osteoclast-like cell formation in mouse marrow culture in the presence of monocyte colony stimulating factor (M-CSF)<sup>(44)</sup>. Moreover, culture of osteoblastic cells with zinc has been shown to have stimulatory effect on the expression of osteoprotegerin (OPG) that can suppress osteoclast development in pre-osteoclasts<sup>(41)</sup>.

Histomorphometrical analysis of our study revealed that zinc supplementation resulted in relative increase in bone surface area relative to the calcium deficient diet group. This is in agreement with Ovesen et al<sup>(45)</sup> who assessed the skeletal effects of alimentary zinc supplementation in growing rats. They found that zinc exerted its main effect on the periosteal envelope, thereby increasing bone mass.

In contrary to our findings, Kenney and McCoy<sup>(46)</sup> investigated the effect of zinc supplementation on male weanling rats fed low calcium diet. They found that calcium deficiency reduced bone mineral content and adding zinc to the low calcium diet further reduced bone strength and elasticity. This may be attributed to the dose of zinc used in this study which was 72 mg Zn/kg diet. Zinc is essential to produce and maintain organic components of bone matrix as well as for normal calcification. On the other hand, a very high concentration of Zn can interfere with hydroxyapatite crystal growth<sup>(47)</sup> and accelerate bone resorption.

Concerning the energy dispersive X ray microanalysis, the zinc supplementation resulted in relative regaining of the normal percentage of calcium and phosphorus. This is in accordance with Hyun et al<sup>(48)</sup> who found that dietary zinc intake and plasma zinc have a positive association with bone mineral density in men. Bone mineral densities for the hip, spine, and wrist were significantly lower in men with the lowest plasma zinc concentrations than in men with higher plasma zinc

concentrations. In addition, Siddapur et al<sup>(49)</sup> noted a positive correlation between serum zinc and bone mineral density in both diabetic and non-diabetic postmenopausal women.

## CONCLUSION

In conclusion, dietary zinc supplementation can improve the condition of the alveolar bone affected by calcium deficiency in rats. This is probably due to its stimulatory effect on osteoblasts and its inhibitory effect on osteoclast development and activity. There is therefore an evidence for zinc being a potent factor in bone metabolism.

Further researches should be done to test the efficiency of zinc in prevention and treatment of osteoporosis induced by different approaches like corticosteroids administration and to investigate its efficiency in bone healing.

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