Changes in bone components of newborn rats after maternal treatment with cytarabine

Zofia Drzazga, Katarzyna Michalik, Michał Kaszuba, Hanna Trzeciak

Abstract—**Concentration of abundant elements like calcium as well as elements present in trace amount like zinc, iron, silicon, potassium in mandibles of 7, 14 an 28 day old newborn rats after maternal administration with cytarabine were determined by X-ray fluorescence analysis.**

The studies were carried out using measurement system containing X-ray tube ECLIPSE-III and X-ray and gamma ray detector XR-100T-CdTe (Amptek Inc.).

Concentrations of studied elements (Ca, Zn, Si, K, Fe) obtained for bones from cytarabine treated rats (3 mg/kg sc.) markedly differ from those obtained for bones from control group. Cytarabine administration during pregnancy disrupts bone ontogenesis and mineralization of new born rats. Remarkable changes in Ca, Si and Fe content strived for concentration level compared with this characteristic for the control group with time.

I. Introduction

CYTARABINE, an analog of deoxycytidine, is an important agent in the treatment of ovarian carcinoma, acute myeloid and lymphoblastic leukaemia. Its mechanism of action has been attributed to an interference with DNA replication [1]-[2]. Efficiency of anticancer treatment depends on systematic therapy, which is recommended also to pregnant women. However, cytarabine pharmacotherapy is not free from adverse effects which include leucopenia, thrombocytopenia, anaemia, fever, anorexia, nausea, vomit, acute cerebellar syndrome and aseptic meningitis [3]. Thus an experiment estimating influence of cytarabine treatment, during pregnancy on bone mineralization and development in newborn rats has been performed. Animal models are useful with regard to developing methodologies that can be used to elucidate the biochemical and morphological properties of tissue and to differentiate between healthy and non-healthy tissues.

Many trace elements are required for the growth and maintenance of healthy bones. The hardness and rigidity of this tissue depends on incorporation of the mineral into protein matrix containing crystals of hydroxyapatite.

Manuscript received June 3, 2008. This work was supported in part by Polish Ministry of Education under project No. 2P05D085.

Z. Drzazga is with the A. Chełkowski Institute of Physics, Department of Medical Physics, University of Silesia, ul. Uniwersytecka 4, 40-007 Katowice, Poland, (e-mail: drzazga@us.edu.pl)

K. Michalik is with the A. Chełkowski Institute of Physics, Department of Medical Physics, University of Silesia, ul. Uniwersytecka 4, 40-007 Katowice, Poland, (corresponding author phone: +48 032 3591746; e-mail: kasia_michalik1@wp.pl).

M. Kaszuba is with the Institute of Stomatology, Łabędzia 2, 40-534 Katowice, Poland

H. Trzeciak is with the Medical University of Silesia, Department of Prosthetic Dentistry, pl. Grunwaldzki 1, 41-902 Bytom, Poland

Calcium (Ca) is responsible for the stimulation of bone formation and the inhibition of bone resorption [4]. Crystallization of bone salt occurs in several stages, proceeding from amorphous calcium phosphate through intermediate crystalline structures, such as octocalcium phosphate [5]. Sufficient calcium has a positive effect upon bone quality however, other minerals also may affect bone biology.

Iron (Fe) is a component of the enzymes procollagen proline hydroxylase and procollagen lysine hydroxylase which are essential for the hydroxylation of proline and lysine residues respectively in biosynthetic precursors of collagenis. Fe deficiency in young rats leads to decreasing mechanical strength bone. Its deficiency negatively affects bone microarchitecture and has significant impact on bone mineral density, content and fragility [6]-[8].

Zinc (Zn) concentrated mainly in the layer of osteoid plays a physiologically important role in bone tissues especially in the regulation of bone metabolism and bone protein synthesis [9]. Its supplementation could have beneficial effects on the bone density [10]. Zn deficiency is associated with many kinds of skeletal abnormalities in foetal and postnatal development. One of teratogenic effects of Zn deficiency is micrognathia (undersized mandible) in the rat. Zn is a cofactor for enzymes such as alkaline phosphatase, collagenase carbonic anhydrase essential for bone resorption and remodelling. In contrast to Ca the role of Zn as a bone growth factor in growing animals and humans has not been fully clarified [11]-[12].

Bone silicon (Si) is located mainly in osteoblast mitochondria and is required for connective tissue matrix formation [13].

Deficiency of potassium (K) may result in reduced bone calcification [14].

Several techniques have been used in trace elements analysis of bones, e.g. atomic absorption spectrophotometry, neutron activation analysis, X-ray microanalysis [15]-[18]. X-ray fluorescence (XRF) is an useful non-destructive analytical technique for study biological materials such as bones, teeth, blood, head hair, as well as kidney stones and for determination the concentrations of various elements containing in tissues [19]. This paper is focused on the calcium, zinc, iron, silicon and potassium concentration analysis by means XRF method.

II. Materials and Methods

The study was performed in female Wistar rats (220-230g) in the Department of Pharmacology of the Medical University of Silesia in Katowice. After fertilization rats

were treated with cytarabine in a dose of 3 mg/kg sc. between 5th and 15th day of pregnancy. Pregnant control rats were administrated by 0.9% NaCl solution in volume of 0.5 ml/kg sc. The mandible (left and right) of 7, 14 and 28 day old newborn rats were skeletonised and cleaned from soft tissue, brushed in purified water and air-dried before X - ray fluorescence measurements. The samples were divided into groups regarding the age and maternal administration of cytarabine. The numbers of newborn animals treated with cytarabine (designated further as cytarabine group) were following: 4 (4), 13 (7) and 7 (8) for 7, 14 and 28 day old, respectively, numbers in bracket concerning the control group. Three areas A, B and C on mandible surface were chosen for examination (Fig. 1.). The size of the analysis region of these areas was 1.0 mm^2. Measurements were repeated three times for each bone.

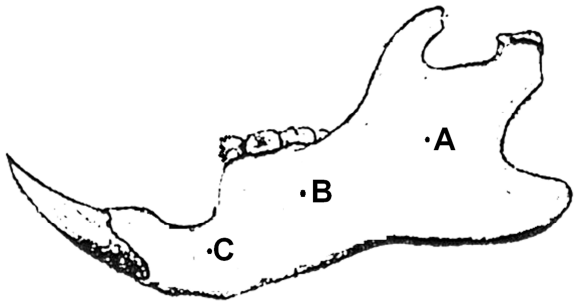

Fig. 1 Regions of interest on rat mandible

The study was carried out with use of: an ECLIPSE-III X-Ray tube system with silver target and beryllium window, power supply and control electronics, XR-100T-CdTe X-ray and gamma ray detector with preamplifier (sensitivity 0.82 mV/keV) and cooler system (Amptek Inc). The detection accuracy of CdTd detectors for quantitative X-ray spectroscopy is discussed in [20].

The X-ray detector and tube were mounted on a bracket to create a fixed and reproducible geometry (45^o incident and take-off angles; tube to sample 4 mm and sample to detector 65 mm). All measurements were performed at room temperature (~25 ^{o}C) and under atmospheric pressure (~1013 hPa). The spectra were collected at acquisition time 180 seconds, 20 kV and 10 μA. Calibration was done using pure lead, three stainless steel standards (D845-847, containing Mn, Si, Cu, Ni, Mo, Nb) and Calcite (Kalkstein KH-2) in the same geometry.

The results were analyzed with use of Fundamental Parameters program XRF-FP software. The spectrum deconvolution was performed automatically after escape-peaks and background removal as well as peak intensities extraction for the Compton scatter peaks. The Ca, Si, Fe, K and Zn concentration in the bones were calculated.

Statistical analysis of the results was done with Statistica 7.1 using Shapiro-Wilk's test to check the normality of the distributions together with ANOVA and post hoc Tukey's test. Differences with a $p < 0.05$ were regarded as significant.

III. Results and Discussion

The X-ray fluorescence spectra of rats mandibles could be divided into two parts: one part associated with high, characteristic peak with maximum about 3.69 keV mainly due to the calcium and second one connected with braking radiation with two small peaks appearing against the background characterized as Fe, (6.4 keV) and Zn (8.63 keV). Complicated and asymmetric peak in the range 1.2 – 5.0 keV pointed at presence of aside from Ca other elements. Therefore the deconvolutions of X-ray fluorescence spectra were performed. The results of deconvolution for mandible of 28 day old newborn rats (control and cytarabine group) are presented in Fig. 2.

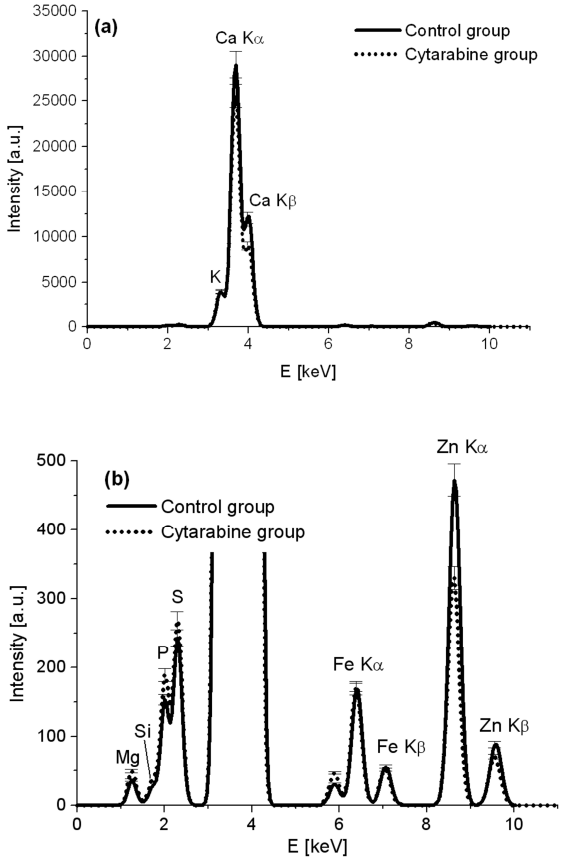

Fig. 2 Averaged X-ray fluorescence spectra (with marked standard deviation) at area A of rat bones from control and cytarabine group after XRF-FP processing. (b): zoom of Mg, Si, P, S, Fe and Zn spectrum

Deconvolution of main peak revealed following contributions originated from Si, S, Cl, K, Ca K_α and Ca K_β. The energy range 5.5 – 7.5 keV and 8.2 – 9.8 keV consisted of peaks characteristic for Fe and Zn, respectively. One can see fluorescence spectra intensities differ between mandibles from control and cytarabine group.

Although X-ray fluorescence spectra of studied mandibles were influenced by Mg, S and Cl, the detailed analysis was focused only on Si, K, Ca, Fe and Zn.

The statistical formulations of the quantitative results for Ca at different areas (A, B and C) for mandibles from cytarabine group of rats 7, 14 and 28 day old are presented in Table I.

TABLE I
AVERAGED CA CONCENTRATIONS (±SEM) IN BONES FOR CONTROL AND CYTARABINE GROUP OBTAINED AT A, B AND C AREAS FROM 7, 14 AND 28 DAY OLD NEWBORN RATS

Group	Area	Ca concentration [wt%]		
		7 days	14 days	28 days
Control	A	25.1 ± 0.9	26.6 ± 1.0	27.1 ± 0.3
	B	22.7 ± 1.2	25.3 ± 1.4	26.1 ± 1.4
	C	21.4 ± 1.2	20.8 ± 1.3	21.3 ± 1.1
Cytarabine	A	18.8 ± 1.1	25.3 ± 1.8	26.7 ± 0.8
	B	18.6 ± 0.7	22.5 ± 1.5	24.5 ± 1.7
	C	17.1 ± 1.2	22.5 ± 2.9	22.7 ± 1.6

One can see the highest concentrations were obtained for A area and the lowest for C area and they differed with signification level ($p<0.0001$) for bones from 28 old day rats. There were no significant differences between A area and B area for bones from 7 old day rats and between B and C area for bones from 14 old day rats. It is noteworthy that maternal treatment with cytarabine caused marked Ca concentration decrease especially seen in 7 day after birth.

Ca concentrations clearly increased in 14th day after birth. It was found that organism strive for comparable Ca concentration level with this characteristic for the control group. It was previously concluded [21] that differences in concentration between areas were connected with mineralization degree on individual mandible areas due their function what was also suggested in [22]. However maternal administration with cytarabine effaced this diversity what could lead to some irregularity in mandible functions.

Fig. 3. (a) and (b) showed the statistical formulations of the quantitative results for Si and Fe respectively at different areas (A, B and C) for mandibles from cytarabine group of rats 7, 14 and 28 day old.

The highest Si concentration in mandible of cytarabine treated rats as well as in control group was calculated at C area (9.6 ± 1.2 wt% and 5.3 ± 1.4 wt%, respectively).

It was found that Si and Fe concentration are significant higher in cytarabine group bones than in control bones of 7 day old newborn rats.

Concentration of Fe in bones from control rats increased with age. Moreover differences between Fe content at areas A, B and C became higher and statistically essential ($p<0.001$) in 14th and 28th day after birth. This increase was reverse than those observed for Si and Ca. Administration of cytarabine during embryonic and foetal period caused significant increase of Fe concentration observed for bones from 7 day old rats. Between 14^{th} and 28^{th} day after birth Fe proportions were inversely related to control mandibles. Differences between areas became statistically essential not until for samples from 28 day old rats. It could be concluded that for Fe as well as for Ca and Si disorder of mineral metabolism caused by cytarabine administration during pregnancy levelled with age.

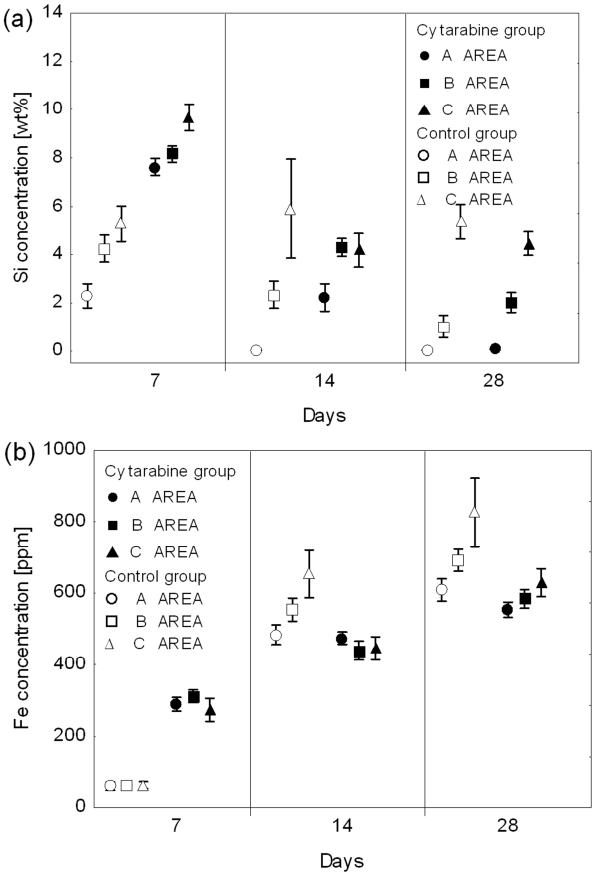

Fig. 3 The comparison of averaged Si (a) and Fe (b) concentrations in bones from control and cytarabine group obtained at A, B and C areas for 7, 14 and 28 day old newborn rats.

Analysis of K concentration in bones revealed different behaviour that was observed for other studied elements (Fig. 4.). There were no significant differences between areas for cytarabine and control groups of mandibles. Moreover in

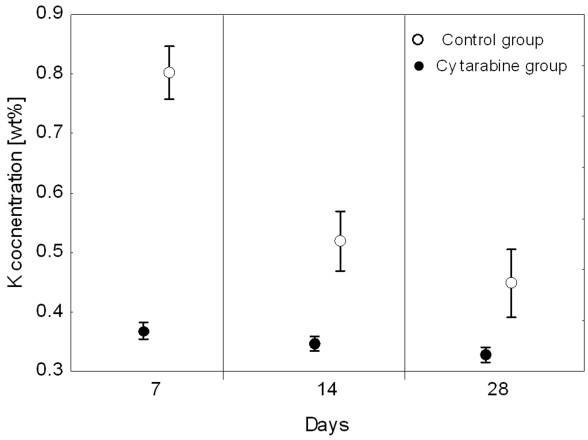

Fig. 4. The comparison of averaged K concentrations in bones from control and cytarabine group obtained for 7, 14 and 28 day old newborn rats.

control group the K content was markedly higher than in cytarabine group and decreased with age (from 0.8 to 0.4 wt%). It is noteworthy that in cytarabine group K concentration kept on constant level (0.36 wt%).
It was previously reported that Zn concentration is dependent on region of interest and time changes were statistically essential with $p < 0.001$ [21]. For bones from cytarabine group Zn concentrations were at about 2.3 times lower than in bones from 7 and 14 day old control group rats and about 1.5 lower in bones from control group rats 28 day old. Moreover Zn content was comparable in the range of error for all age cytarabine group (Table II).

TABLE II
AVERAGED ZN CONCENTRATIONS (±SEM) IN BONES FROM CYTARABINE GROUP OBTAINED AT AREAS A, B AND C FOR 7, 14 AND 28 DAY OLD NEWBORN RATS

Area	Zn concentration [ppm]		
	7 day	14 day	28 day
A	96 ± 11	113 ± 14	91 ± 8
B	83 ± 5	90 ± 18	86 ± 12
C	99 ± 21	91 ± 27	90 ± 18

For better insight into problem, the values of Zn/Ca ratio for 7, 14 and 28 day old rats treated with cytarabine were calculated and compared with those obtained for control group (Fig. 5.).

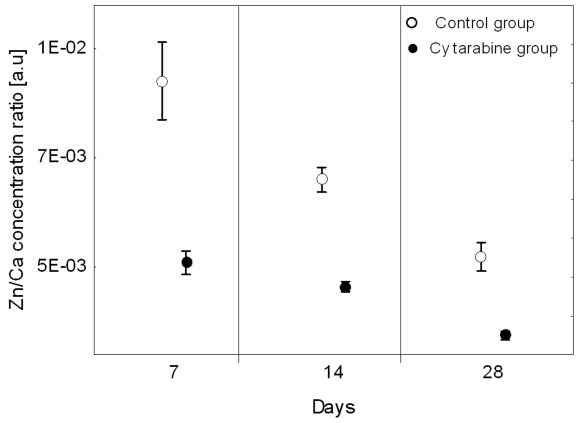

Fig. 5. The averaged Zn/Ca concentration ratio at areas A of mandibles for 7, 14, 28 day old newborn rats obtained for control and cytarabine group

The most significant decrease ($p < 0.001$) of Zn/Ca ratio with age of newborns was observed between 7 and 14 day old rats at A and B mandible areas. It was reported [11], [23] that Zn plays a physiological role in the regulation of bone metabolism and stimulation protein synthesis in newborn rats. Moreover it was shown that calculated Zn/Ca ratio decreased with age indicating the important role of zinc at the beginning of bone ontogenesis [21]. In present studies it was found that during 28 first days of life this ratio decreased about 1.5 times after maternal treatment with cytarabine indicating negative influence of cytarabine on the zinc concentration in mandible development. It was interesting that in contrast to Ca, Si and Fe concentration of Zn and K were significant lower even in 28th day after birth. The effect of zinc supplementation on bone morphological changes should be taken into consideration analogically to ovariectomized rats [23].

IV. CONCLUSION

X - ray fluorescence with fundamental parameters analysis was used to calculate concentration elements responsible for correct bone mineralization and development such as calcium, zinc, silicon, potassium and iron in bones of new born rats (7-, 14-, 28- day old). It was found that cytarabine maternal administration during pregnancy caused significant changes in their concentrations in comparison with control mandibles especially for zinc. It was noted that organism aiming at obtain proper content of studied elements with age. However concentration of zinc remaining still significant lower even in 28th day after birth. Obtained results showed that maternal administration of anticancer drug - cytarabine decreased the endogenous zinc known as essential element for the bone growth of newborn rats.

REFERENCES

[1] M. Suwalskya, P. L. Hernandeza, F. Villenab, C. P. Sotomayorc, "The anticancer drug cytarabine does not interact with the human erythrocyte membrane" Z. Naturforsch vol. 58c, pp. 885-890, 2003

[2] S. I. Marchenko, T. V. Trukhacheva, L. P. Gubina, D. V. Moiseev, P. T. Petrov, A. I. Zhebentyaev, "HPLC-based quality control of cytarabine preparations" Pharm. Chem. J. vol. 40 no.12, 2006

[3] K. W. L. Yee, J. Cortes, A. Ferrajoli, G. Garcia-Manero, S. Verstovsek, W. Wierda, D. Thomas, S. Faderl, I. King, S. M. O'Brien, S. Jeha, M. Andreeff, A. Cahill, M. Sznol, F. J. Giles, "Triapine and cytarabine is an active combination in patients with acute leukemia or myelodysplastic syndrome" Leukemia Res. vol. 30 pp. 813–822, 2006

[4] J. J. B. Anderson, S. C. Garner, "Calcium and Phosphorus in Health and Disease" Chap. 7, CRC Press, 1996

[5] R.G.G. Russell, A.M. Caswell, P.R. Hearn, R.M. Sharrard, "Calcium in mineralized tissues and pathological calcification" Br. Med. Bull. vol. 42, pp. 435 – 446, 1986

[6] D. M. Medeiros, B. Stoecker, A. Plattner, D. Jennings, M. Haub, "Vertebrae from calcium and iron restricted rats exhibit significant compromise in trabecular bone" J. Nutr. vol. 134 pp. 3061–3067, 2004

[7] J. H. Beattie, A. Avenell, "Trace element nutrition and bone metabolism" Nutr. Res. Rev., vol. 5, pp. 167-188, 1992

[8] M. Parelman, B. Stoecker, A. Baker, D. Medeiros, "Iron restriction negatively affects bone in female rats and mineralization of hFOB osteoblast cells" Exp Biol Med vol. 231, pp. 378–386, 2006

[9] Masayoshi Yamaguchi, "Role of zinc in bone formation and bone resorption" J Trace Elem. Exp. Med. vol. 11 no. 2-3, pp. 119–135, 1998

[10] A. Higashi, T. Nakamura, S. Nishiyama, M. Matsukura, S. Tomoeda, Y. Futagoishi, M. Shinohara, I. Matsuda, **"**Zinc kinetics in patients with bone demineralization due to physical immobilization**"** J Am. Coll. Nutr. vol. 12 no.1, pp. 61-65, 1993

[11] Zhong Jie Ma, Masayoshi Yamaguchi, "Alternation in bone components with increasing age of newborn rats: role of zinc in bone growth" J Bone Miner. Metab. vol. 18 pp. 264–270, 2000

[12] Zhong Jie Ma, Masayoshi Yamaguchi, "Role of endogenous zinc in the enhancement of bone protein synthesis associated with bone growth of newborn rats" J Bone Miner. Metab. vol. 19 pp. 38 – 44, 2001

[13] H. Beattie, A. Avenell, „Trace element *nutrition*. and bone metabolism" Nutrition Nutr. Res. Rev. vol. 5, pp. 167-188, 1992
[14] M. B. Gillis, "Further studies on the role of potassium in growth and bone formation" J. Nutr. vol. 4, pp. 45-50, 1950
[15] Z. Jie Ma, M.i Yamaguchi, "Alteration in bone components with increasing age of newborn rats: role of zinc inbone growth" J Bone Miner. Metab. vol. 18, pp. 264–270, 2000
[16] F. A. El-Amri, M. A. R. El-Kabroun, "Trace element concentrations in human bone using instrumental neutron activation analysis" J Radioanal. Nucl. Chem. vol. 217 no. 2, pp. 205 – 207, 1997
[17] G. Boivin, Ć P.J. Meunier, "The mineralization of bone tissue: a forgotten dimension in osteoporosis research" Osteoporos. Int. vol. 14 no. 3, pp. 19–24 2003
[18] M. Grynpas, "Age and disease-related changes in the mineral of bone" Calcif. Tissue Int. vol. 53 no. 1, pp. 57 – 64, 1993
[19] P. J. Pottas, A. T. Ellis, P. Kregsamer, J. Marshall, C. Streli, M. West, P. Wobrauschek, "Atomic Spectrometry Update- X-ray Fluorescence Spectrometry" J. Anal. At. Spectro., vol. 18, pp. 1297-1316, 2003
[20] R. Redus, J. Pantazis, T. Pantazis, A. Huber, B. Cross, "Characterization of CdTe detectors for quantitative X-ray spectroscopy", IEEE Trans Nucl Sci, 2008 in press
[21] Z. Drzazga, K. Michalik, K. Maciejewska, H. Trzeciak, M. Kaszuba, "Role of endogenous zinc in bones of newborn rats" BioFactors vol. 29, pp. 1-6, 2007
[22] M. J. Glimcher, "Molecular Biology of Mineralized Tissues with Particular Reference to Bone" Rev. Mod. Phys, vol. 31 no. 2, pp. 359-393, 1959
[23] S. Kishi, Y. Segawa, M. Yamaguchi, "Histomorphological confirmation of the preventive effect of β-ananyl—L-histidinato zinc on bone loss in ovariectomized rats", Biol Pharm Bull, vol. 17 pp. 862-865, 1994.