

ULTRA STRUCTURAL AND HISTOLOGICAL STUDIES ON MICE BONE UNDER THE INFLUENCE OF NICKEL SALT TOXICITY

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ABSTRACT

Bone is essentially a heterogeneous system consisting of water, mineral and collagen which determine the physical properties of bone composite material. The decrease in bone weight was dose dependent after treatment with all the two salts of metal *i.e.* NiNO_3 and NiSO_4 . Bone provides essential framework and rigidity to the body. The toxic substances affect the development of bone by impairing the availability of essential nutrients resulting in abnormal bone development and toxicity. The impact of nickel toxicity on bone of mice has been studied histochemically (under light and electron microscope) by using two nickel salts. Histopathologically, the necrosis to layers of decalcified bone *i.e.* periosteum, matrix and endosteum was observed

with all two salts. The bone forming cells, lamellae and Haversian canals were also affected. The cortical width of bone section decreased as the dose of salts increased. These changes were also observed on samples of powdered dried bone of all groups with SEM. These changes were dose dependent and of the same order as mentioned above. The toxicity of nickel salts was in the order of $\text{NiSO}_4 > \text{NiNO}_3$.

KEYWORDS: mice, nickel salts, SEM.

INTRODUCTION

Bones are highly specialized tissues providing an essential framework and functional rigidity to the body. Structurally, they are complex tissues with osteocytes and chondrocytes embedded in prominent matrices which vary in form and chemical composition according to the age of the individual, although bone seems to be more resistant to toxic effects than the liver or kidney,^[1] the toxic substances are capable of influencing bone development by

impairing the availability of essential nutrients and their metabolic pathways, or by inhibiting essential biosynthetic events. Studies have been done to find the differences between the normal and intoxicated bone by xenobiotics.^[2-5]

Trace elements and essential nutrients are known to be important in mammalian development^[6-10] and some of these are essential in the structural and functional development of bone. They may contribute to the structure of the tissues, serve in physiological roles or contribute to metalloenzymes system.

The elements present in the environment and in the diet which impair the availability of these essential nutrients by chelating them or impairing their absorption at the intestinal mucosa are potential cause of abnormal bone development and toxicity.^[11] Imbalances between elements of nutritional importance and interactions with xenobiotic ions are significant causes of structural deformity in growing bone and in the homeostatic events present in adult tissue.^[12] The mechanisms involved may be more complex than a simple competitive binding at intestinal receptor sites and they may involve carrier protein, metalloenzyme complexes and unspecified physiological effects. 50 mM to 5 mM Ni^{2+} elicits a similar increase in cytosolic Ca^{2+} in the presence of 1.25 mM Ca^{2+} and 0.8 mM of Mg^{2+} (Shankar *et al.*),^[13] it apparently binds to a single site to stimulate release of Ca^{2+} from internal stores.

In the absence of Ca^{2+} and Mg^{2+} , higher nickel sensitivity and a “hooked” Ni^{2+} dose response (with inhibition of a higher concentration) are seen. Addition of Ca^{2+} or Mg^{2+} shifts the dose responses to higher concentrations. In the complete absence of Ca^{2+} nickel stimulates increase in cytosolic Ca^{2+} at concentrations as low as 5mM.

MATERIAL AND METHODS

Adult male mice, weighing 32-35 gm of Balb/c were orally administered different doses of nickel compounds daily for 40 days through gavage i.e. 5.0 mg/kg b.wt., 15 mg/kg b.wt. and 40 mg/kg b.wt. of NiSO_4 ; 5.0 mg/kg b.wt., 20 mg/kg b.wt. and 40 mg/kg b.wt. of NiNO_3 . The animals were divided into seven groups each group having 5 mice (one control group on normal diet and water). The weight of femur bone from treated groups was taken along with control after 40 days. The femur bone from all these groups was incubated in EDTA (5.5%) for seven days, the solution being changed every 24 hours and softness of bones tested. As soon as bone became soft, it was fixed into Bouins. The tissues were dehydrated in different grades of alcohol, cleared in benzene and embedded in paraffin wax (60-62°C).

Sections of 8 thick were cut on microtome and stained in haematoxylin /eosin (H/E).The dried femur bone from control and experimental groups was taken and made its powder, fixed on stubs for gold coatings with ion sputter, JFC 1100. Then examined using JSM 6100 JEOL, Scanning Electron Microscope (SEM).

RESULTS

The intake of feed and water by treated mice reduced as compared to control. Moreover, the decrease was dose dependent. The femur bone weight decrease after low doses of salts was non-significant whereas after moderate and high doses the decrease in weight of femur bone was significant as shown in Table 1.

Table 1: *In vivo* effect of nickel compounds administration on femur Bone weight of male mice

Compound	Control	Low Dose	Mod. Dose	High Dose
Ni NO ₃	-	-	-	-
Bone Weight (mg)	4.0±0.9	3.6±0.7*	3.0±0.71**	2.8±0.61**
% change	-	-10.0	-25.0	-30.0
NiSO ₄	-	-	-	-
Bone Weight (mg)	4.0±0.9	3.5±0.7*	2.9±0.8**	2.6±0.5**
% change	-	-12.5	-27.5	-35.0

* Non-significant different from the control value $p > 0.05$.

** Significant different from the control value $p < 0.05$

The femur bone of various groups has been decalcified with 5.5 % EDTA. Its inorganic part got dissolved and organic part was left behind. The substance of the bone was distinguished into three regions, viz. periosteum (PO), matrix (MA) and endosteum (EO) (Fig.1). Periosteum, covers the bone externally which is made of fibrous tissue. It has osteoblasts and a few blood vessels. Matrix is made of collagen fibres. It has the spaces showing the dissolved inorganic salts due to EDTA. The lamellae (LA) are present around the Haversian canals (HC). Endosteum, lines the marrow cavity. It is made up of fibrous tissue and contains bone forming cells which show spaces due to digestion of inorganic salts with EDTA.

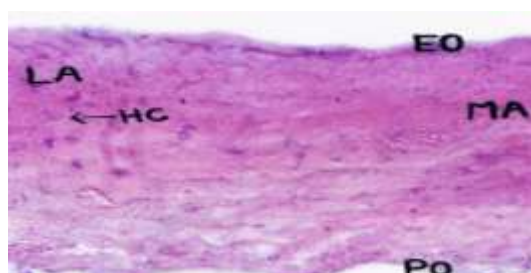


Figure1: Transverse section of femur bone of mice (H / E 400X)
(Showing periosteum (PO), matrix (MA) and endosteum (EO) covering the bone marrow.

Note the Haversin canals (HC) surround by Lamellae (LA) in matrix (control)).

After treatment with low dose of NiNO_3 the periosteum and endosteum was slightly damaged. The number of Haversian-canals and lamellae was slightly decreased. The width of bone was almost same as in control (Fig.2).

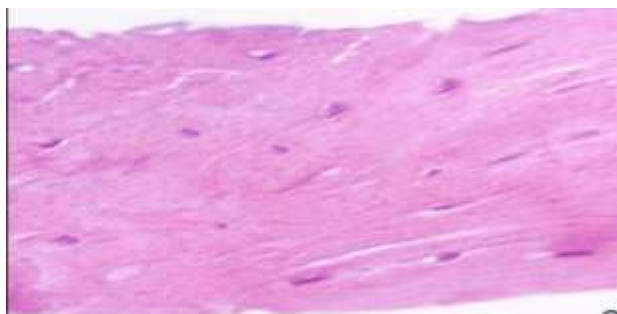


Figure 2: Showing negligible effects with low dose of NiNO_3 .

With moderate dose, the periosteum and endosteum had reduced in size. The matrix was also reduced with scattered lamellae, instead of encircling the Haversian canals. The bone in T.S. was about of half size as compared to control (Fig.3).



Figure3: Decrease in bone width and damaged layers of bone with moderate dose of NiNO_3 .

With high dose, all the three layers badly reduced in size, the lamellae were also damaged as compared to control. The size of bone in T.S. was about 1/3 of control (Fig.4).

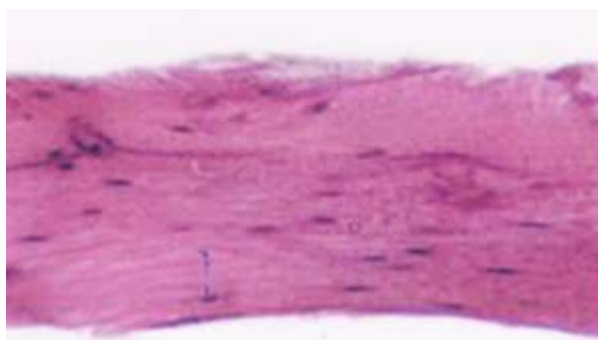


Fig.4.Further decrease in bone width and damaged bone layers with high dose of NiNO_3

The histopathological effect of low and moderate dose of NiSO_4 on bone was of similar nature as that of low and moderate doses of NiNO_3 . With high dose of NiSO_4 the histopathological effects were like that of NiNO_3 but the decrease in width was much more than that of NiNO_3 (Fig.5).



Fig.5. Damaged bone layers and decrease in width of bone to $\frac{1}{4}$ with high dose of NiSO_4 .

SEM of bone sample of control dried femur of mice showed the presence of Haversian canals surrounded by layers of calcium (Fig.6), with low dose of NiSO_4 the erosion of Haversian canals and damage of a part of bone was observed with moderate and high doses badly damaged bone sample.

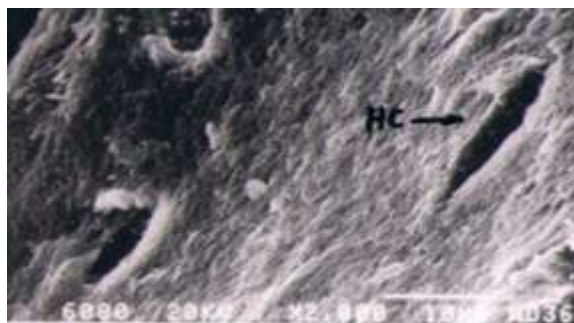


Figure 6: Scanning electron micrograph showing of femur bone of mice showing, Haversian canals surrounded by layers of calcium in control.

Haversian canal were completely chalked with eroded debris (Fig.7,8). While with NiNO_3 a part of bone was damaged and the Haversian canals showed some erosion with low dose.



Figure 7: Damaged Haversian canals with moderate dose of NiSO_4 .

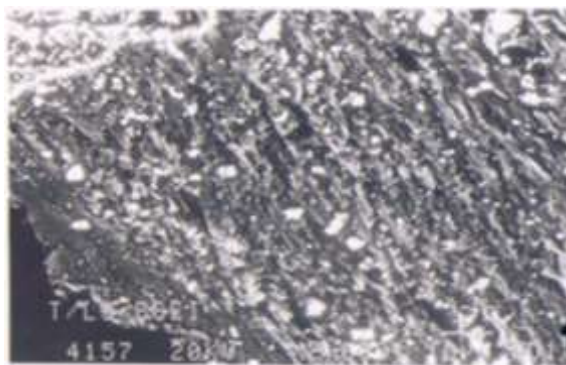


Figure 8: Highly damaged Haversian canals with high dose of NiSO_4 .

With moderate and high dose, contracted Haversian canals with damaged calcium layers were observed (Fig.9.10).

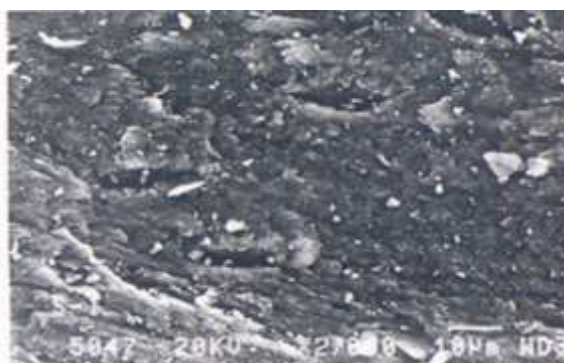


Figure 9: Contracted Haversian canal and damaged calcium layers with moderate dose of NiNO_3 .



Figure 10: Highly damaged Haversian canals and calcium layers with high dose of NiNO_3 .

The decrease in thickness of the femur bone after low doses of all the two salts was non-significant whereas after moderate and high doses the decrease in femur bone was significant / highly significant.

DISCUSSION

In the present study there were no histological changes observed in bone treated with low doses of nickel compounds. However, with moderate and high doses a significant effect on thickness of bone was observed. Similarly, Scanning Electron Microscopic (SEM) studies revealed no morphological changes in bone with low doses while moderate and high doses resulted in degenerating damaging effects on the bone in the form of necrosis. Nickel is known to have toxic effects with cellular damage in cell cultures at high concentrations ^[14]. Pure nickel implanted intramuscularly or inside bone has been found to cause severe local tissue irritation and necrosis and have high carcinogenic and toxic potencies ^[15]. Bone is essentially a heterogeneous system consisting of water, mineral and collagen which determine the physical properties of bone composite material. Water constitutes about 26% of bone volume and is believed to facilitate interactions between the other phases of bone extracellular matrix, viz., the minerals and the organic matrix. ^[16]

The organic matter accounts for one-third (30-35%) of dry weight of bone while the remaining two-third (65-70%) is constituted by inorganic matter. ^[17,18] The interaction of water phase with collagen and bone mineral results in a thin layer, which facilitates the interaction between the organic and inorganic phase of bone. This indicates that nickel replaces the water and lost mineral. ^[19-22] These results suggest that nickel compounds have toxic effect on structural changes and mineralization in the bone. The rate of calcium absorption seems to be directly proportional to the quantity of the calcium-binding protein in the intestinal epithelial cells. This protein functions in the brush border of these cells to transport calcium into the cell cytoplasm. ^[23] These changes seem to impair the conversion of Vit.D₃ to 1,25 (OH)₂ by the liver and kidney and impairment of this hormone will affect the intestinal absorption of calcium and thus be responsible for the decrease in thickness of bone and other changes in its structure observed with SEM. These studies are in conformity with the following reports which state that trace elements and essential nutrients are known to be important in mammalian development and some of these are essential in the structural and functional development of bone. ^[6] The elements present in the environment and in the diet which impair the availability of these essential nutrients by chelating them or impairing their absorption at the intestinal mucosa are potential cause of abnormal bone development and toxicity. ^[11] Imbalances between elements of nutritional importance and interactions with xenobiotic ions are significant causes of structural deformity in growing bone and in the hemeostatic events present in adult tissue. ^[12]

From the study of effect of nickel compounds on the bone of mice it is effected that NiSO_4 , NiNO_3 are toxic to bone. The width of bone decreases to about 1/3 of normal after high doses of nickel salts.

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