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INHIBITORY EFFECTS OF NICKEL NITRATE ON BONE COMPOSITION

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ABSTRACT

A number of biophysical and biochemical parameters have been made use of, in order to study the effect of nickel chloride on bone composition of mice. The animals were divided into two categories- control and experimental. The latter were further subdivided into three groups I, II and III according to the dose of nickel nitrate (NiNO3) administered to them i.e. 6.8, 13.8 and 30.1 mg/kg body weight, respectively. Femur bones were obtained by sacrificing the animals thirty days after weaning them once a week. The percentage loss between the wet weight and dry weight of femur in control animals was found to be 31.5±1.5 .In the three experimental groups I,II and III, the percentage loss was 31.5±1.4, 34.3±2.4 and 37.9±2.8 respectively. The percentage loss between the wet weight in wet water and dry weight in wet water was 37.1±2.3 in the controls and 37.3±1.9, 38.4±2.2 and 53.1±3.1 respectively in the three experimental groups. The percentage weight loss at 50°C, 200°C, 400°C and 600°C temperature was 31.5±1.3, 51.4±3.2, 53.2±4.1 and 62.5±4.9 respectively in the control animals. In the group I, the percentage weight loss at these temperatures were 43.4±3.3, 53.3±5.7, 57.5±4.9 and 63.1±5.2; group II animals showed the percentage weight loss as 52.3±5.3, 54.2±4.9, 58.7±5.7 and 66.0 and the III group showed the loss of 53.1±4.8, 55.1±6.2, 75.0±6.3 and 75.8% respectively .

Key words: Bone, Nickel Nitrate($NiNo_3$), femur.

Introduction

There has been an increasing concern about the entry of potentially harmful substances and trace elements into the food chain destined for human consumption [1, 2]. Heavy metals might be responsible for a variety of acute and chronic toxic effects in vertebrates [3]. Trace metals are thought to play several roles in synthesis of bone, cross-linking, calcification and diseases of the connective tissue [4]. Various authors have considered bone as a component of extracellular matrix [5, 6]. Water constitutes about 26% of bone volume [7] and is believed to facilitate interactions between the other two phases of the bone extracellular matrix viz., the minerals and the organic matrix. The organic matter accounts for one-third (30-35%) of dry weight of bone and the rest fit constituted by inorganic matter [8, 9].

Though the effects of temperature on bone have been investigated, but the mechanism of thermal interaction is not clearly understood [10, 11]. With the different components with change in temperature the physical properties such as stability and interaction of various component gets altered [12, 13] and there is also mineral loss under these conditions [14, 15]. These findings are consistent with the existence of membrane receptor which is strongly sensitive to Ni^{2+} as well as Ca^{2+} and Mg^{2+}.

The aim of the present investigation was to determine the effect of NiNO3 both under normal and altered temperature on the composition of organic and inorganic components of bone.

MATERIAL AND METHODS

Twenty adult male mice Balb/C weighing 30-35 gm. The animals were divided into two groups, viz., the control and the experimental. The experimental animals were further subdivided into three groups and were daily administered $NiNO_3$ doses of 6.8 ,13.8 and 30.1 mg/kg of body weight. The weight of each mice was recorded once a week for one month. They were then sacrificed and the femur bone was taken out. The bone marrow was flushed out with normal saline after careful removal of soft tissues. The wet weight and dry weight of bone were taken within 6 hours of sacrifice.

The femur from control and treated mice was crushed to powder form. The powdered samples prepared from control and treated groups were placed in a crucible and subjected to different temperatures of 50^{o}C, 200^{o}C, 400^{o}C and 600^{o}C for overnight in a muffle furnace. The weight loss at these temperatures was recorded.

The significance of the difference between values was estimated by Student's t-test, p-values of less than 0.05 were considered to indicate statistically significant differences.

Results and Discussion

The changes in bone composition and thermal effect on the bone collagen matrix have been studied with three different doses of $NiNO_3$ (6.8, 13.8 and 30.1 mg/kg). There was a decrease in wet and dry weight of the femur bone in control and experimental groups , the percentage decrease in control group was found to be 31.5±1.5 , while in the experimental groups, the decrease was 31.5±1.4 in group I (6.8 mg/kg $NiNO_3$), 34.3±2.3 in group II (13.8 mg/kg NiNO3) and 37.9±2.8 in group III (30.1 mg/kg $NiNO_3$) , the same observations were made by reheating the wet and dry samples , the percentage weight loss was 37.1±2.3 in control and 37.3±1.9 (group I), 38.4±2.2 (group II) and 53.1±3.1(group III) in experimental mice (Table 1). The percentage change in dry weight and dry wet weight indicate that there is an increase in weight loss with $NiNO_3$ dose administered. The percentage loss was 17.8±1.2 in control, 18.4±1.3 in group I, 32.3±2.5 in group II and 40.0±3.1 in group III (Table.1). The percentage increase in weight loss is suggestive of low cellular synthesis of bone mass in $NiNO_3$ treated animals as compared to controls. These results support the earlier findings in lead treated animals observed under various stimulating conditions and show that there is a low deposition of bone mass [16-21].

The results of thermal analysis of control and experimental samples, the percentage loss of weight at 50°C was 31.5±1.3 in control and 43.4±3.3, 52.3±5.3 and 53.1±4.8 in the three experimental groups respectively Table (2) . The percentage loss in the four categories of mice at 200°C was 51.4±3.2, 53.3±5.7, 54.2±3.9 and 55.1±6.2,(Table 2A) at 400°C it was 53.2±4.1, 57.5±4.9, 58.7±4.9 and 75.0±5.7 (Table 2B) while at 600°C the percentage of weight loss was observed to be 62.5±4.9, 63.1±5.2, 66.0±5.7 and 75.8±6.3 respectively (Table 3). Thus, it is clear from the table (Table 2, 3), that the weight lost by the samples increases with the increase in temperature. Also, the weight loss increases with the increase in $NiNO_3$ dose administered to the animals . This is suggestive of the fact that the bone material loses its weight because of thermal oxidation of bone. Temperature affects the thermal stability of bone collagen and the results comply with those of previous studies [22]. At 400 and 600°C, the percentage weight loss was due to evaporation of water and oxidation of organic content of bone. The loss was significant ($p<0.05$) in higher doses of NiNO3 (13.8 and 30.1 mg/kg) and non-significant ($p>0.05$) in small dose (6.8 mg/kg) of $NiNO_3$ in the experimental animals.

Table 1: Variations in wet weight and dry weight of femur in control and experimental groups

Sample	Wet weight (mg) (1)	Dry weight (mg) (2)	Wet weight wet water (mg) (3)	Dry weight wet water (mg) (4)	% weight (mg) loss between 1&2	% weight (mg) loss between 3 &4
Control	38.0±2.3	26.0±1.7	35.0±2.1	22.0±1.2	31.5±1.5	37.1±2.3
6.8 $NiNO_3$	36.0±3.2	25.0±1.6	33.0±2.3	21.0±1.4	31.5±1.4	37.3±1.4
13.8 $NiNO_3$	32.0±2.4	21.0±1.2	31.0±1.9	20.0±1.3	34.3±2.3	38.4±2.2
30.1 $NiNO_3$	29.0±1.9	18.0±1.1	28.0±1.6	13.0±0.9	37.9±2.8	53.1±3.1

Table 2: Percentage weight lost in control and experimental bone at 50 and 200°C temperatures.

Temp.	50°C			200°C		
Sample	Initial wt. mg	Final wt. mg	% lost	Initial wt. mg	Final wt. mg	% lost
Control	38.0±3.3	26.0±2.5	31.5±1.3	35.0±3.2	17.0±1.3	51.4±3.2
6.8 NiNO3	33.0±3.2	23.0±3.7	43.4±3.3	32.0±2.9	15.0±1.1	53.3±5.7
13.8 NINO3	32.0±2.9	21.0±1.5	52.3±5.3	31.0±2.5	14.0±0.9	54.2±3.9
30.1 NiNO3	32.0±3.1	15.0±0.9	53.1±4.8	29.0±1.9	13.0±0.9	55.1±6.2

Table 3: Percentage weight lost in control and experimental bone at 400 and 600°C temperatures.

Temp.	400°C			600°C		
Sample	**Initial wt. mg**	**Final wt. mg**	**% lost**	**Initial wt. mg**	**Final wt. Mg**	**% lost**
Control	38.5±2.7	18.0±1.2	53.2±4.1	40.0±2.7	15.0±0.9	62.5±4.9
6.8 NiNO3	33.0±2.9	14.0±1.1	57.5±4.9	35.3±3.1	13.0±0.5	63.1±5.2
13.8 NINO3	31.5±2.5	13.0±0.9	58.7±4.9	32.4±2.9	11.0±0.7	66.0±5.7
30.1 NiNO3	32.0±2.8	8.0±0.5	75.0±5.7	33.1±3.1	8.0±0.6	75.8±6.3

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