Influence of oral zinc sulphate supplementation on the development of the teeth and jaws of growing rat (Histological study)

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ABSTRACT
Background: This study was carried out to determine the effect of oral zinc supplementation on the growth of molar teeth and jaws on growing rat.

Materials and methods: Fifteen western albino pregnant rats subjected to zinc deficiency diet from time of 16th of gestation till day 21 postnatal days. Oral Zinc supplement in different dose (0.25 and 0.5 mg) were added to the normal zinc diet of the mothers from one day to 21 day postnatal periods. Neonate rats were sacrificed at the following postnatal periods (7, 10, 14, 18 and 21 day). Histological evaluation for the development of 1st molar tooth with development of the jaws were estimated under light microscope. In addition, serum zinc level in all studied periods were determined with atomic absorption spectrophotometer.

Results: Histological examination for experimental group (0.5) showed acceleration in the growth of molar teeth and the bones of the maxilla and mandible illustrates in early apposition of dental hard tissue and bone trabeculae. Declination in zinc levels were observed approximately in all studied groups with the increment of the period of the growth.

Conclusion: This study was illustrated that there are obvious effects of oral supplementing zinc on the growth of teeth and jaws of the rat suffered from zinc deficiency in gestation period and ameloblast showed to be the most affected dental cells.

Keywords: zinc, molar teeth and jaws, Neonate rats. (J Bagh Coll Dentistry 2011; 23(sp. issue):53-58).

INTRODUCTION
Zinc has the symbol Zn. It is a bluish-white metal and brittle at ambient temperatures but is malleable at 100 to 150°C. It is a reasonable conductor of electricity. It is a micro mineral needed in the diet on a daily basis, but only in very small amounts (50 milligrams or less) (1).

It is an essential trace element required for the growth of humans and other animals (2). Zinc-deficiency in humans and animals causes a wide variety of symptoms, including impaired growth, alopecia, anemia, dwarfism, impaired sexual development, and dermatitis, loss of hair, poor appetite, abnormal dark adaptation, delayed wound healing and mental lethargy (3). Its functions are growth, cell division, fertility, Immune system, taste, smell, appetite, Skin, hair, nails, vision, regulating genetic activities, supporting blood sugar balance and metabolic rate (4).

Zinc may add an unwanted flavor to water. This occurs at concentrations of about 2 mg Zn/ L (3).

MATERIALS AND METHODS
Material: Zinc Sulphate
Methods: Fifteen western albino pregnant rats subjected to zinc deficiency diet from time of 16th of gestation till day 21 postnatal day. Oral Zinc supplement in different dose (0.25 and 0.5 mg) were added to the normal zinc diet of the mothers from one day to 21 day postnatal periods. Neonate rats were sacrificed at the followings postnatal periods (7, 10, 14, 18 and 21 day). Histological evaluation for the development of 1st molar tooth with development of the jaws were estimated under light microscope. In addition, serum zinc level in all studied periods were determined with atomic absorption spectrophotometer.
Collection of specimens
The experimented animals were subjected to heart puncture to obtain blood biochemical of serum zinc level for analysis and then the head of the of the rats were separated from the body and cut sagitally into two halves, and the specimens (concerning maxilla, mandible and the molars tooth only) were preserved in 10% buffered formalin for 72 hours for histological examination.

Histological preparation:
After fixation, the specimens were decalcified in 10% formic acid for 10-15 days; solution was changed every 48 hours. After decalcification, the specimens were washed for 24hours in running water; the dehydrated specimens pass through a series of alcohol concentration 40%, 60%, 80%, 95% and absolute alcohol.
Specimens were passed through two changes of xylol (xylene) for 15 minutes to get rid of any excess of ethanol. Specimens were cleared and embedded in paraffin wax. Serial cross sections of 5-micrometer thickness were obtained by microtome and stained with Hematoxylin and Eosin (H&E).
All these sections were examined under light microscope to evaluate tissues changes according to zinc supplementations.

Measurement of serum Zinc
Two ml of blood samples were taken through cardiac puncture and evaluated by atomic absorption spectrophotometer.

RESULTS
Control group at the 7th postnatal day: Tooth germ of upper and lower first molar show tooth development at advance bell stage. Appositions of predentin, dentin were illustrated.

Experimental group at the 7th postnatal day (0.25 mg):
Tooth germs of upper and lower first molar show tooth development at bell stage which were illustrated dental papillae, dental sac and enamel organ.

Experimental group at the 7th postnatal day (0.5 mg):
Tooth development at advance bell stage for first molar (upper and lower) teeth showing apposition of hard tissue, dentin and enamel and showed maxillary bone trabecula formation.

Control group at the 10 days postnatal: Upper and lower teeth germ shows tooth development at advance bell stage recognized by apposition of hard.
Experimental group at the 10 days postnatal (0.25): Tooth development at advance bell stage for upper and lower teeth was detected with apposition of hard tissue. Thin bone trabecula of maxillary and mandibular jaws were recorded too.

Figure 5: Experimental group at the 10 days postnatal (0.25)

Experimental group at the 10 days postnatal (0.5mg): Tooth development at eruptive stage for upper and lower teeth showed pulp, dentin, and mature enamel detected as enamel space. Fusion of oral epithelium with reduced enamel epithelia can be recognized easily with mandibular and maxillary bone trabeculi.

Figure 6: Experimental group at the 10 days postnatal (0.5mg).

Control group at the 14 days postnatal: Tooth germs at maturative stage for the upper and lower teeth were detected in control group after 14 days postnatal period.

Figure 7: Control group at the 14 days postnatal.

Experimental group at the 14 days postnatal (0.25mg): Histological feature of tooth germ of upper and lower showed a retardation in enamel apposition especially in upper as ameloblast illustrates different feature view from typical ameloblast.

Figure 8: Experimental group at the 14 days postnatal (0.25mg).

Experimental group at the 14 days postnatal (0.5 mg): Histological feature for upper and lower teeth shows eruption stage with destruction of all connective tissue overlies the newly formed enamel.

Figure 9: Experimental group at the 14 days postnatal (0.5 mg).

Control group at the 18 days postnatal: The tooth germ of upper and lower jaw of a rat at 18 days old shows full crown formation, full thickness
formation of dentin with maturation of enamel appeared as enamel space. Fusion of oral epithelia with reduced enamel epithelia (which represented by fusion of ameloblast, stratum intermedium and stellate reticulum) can be detected with mandibular and maxillary bone trabeculi.

Experimental group at the 18 days postnatal (0.25 mg): tooth development at advance bell el represented by apposition of hard tissue included dentin and enamel. Enamel thickness still incomplete stage and even its maturation, whereas ameloblast can’t be identify, still primitive bone trabeculi appeared as woven bone filled with osteoblast rimming its surface.

![Figure 10: Control group at the 18 days postnatal](image1)

![Figure 11: Experimental group at the 18 days postnatal (0.25 mg)](image2)

Experimental group at the 18 days postnatal (0.5 mg): tooth development at eruptive stage for upper and lower jaw (full crown formation). Root formation, epithelial diaphragm, apposition of radicular dentin and even cementoblast cell and well developed maxillary and mandibular bone formation.

![Figure 12: Experimental group at the 18 days postnatal (0.5 mg)](image3)

Control group at the 21 days postnatal: full mature crown, full dentin thickness, and sulcular epithelia were detected too.

Experimental group at the 21 days postnatal (0.25 mg): Histological feature for tooth germ of upper and lower first molar show tooth development at eruptive stage, reduced enamel epithelia fused with oral epithelia.

![Figure 13: Control group at the 21 days postnatal](image4)

![Figure 14: Experimental group at the 21 days postnatal (0.25 mg)](image5)

Experimental group at the 21 days postnatal (0.5 mg): eruption of the crown with root formation. Formation of cemento-enamel junction, formation of sulcular epithelia, well developed formation of alveolar bone with well-developed periodontal ligament with its grouping obviously detected.
Well-developed bone for maxilla and mandible reported too.

Serum zinc level
The mean of serum zinc level in control from 120 to 90 µg/dl in 7 days and 21 days respectively. For group (0.5mg) it’s reported to be 150 to 130 for 7 days and 21 days respectively, while group (0.25mg) reported to be the lowest in serum zinc level and it recorded to show 90, 85 as for 7 days and 21 days respectively. Also significant different values between group 0.5 and 0.25 in all studied periods.

**Figure 16: Serum Zinc level in different groups and in different periods**

**DISCUSSION**
In the present study, the effects of zinc deficiency on the maxilla, mandible and teeth during growth were evaluated in rats. Zinc has been demonstrated to be essential for normal growth of human skeleton and for skeletal growth in many animals. It is the second most abundant trace metal in the human body and is present in all living cells and body secretions (8). Although zinc is an essential trace element, different concentrations are toxic to cells, and zinc uptake, intracellular storage and efflux are carefully maintained (9). The presence of morphological changes in ameloblast may related to the fact that zinc are essential components of many enzymes and transcription factors and the deficiency of zinc results in reduced food intake and growth, impaired synthesis of DNA and as a result cell architecture will be impaired too (9). It is known that rats are more susceptible than other animals to zinc deficiency; it therefore provides a useful model for the study of the effects on craniofacial structures of dietary zinc deficiency and its supplements during growth (2). The present materials used zinc sulfate rather than other zinc form like zinc methacrylate because of its adequate absorption which lead to elevation of serum zinc level. Our observations concerned on growth sequences of (molar teeth and jaws) in zinc deficiency rat supplemented with different dose (0.25, 0.5mg) in periods include 7, 10, 14, 18, 21 days(postnatal). Choosing these periods represented:
- a. Time of dentinogenesis.
- b. Time for amelogenesis.
- c. Formation of root.
- d. Formation and development of jaw bone.
- e. Appearance of the crown in oral cavity (eruption process).

Acceleration in teeth growth and early apposition of hard dental tissue and jaw bones enhance the process of eruption in experimental group (0.5 mg) Which retarded in the growth of teeth, eruption and bone formation in experimental group (0.25 mg) may related to its low dose that can’t be reestablish and reenhance the activity of formative cell, their growth, differentiation and division and also zinc is essential for synthesis of hydroxypapatite that is important in mineralization process.

**REFERENCES**

