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In Vivo Effect of Calcitonin Hormone on Rat Embryonic Dental Tissue (Histological, Biochemical, And Radiographical Studies)

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Abstract

Background: Calcitonin is a hypocalcemic factor secreted by the parafollicular cells of the mammalian thyroid and it plays a role in the calcification of the dental matrix.

Aim of the study: To evaluate the role of Calcitonin in the dental tissue

Material and Method: Sixty albino wister female rats (2-3 months of age, 200-250 gm of weight), were used in the present experiment. The rats were divided into:

1. The control group consists of 20 rats that received distilled water (I.M.).
2. Experimental group: consists of 40 rats, 20 rats received Calcitonin in 0.1 IU and remain 20 rats received 0.5 IU of Calcitonin (I.M).

From all pregnant rats, prenatal embryos (16 days I.U.L and 18 days I.U.L and postnatal embryos (1 day and 10 days old were studied radiographically using Promax X-ray and histologically (by Hematoxylin and eosin stain), biochemical analysis for alkaline phosphatase was done to blood sample obtained from prenatal group and delivery rats.

Results: The present study showed that embryos with Calcitonin 0.1 IU illustrate histologically:

a. Tooth germ in cap stage of 16 days of I.U.L.
b. Tooth germ in bell stage with cervical loop development at 18 days of I.U.L.
c. Tooth germ showed deposition of hard tissue enamel and dentin at one day old rat.
d. Tooth germ showed maturation of hard tissue enamel and dentin at 10 days old rat.

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Histologically: Embryos with 0.5 IU illustrate retardation in tooth development and impairment in calcification and maturation of enamel and dentin, with a wide zone of predentin formation. Radiographically: Cephalic and lateral views of embryos treated with 0.1 and 0.5 IU showed low density of skeletal bone and head. Biochemically: The mean concentration of alkaline phosphatase enzyme was recorded to be less in experimental groups (0.1 and 0.5 IU of calcitonin) in comparison to the control group and they are less in the 0.5 IU group in comparison to 0.1 IU group.

Conclusion: Calcitonin hormone of 0.1 IU dose showed to be the initiator and illustrates premature tooth development. Whereas 0.5 IU dose showed to be retarder for tooth germ development and effects on mineralization and maturation of bone and dental hard tissue.

Keywords: calcitonin, osteoclast, alkaline phosphatase

Introduction

Calcitonin is a hormone that received its name because of its secretion in response to induced hypocalcemia and hypocalcemia effect [1] therefore Calcitonin is known to participate in calcium and phosphorous metabolism in mammals. The major source of calcitonin from the parafollicular or C cells in the thyroid gland [2]

Calcitonin is an inhibitor of bone resorption, whose function is to prevent bone loss at times of stress on calcium conservation. This includes pregnancy, lactation, and growth [3]

Many studies have examined the effects of calcitonin on alkaline phosphatase enzymes and suggested changes in effect on bone development [4]

Other researchers studied the in vitro effects of calcitonin in the cultured tooth germ and its influence on the differentiation of odontoblast and the formation of hard tissue predentin and dentin [5,6].

Others studied the role of calcitonin in the calcification of dental matrix in vivo (rats) with chronic calcitonin deficiency only [7]

As incisor teeth of rats grow continuously and represent the rapidly growing calcified structure, and as there are no studies concerning the role of calcitonin dose in developing tooth germ, the present study was designed to investigate its effect [8].

Materials and Methods

Sixty Albino Wister female rats (2-3 months of age, 200-250 gm of weight), were used in the present experiment. Those rats were divided into two groups:

1. Control group: consist of 20 rats that received distilled water as an intramuscular injection (I.M).
2. Experimental group: consisting of 40 rats, 20 rats received Calcitonin in a concentration of 0.1 IU/ml, and the remaining 20 rats received 0.5 IU/ml as intramuscular injection.

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From all pregnant rats of both (experimental and control), groups of rats were sub-divided according to period of gestation life into:

1. Prenatal rat group includes: 16 days I.U.L. (41 embryos), and 18 days I.U.L. (42 embryos)
2. Postnatal rat group include One day old rats (15 rats) and 10 days old rat (15 rats).

Blood samples were obtained (before the scarifying date) from pregnant rats for the prenatal group and from delivery rats for the post-natal group for biochemical analysis. The prenatal group (16 days and 18 days of gestation I.U.L) and the post-natal group (1-day and 10-day old rat) were examined radiographically and histochemically.

Calcitonin Hormone: Miacalcin (Calcitonin - salmon) of 100 I.U/ml administered by intramuscular injection. It was injected in a dose of 0.1 and 0.5 I. U (80 and 200 mg/kg. B.W. alternatively) given by (1 ml) disposable sterilized syringe.

Distilled Water: The distilled water was injected into the control group in the same way as in the experimental group

Calcitonin Injections to Pregnant Rats: Sixty female Albino Wister rats with an age range of 2-3 months were first isolated from the rest, for two weeks to exclude any previous pregnancy. After examination and confirmation of non-pregnancy by the resident veterinary doctor, each female was paired with a male in separate cages which were checked daily for the presence of a vaginal plug. By calcitonin, the evidence of mating in the following morning was designated as day zero of gestation. Intramuscular injections were started on zero days of gestation daily for one week. Control females were injected by distilled water and the experimental ones injected

Histological Evaluation: After the experiment animals were scarified using ether vapour. The embryos from the uterus of the pregnant rats were obtained, separated the head from the body, and cut sagitally into two halves, and the specimens (concerning the incisors tooth only) were preserved in 10% buffered formalin for 72 hours for the histological process.

Histological Process:

Dehydration of specimens in a series of alcohol concentrations 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 ethanol. Embedding of specimens in paraffin blocks. Serial sections of 5-micrometer thickness were obtained by Microtome and mounted on microscopic slides 213455555555555555555555

Radiographical Evaluation: Radiographical evaluation for osteogenesis (bone formation) and density of the head of embryos in both groups was done in digital radiograph in both lateral view and cephalic view with exposure values of: 68 KV, 5 mA, 13.000 MS, with a spot size of 0.25 mm2, and the target to sensor distance was 23cm. The x-ray machine used was Promax x-ray (Dimax 3, Planmeca).

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Biochemical Analysis: 2 ml of blood samples were taken through cardiac puncture, from all pregnant rats included in the present study, the blood samples were centrifuged in a universal 16 A centrifuge, and serum was obtained for ALP analysis.

Enzymes Analysis -Alkaline Phosphatase

Serums of studied groups (control and experimental) were analyzed by colorimetric method for ALPase activity according to the following reaction:

Phenyl phosphate → ALP/PH 10 → phenol + phosphate

The liberated phenol is measured in the presence of amin-4-antipyrine and potassium ferricyanide. The presence of sodium arsenate in the reagent stops the enzymatic reaction. The procedure was done according to the manufacturer's instructions (Biormeriex phosphate alkaline kit/ France)

Calculation:

(Reading of Sample - Blank)/ (Reading of Standard) ×Concentration of Standard (n)

The concentration of standard 20 KAV/10 ml.

Results

The results of histological features of tooth development are assessed in the experimental group including rats from treated mothers with 0.1 IU and with 0.5 IU of calcitonin) and in the control group coincidental with different periods of tooth development.

1- Prenatal life

a) At 16 days I.U.L.

Control group: shows tooth germ in the bud stage, enamel organ arises from dental lamina which extended from oral epithelium illustrates active basal cell proliferation with presence of basement membrane separates it from the dental papilla. Dental papilla shows proliferation and active mitotic division of ectomesenchymal cells. The dental sac shows active cell proliferation, condensation around bud germ, and active blood vessel formation as shown in Fig 1.

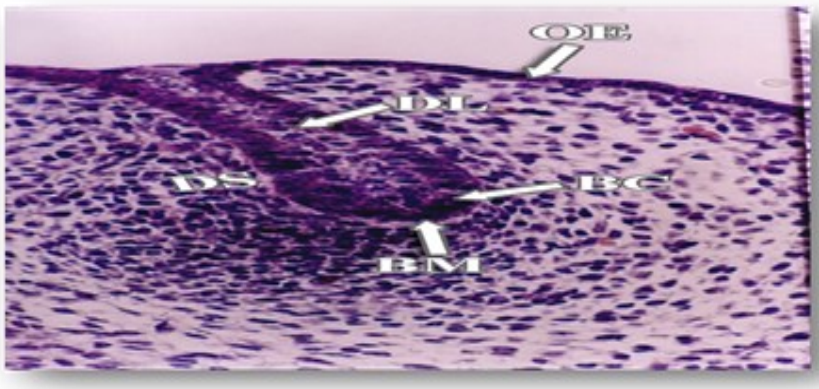

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Fig1. High magnification of the upper tooth germ of embryo rats (16 days I.U.L), control, shows Oral Ectoderm(0E), Dental Lamina (DL), Basal Cell (BC), Basement Membrane (BM) and Dental Sac(DS). H&E X 200.

Experimental group

(0.1 IU of Calcitonin): The histological findings of the coronal section include the upper and lower jaw of embryo rat at 16 days I.U.L. shows tooth germ in the cap stage. The Enamel organ differentiates into 3 layers include Inner enamel epithelium, Stellate reticulum, and Outer enamel epithelium.

The dental papilla shows active proliferation and condensation of ectomesenchymal cells beneath the inner enamel epithelium. The dental sac shows active cell proliferation, fibroblast cells can be detected. Active blood vessel formation can be noticed as displayed in Fig. 2.

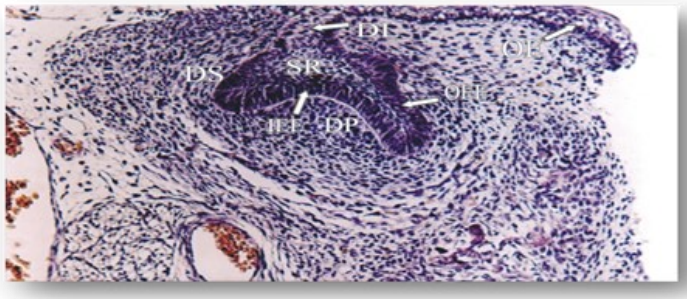

Fig.2: Coronal section in the upper and lower jaw of embryo rat (16 days I.U.L.), mother treated with 0.1 I.U of Calcitonin illustrates: A– Tooth germ of upper central in cap stage, Oral Ectoderm (OE), Dental Lamina (DL), Inner Enamel, Epithelium (IEE), Outer Enamel Epithelium (OEE), Stellate Reticulum (SR), Dental Papilla (DP), Dental Sac (DS). H&E X100

group (0.5 IU of Calcitonin): The microphotographic views of jaws for embryo rats at 16 days I.U.L. show an early stage of tooth germ development represented by the presence of primordium of thickening in certain areas of oral ectoderm. On high magnifying power the histological view illustrates active proliferative cells. Localized as a certain foci in oral ectoderm. The underneath tissue is the ectomesenchymal tissue as shown in bellow Fig. 3

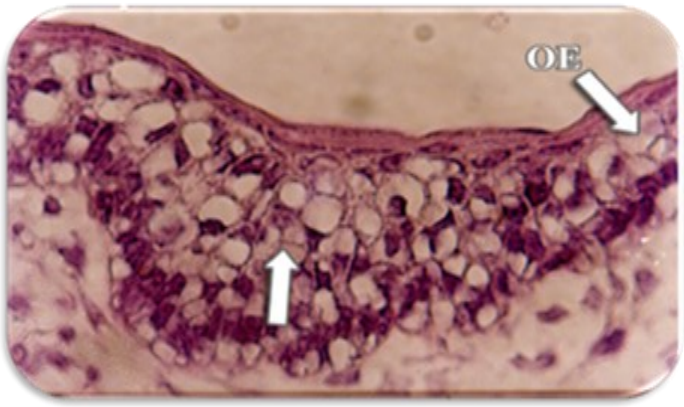

Fig. 3: High magnification shows: Active mitotic basal cells (arrow) representing early stage of tooth germ development, within the Oral Ectoderm (OE). Note theunderneath tissue is Ectomesenchymal Tissue (EMT). H&E X200.

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12b) At 18 days I.U.L.

Control group: histological findings of jaws of embryo rats 18 days I.U.L. shows tooth germ developed in the cap stage. The lower jaw illustrates the early cap stage. Tooth germ shows an enamel organ with 3 layers: Inner enamel epithelium as tall columnar cells in the concave area of the enamel organ. Outer enamel epithelium as cuboidal cells occupy the convex area of the enamel organ. The dental papilla shows active proliferative mesenchymal cells. The dental sac illustrates the condensation of the mesenchymal cell around the enamel organ as displayed in Fig. 4.

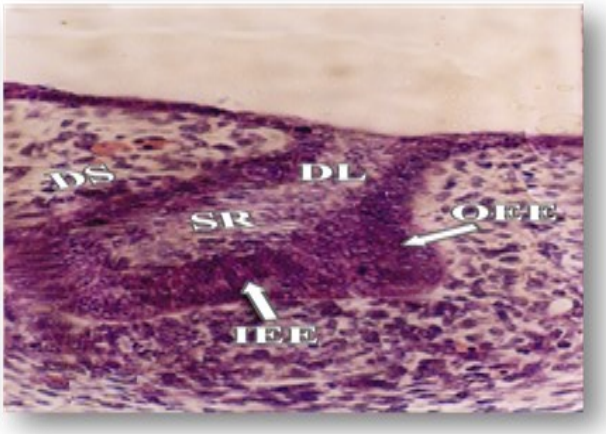

Fig.4: High magnification of tooth germ(control) of the embryo (18 days I.U.L) shows Inner Enamel Epithelium (IEE), Outer Enamel Epithelium (OEE), Stellate Reticulum (SR), Dental Lamina (DL), Dental Papilla (DP), Dental Sac (DS). H&E X200.

Experimental group (0.1 IU of calcitonin):

The microscopical findings of embryo jaws at 18 days I.U.L show tooth germ at the bell stage. The Enamel organ illustrates 4 layers including the inner enamel epithelium, the Stratum intermedium, represented by a few layers of the squamous cell lies over the inner enamel epithelium, the Stellate reticulum showed as stellate in shape and the Outer enamel epithelium. Fig.5 Cervical loop development can be detected, starting with root formation. Vestibule establishment can be also seen.

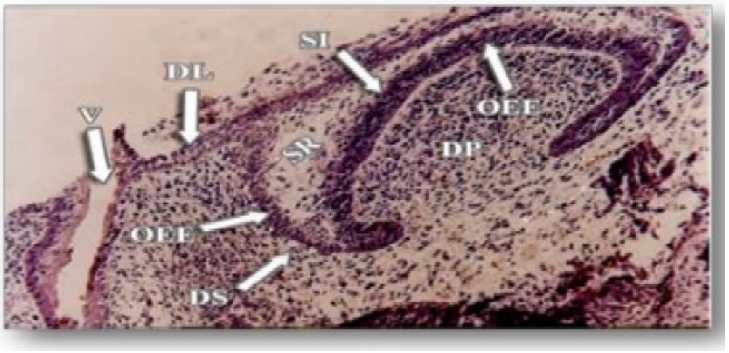

Fig.5: Microphotograph view of tooth germ for embryo rat (18- days I.U.L.), mother treated with 0.1 I.U of calcitonin. Illustrates tooth development at bell stage. Dental Lamina = DL, Inner Enamel Epithelium = IEE, Outer Enamel Epithelium = OEE, Stratum Intermedium = SI, Stellate Reticulum = SR, Dental Papilla = DP, Dental Sac = DS, Vestibule = V, H&E X100

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Experimental group (0.5 IU of calcitonin):

Histological findings of jaws of embryo rat (18 days I.U.L.) shows tooth development, suggested cap stage. The enamel organ shows inner enamel epithelium similar to outer enamel epithelium (tall columnar), stellate reticulum shows active proliferative cell, packed, condensed between inner and outer enamel epithelium. Dental papilla and dental sac show cells closed to inner enamel without separation, the cells also looked to show low proliferative activity. Fig.6.

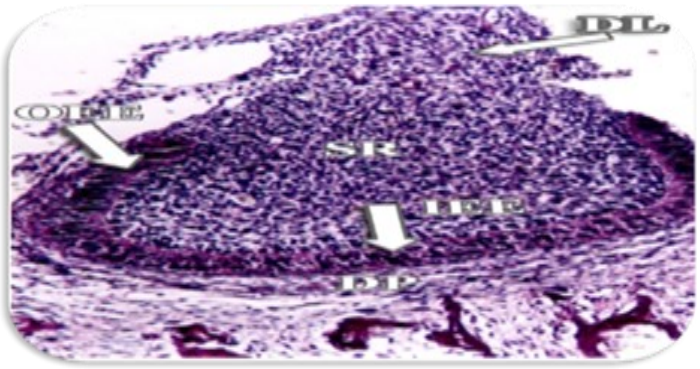

Fig.6: High magnification shows tooth germ layers, Inner Enamel Epithelium (IEE), Outer Enamel Epithelium (OEE), Stellate Reticulum (SR), Dental Lamina (DL), Dental Papilla (DP). H&E x 100

2- Postnatal life

At 1-day old rat.

Control group: Histological findings for tooth germ of one day old rat shows many events:

1. Histodifferentiation represented by odontoblast cell that deposits dentin and ameloblast cell that deposits enamel.
2. Morphodifferentiation represented by output morphology of tooth which shows to be incisors.
3. Fig.7 illustrates ameloblast cell tall columnar with Tom's process represented secretory phase.

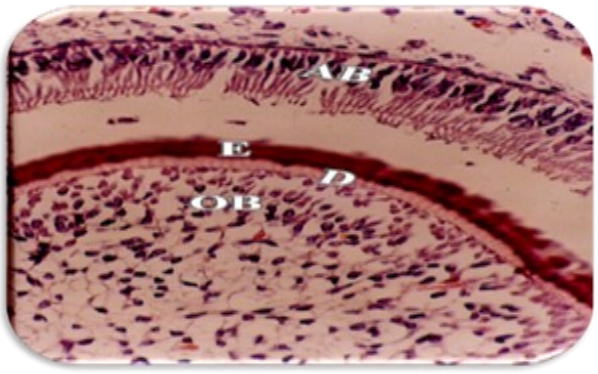

Fig.7: View for tooth germ of 1-day neonatal rat (control) shows Odontoblast (OB), Dentine (D), Enamel (E), Ameloblast (AB) with Tom's Process.

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Experimental group (0.1 IU of calcitonin):

Microphotograph view of tooth germ for embryo one day old shows hard tissue apposition (dentin and enamel). Fig.8 shows odontoblast cells occupy pulp surface, ameloblast cells occupy outer surface.

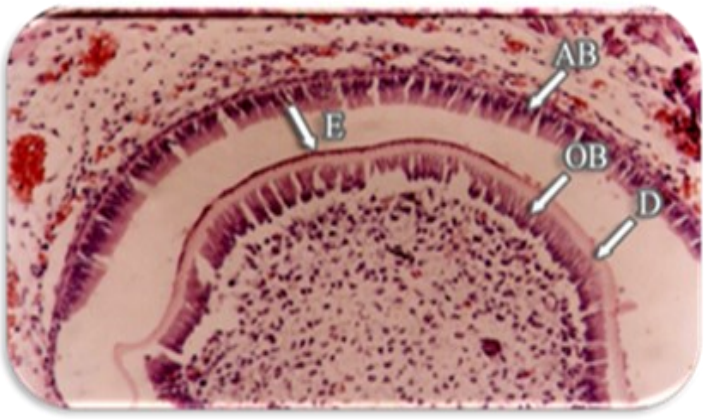

Fig.8: Microphotograph view of tooth germ for embryo rat 1-day old mother treated with 0.1 IU of Calcitonin illustrates hard dental tissue apposition (Dentine and Enamel) Pulp = P, Odontoblast = OB, Dentine = D, Enamel = E, Ameloblast = AB. (H& E X 100)

Experimental group (0.5 IU of calcitonin):

The histological findings of tooth germ of one day old rat shows, odontoblast cell differentiated from dental papilla, deposits of dentine (narrow zone). ameloblast cells show to be in their Presecretory stage. The pulp shows active mesenchymal cell, fibroblast cell, formation of new blood vessels. Fig. 9

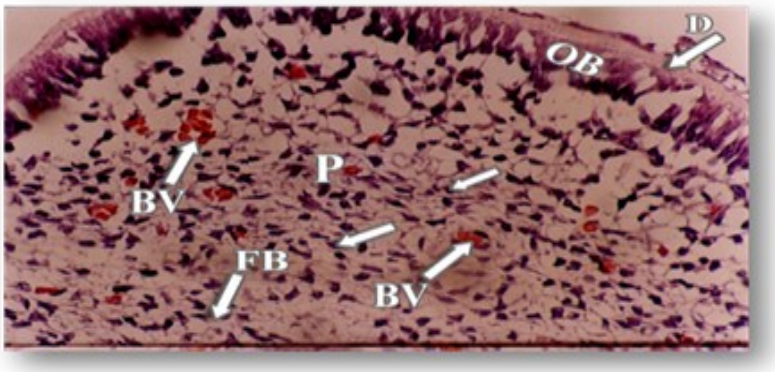

Fig.9: High power view for tooth germ related to embryo rat 1 day old treated with calcitonin (0.5 I.U) shows: Odontoblast (OB), Dentine (D), Pulp (P), active Mesenchymal Cell (arrow), Fibroblast Cell (FB), Blood Vessels (BV), H&E x 200.

b) At 10 days old rat:

Control group: the histological feature of tooth of rat (10 days old) shows complete deposition of hard tissue. The tooth surround by bony crypt, well developed bone formation which represented

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bone of the jaws. On high magnifying view, shows odontoblast, predentin, dentin, enamel, ameloblast in its Maturative stage as it represented microvilli in its distal end close to enamel. Fig.10.

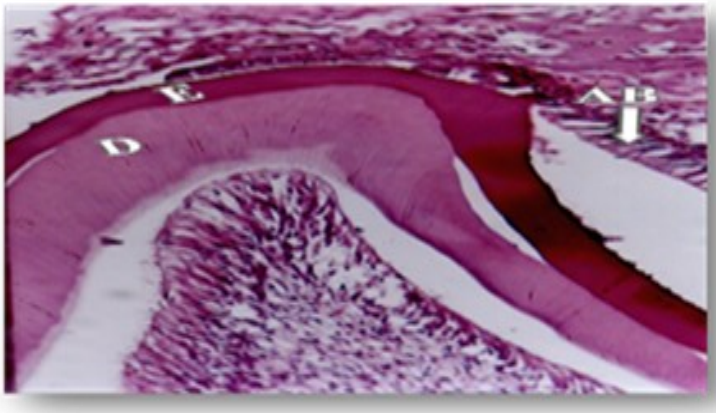

Fig.10: Microphotograph of the tooth germ of rat (10 days old) control, illustrated deposition of Dentin (D), Enamel (E), Ameloblast (AB) shows reduction in its size, H&E X 100.

Experimental group (0.1 IU of calcitonin):

Microscopical evaluation of tooth for rat (10 day old) shows apposition of hard tissue dentin and enamel. In Fig.11 High magnification shows Ameloblast still tall columnar in shape which still its secretory stage.

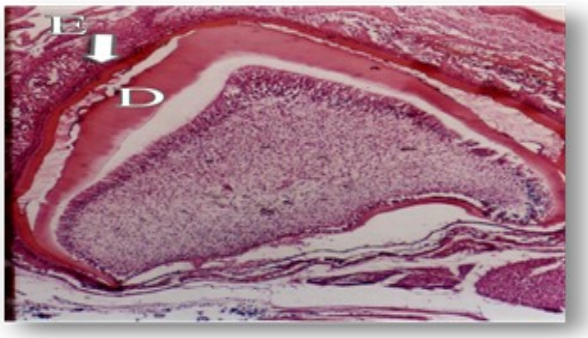

Fig.11: Microphotograph view for tooth germ of embryo rat 10 days old, mother treated with 0.1 calcitonin, illustrates apposition of Dentine (D), Enamel (E). H & E X 100

Experimental group (0.5 IU of calcitonin):

Histological findings of tooth related to rat (10 days old), illustrates deposition of hard tissue dentin and enamel, the tooth germ enclosed with hazy bone crypt represented by a thin trabecula.

High power magnification shows wide zone of predentin with irregular border line between predentin and dentine. Ameloblast secretes thin layer of enamel. Fig.12.

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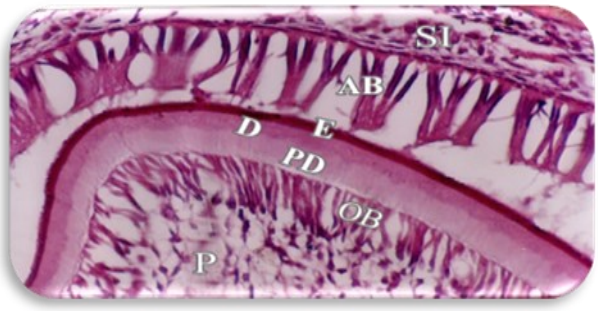

Fig.12: high power view shows Pulp (P), Odontoblast (OB), Wide layer of Predentin (PD), dentine (D), thin layer of Enamel (E) opposed by Ameloblast (AB). Note Stratum Intermedium (SI). H&E X 200.

Statistical analysis of hard structure width of central incisors in different group at 10 days old.

The statistical evaluation of the width of predentin, dentin and enamel were measured for studied groups (control and experimental) at time of calcification included (10 days old only). Statistical analysis shows significant value. No significant value was found in predentin thickness between control and experimental (0.1 IU) group. For dentin thickness, significant value was found in differences of the mean of width dentin between control and experimental (0.5 IU) groups while non significance difference was illustrated between control and experimental (0.1 IU) groups in dentin thickness.

Enamel thickness in experimental (0.5 IU) group shows thin layer deposit at 10 days old rat. statistical analysis for enamel width between control and experimental groups shows significant difference. Non-significant value in enamel thickness between control and experimental (0.1 IU) groups was recorded.

Radiographical Findings

Cephalic radiographic views and lateral radiographic figures are taken to embryos of treated mother with 0.1 IU and 0.5 IU of calcitonin. Age of embryo included 18 day I.U.L, 1-day old rat and 10 days old rat.

In comparison with control group matching same period of age, the results of radiographic film revealed low density of skeletal bone concerning to head region (especially maxilla and mandible, carrying the tooth germ), in the embryos treated (experimental groups). Differences radio opacity of bone illustrates easily between the study groups in different interval periods.

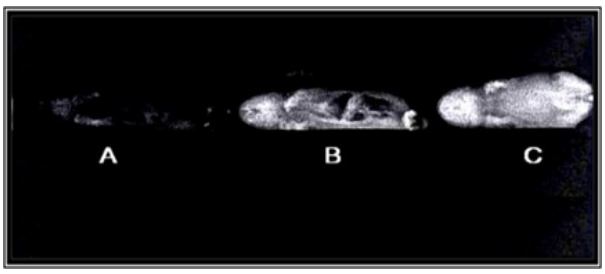

Fig.13: Lateral radiographic view of 1-day old rat represent:

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A- 1-day old rat from mother treated with 0.5 IU of calcitonin.
B- 1-day old rat from mother treated with 0.1 IU of calcitonin.
C- 1-day old rat control.

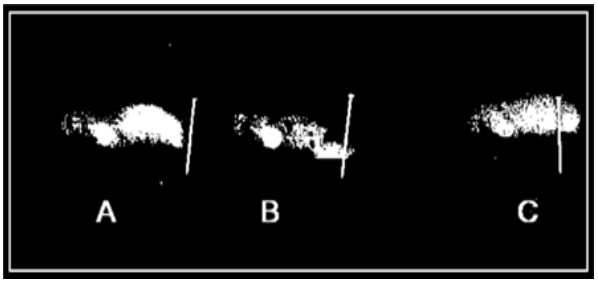

Fig.14: Cephalic radiographic view of 10-day old rat represent:

A- 10-day old rat from mother treated with 0.5 IU of calcitonin.
B- 10-day old rat from mother treated with 0.1 IU of calcitonin.
C- 10-day old rat control

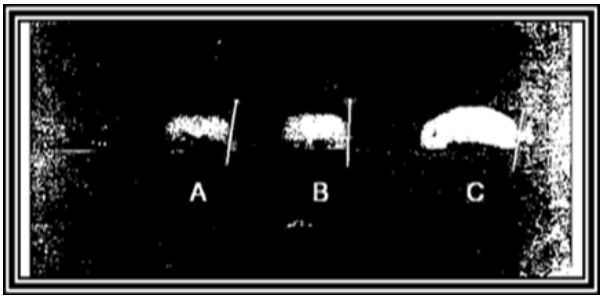

Fig.15 Lateral radiographic view of 18 days I.U.L embryos represent:

A- 18-day I.U.L. embryos from mother treated with 0.5 IU of calcitonin.
B- 18-day I.U.L. embryos from mother treated with 0.1 IU of calcitonin.
C- 18-day I.U.L. embryos control

Biochemical results:

Assessment of Alkaline Phosphatase enzyme (ALP) in serum of pregnant rat.

The mean concentration of ALP in serum of control group at different periods (16 day I.U.L, 18 day I.U.L and 1-day postnatal period) shows little variation in levels as (742.2+ 2.39, 744.8+- 2.3 and 742.8 +2.24) respectively.

In experimental group (0.1 IU) the level of ALP shows to be higher in 1 day period than in 16 day I.U.L and 18 day I.U.L (439.1+- 2.43, 207.9+- 4.06 and 218.8+- 2.19) respectively.

While in experimental (0.5 IU) group, ALP level shows to be higher in 16 day I.U.L than in 18 day I.U.L and 1 day postnatal (350.8+- 2.74, 289.1+-3.41 and 286.7+- 2.8) respectively, (Table 1).

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Table (1) Mean and Standard deviation of AIP. And ANOVA table

Study groups	16 day		18 day		1day		F-test	P-value	Sig
	Mean	SD	Mean	SD	Mean	SD			
Control	742.2	2.396	744.8	2.305	742.8	2.242	5.180	0.010	S
Exp.1(0.1)	207.9	4.061	218.8	2.199	439.1	2.433	280.4	0.000	HS
Exp.2(0.5)	350.8	2.748	289.1	3.411	286.7	2.840	218.4	0.000	HS

***P<0.05 Significant**

****P<0.0001 High significant**

Statistical analysis (ANOVA) of (ALP) mean concentration in serum among study groups of different interval periods

Statistical analysis t-test and p-value for each group between different periods (Table 2) records significant value in control group between 16 and 18 I.U.L periods and between 18 day and 1 day. While non-significant value shows between 16 I.U.L and 1 day in the levels of ALP.

For experimental (0.5 IU) group high significant values in differences of ALP concentration in comparison between 16 IU and 18 IU periods and 16 IU and 1-day periods. Non-significant value record between 18 I.U.L and 1-day periods.

Table (2) t-test between times of ALPase

	Control		Exp.1(0.1)		Exp.2(0.5)	
	t-test	P-value	t-test	P-value	t-test	P-value
16 &18	2.811	0.014 S*	8.055	0.000 HS	57.05	0.000 HS
16&1day	0.770	0.454 NS	182.7	0.000 HS	63.75	0.000 HS***
18&1day	2.523	0.024 S	236.2	0.000 HS	1.680	0.115 NS**

***P<0.05 Significant**

****P>0.05 Non significant**

*****P<0.0001 High significant**

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Statistical analysis (t-test) of serum (ALPase) level in study groups at different interval periods

Using ANOVA test (Table 3) revealed high significant variation among the study groups in serum ALPase level at different interval periods. And significant value in control group among different periods.

Table (3) Mean and Standard deviation of AIP. And ANOVA table

Study groups	16 days		18 days		1day		F-test	P-value	Sig
	Mean	SD	Mean	SD	Mean	SD			
Control	742.2	2.396	744.8	2.305	742.8	2.242	5.180	0.010	S
Exp.1(0.1)	207.9	4.061	218.8	2.199	439.1	2.433	280.4	0.000	HS
Exp.2(0.5)	350.8	2.748	289.1	3.411	286.7	2.840	218.4	0.000	HS

***P<0.05 Significant**

****P<0.0001 High significant**

(Table 4) illustrates t-test and p-value which shows high significant record in difference of concentration of ALPase between control and experimental (0.1 IU) group, control and experimental (0.5 IU) group and experimental (0.1 IU) and (0.5 IU) groups at different interval periods.

Table (4) t-test between times of ALPase

Study groups	16 days		18 days		1day	
	t-test	P-value	t-test	P-value	t-test	P-value
Control&exp.1(0.1)	424.3	0.000 **HS**	698.8	0.000 **HS**	320.2	0.000 **HS**
Control&exp.1(0.5)	386.4	0.000 **HS**	401.2	0.000 **HS**	446.6	0.000 **HS**
Exp.1(0.1)&exp.2(0.5)	114.9	0.000 **HS**	70.81	0.000 **HS**	145.1	0.000 **HS**

****P<0.0001 High significant**

Statistical analysis (t-test) of serum (ALPase) level in study groups at different interval periods

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Discussion

Prenatal Period

At 16 days I.U.L, embryos received 0.1 IU of calcitonin showed premature initiation of tooth germ, as the result illustrates tooth germ at cap stage incomparison to control which showed tooth germ at bud stage. While a deterioration in development of tooth germ was observed in experimental group that received 0.5 IU of calcitonin, represented as localized foci of epithelium thickening in oral ectoderm. This result could be explained by the effect of (0.1 IU calcitonin) as a chemical signal in initiating or participating in premature interaction between the epithelium of enamel organ (oral ectoderm) underneath mesenchyme of neural crest cell in origin, leading to the early development of tooth germ. Versus the action of 0.5 IU calcitonin which may act as an inhibitory factor for epithelial-mesenchymal interaction that deteriorates tooth development.

At 18 days I.U.L, the present findings showed the development of tooth germ in the cap stage in the control group while the experimental (0.1 IU of calcitonin) group developed in a bell stage. Two different stages of development morphogenesis and histogenesis, respectively.

The results may be attributed to the influence of 0.1 IU calcitonin dose on cell differentiation, illustrated by the formation of 4 distinct layers: inner enamel epithelium, stratum intermedium, stellate reticulum, and outer enamel epithelium. Also, the development of the cervical loop which initiates root formation and establishment of the vestibule, is indicated for early and faster evidence of development in comparison with control.

Postnatal period

The present study of embryonic incisor tooth germ of a one-day neonatal rat showed deposition of hard tissue, dentin, and enamel for both control and experimental (0.1 IU calcitonin) groups with histodifferentiation of both cell odontoblast and ameloblast cell, while in experimental (0.5 IU of calcitonin) group, only dentin deposition is illustrated, and the ameloblast seems to be in presecretory stage. Delay in histodifferentiation leads to delay in the deposition of specialized tissue, as a biological sequence due to the influence effect of that dose of calcitonin. [9]

At 10 days old rat, histological results for control showed complete deposition of hard tissue (enamel and dentin). The presence of microvilli in its distal end indicated of maturation stage of ameloblast in which typical morphology of absorptive cells was demonstrated in histological figures for transporting organic components as well as water from the matrix with the addition of minerals and growth of crystals [10].

For the experimental (0.1 IU calcitonin) group the histological examination revealed a complete deposition of hard tissue, enamel, and dentin, although the mean thickness of (predentin, dentin, and enamel) showed to be more than in control, but statistically with no significant value. The results may show that 0.1 IU dose, could not interfere with amelogenesis and dentinogenesis processes. [11].

While the experimental (0.5 IU calcitonin) group illustrated a wide zone of predentin with an irregular borderline, ameloblast secretes a thin layer of enamel as histological records revealed a

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significant difference in mean thickness of predentin, dentin, and enamel, in comparison to the control group. This result indicated impairment in the maturation process including the deposition of organic matrix represented by a thin layer of enamel formation and the mineralization process illustrated by decalcified dentin which is the predentin layer.

Radiographical Findings

Results of cephalic and lateral radiographic view showed low density of skeletal bone in treated embryos with calcitonin specially and severely with 0.5 IU, in comparison to control. These results supported the previous histological findings.

Biochemical Results

The present study provides data on levels of alkaline phosphatase enzyme: - serum of pregnant rat at 16 days I.U.L, 18 day I.U.L, and 1 day (equal to 21 day I.U.L). Unfortunately, we failed to get blood sample from rats of 10 days old because of their small size in a time concerning with mineralization process and the data above.

The present results showed that ALPase concentration in the pregnant rat of control illustrated a significant difference at interval periods 16 I.U.L, 18 I.U.L, and 1 day old while it showed a high significant value in differences of ALPase concentration between control and experimental group 0.1 IU and experimental group 0.5 IU.[12] reported that ATPase is essential for the deposition of minerals in the bone and teeth [13] revealed that developing teeth and bone showed a high enzyme activity, and noted differentiating odontoblasts, stratum intermedium, and in osteoblast cells.

This fundamental information explains the present result as the calcitonin dose (0.1 and 0.5 IU) used in this study showed a lower level of ALPase, therefore, it illustrated an impairment in bone formation as appeared histologically and radiographically.

Conclusion

1. Calcitonin hormone has an effect on both bone and teeth as the results showed histologically, radiographically, and biochemically.

2. Calcitonin of 0.1 IU dose showed an effect on developing bone more than teeth, concerning the impairment of the mineralization process.

3. Calcitonin of 0.1 IU dose was found to be an initiator for the tooth-developing germ as the results showed advanced stages of development in comparison to the control.

4. Calcitonin of 0.5 IU dose was found to be an inhibitor and showed deterioration effects to developing bone and tooth. As the results showed failure of complete maturation and calcification of tooth and bone in comparison to control and to Calcitonin of 0.1 IU dose.

5. Calcitonin showed to play role in alkaline phosphatase enzyme level, may affects its metabolism or/and its function and later on developing bone and teeth.

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