The Effect of Thymosin Beta 4 on Developing Dental Tissue (Experimental Study on Rats)

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Athra'a Y. Al-Hijazi, B.D.S., M.Sc. Ph.D. [(2)]

ABSTRACT

Background: Odontogenisis is a complex process controlled by dynamic and reciprocal interactions that regulated by various molecules. Thymosin β4 is a small bioactive peptide with wide spectrum biological effects on much cell types. The present study was designed to highlight the effect of synthetic exogenous Tβ4 on developing dental tissue of the upper central teeth of rats, by histological examination and immunohistochemical evaluation of TGFβ1.

Materials and method: Thirty six Albino Wister pregnant rat 18control group received intraperitoneal injection of normal saline and the others are experimental group received 50µg/300µl of Tβ4 injection. The animals were sacrificed at periods 16th and 18th day I.U.L and one day post natal, as six animals for each period. Histological and immunohistochemical evaluation for expression of TGF β1 in dental tissue of upper central teeth of the rat were done

Results: In-vivo results showed that experimental group had accelerated stages of tooth development with acceleration in deposition of dental hard tissue (enamel and dentin) with high positive expression of TGF β1by enamel organ, dental papilla and dental sac cells.

Conclusion: these data suggest synthetic exogenous Tβ4 act as bioactive initiator enhances tooth development by stimulating proliferation and differentiation of both epithelial and mesenchymalcells.

Keywords: Tooth development, ThymosinTβ4 and TGF β. (J Bagh Coll Dentistry 2016; 28(3):69-74).

INTRODUCTION

Tooth development is a complex physiological process includes different stages bud, cap and bell stage [(1)], it is characterized by a series of reiterative molecular interactions occur between odontogenic epithelium and ectomesenchymal which in neural crest in origin. Signaling molecules of several conserved families mediated the communications between cells also they mediate the communications within the same tissue layer most of these molecules belong to TGFβ superfamily and other families [(2)]. TGF β1 is amember of TGFβ superfamily of cytokines,it plays an important role in regulating crucial biological processes such as cell proliferation, differentiation, apoptosis, and extracellular matrix deposition and remodeling so it acts as a positive and negative regulator of cellular growth[(3)].

Thymosin beta4 is a small bioactive peptide consist of 43 amino acids residue and has multiple biological functions during embryonic development as it has prominent role in sequestration of G-actin monomers and actin cytoskeletal organization that necessary for cell motility and survival [(4,5)].

Tβ4 is highly associated with tooth morphogenesis as it related with differentiation of dental epithelium through up-regulation of number of biological effectors such as Laminin5, Vascular endothelial growth factor and TGFβ [(6-8)].

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Other important biological properties of Tβ4 including recruitment and differentiation of stem cells, angiogenesis [(9)], and antiapoptic activity [(10)].

MATERIALS AND METHODS

Thirty six Albino Wister pregnant female rats aged (2-3months) and weighting (0.25-0.3 Kg). All animals kept under controlled conditions of temperature, drinking and food consumption and all experimental procedures were carried out according to ethical principles of animal experimentation.

The study groups include:

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1. Thymosin beta 4 synthetic peptide from Abcam Company UK (ab42265).
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After scarifying the animals at periods 16th and 18th day I.U.L and one day post natal. The specimens include upper jaw with two central incisors are fixed in 10% buffered formalin for 73 hours then the samples dehydrated and embedded in paraffin. 5µm section was stained

with heamatoxylin and eosin (H&E). Other sections for immunohistochemical identification of TGF β1for all periods and in all studied groups.

Determination of immunohistochemical results for TGFβ1

For each specimen, the number of positive expression of TGF β1 was determined by evaluating 100 cells for eachslide in five high power fields (HPF) counting the positive one, then the mean of count for eight slides for each sample[11].

Statistical Analysis

A. Descriptive data analysis. B. Inferential data analysis.

RESULTS

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One day post natal shows advanced bell stage with accelerated deposition of dental enamel and dentin with primitive bone (Fig 1, 2)

Immunohistochemical results of TGFβ1

For all studied periods: positivity for expression of **TGFβ1**illustrated by dental epithelial and mesenchymal cells (Fig 3, 4)

Experimental group including all three periods records a strong positive expression for **TGFβ1**.(Fig 4).

Statistical analysis revealed that the experimental group including all studied periods illustrated highly significant value in comparisons to control group (Table 1).

Table 1: Statistic analysis of different type of positive cells expressed TGF B1 in the studied groups distributed in each period with comparisons significant

Periods	Cells	Groups	No.	M	SD	SE	95% C.I. for Mean		Min.	Max.	ANOVA F-test
							L.B.	U.B.			
16 days	Enamel organ	Control	6	9	0.52	0.21	8.8	9.9	9	10	F=31.937 P=0.000 HS
		Exp.	6	11	0.98	0.40	9.8	11.9	10	12	
	Dental papilla	Control	6	7	1.21	0.49	6.1	8.6	6	9	
		Exp.	6	12	0.84	0.34	10.6	12.4	10	12	
	Dental sac	Control	6	11	2.73	1.12	8.5	14.2	8	16	
		Exp.	6	17	1.17	0.48	15.9	18.4	15	18	
18 days	Enamel organ	Control	6	12	0.84	0.34	10.6	12.4	10	12	F=31.409 P=0.000 HS
		Exp.	6	11	0.98	0.40	9.8	11.9	10	12	
	Dental papilla	Control	6	15	2.43	0.99	12.0	17.1	11	18	
		Exp.	6	18	1.33	0.54	16.8	19.6	16	20	
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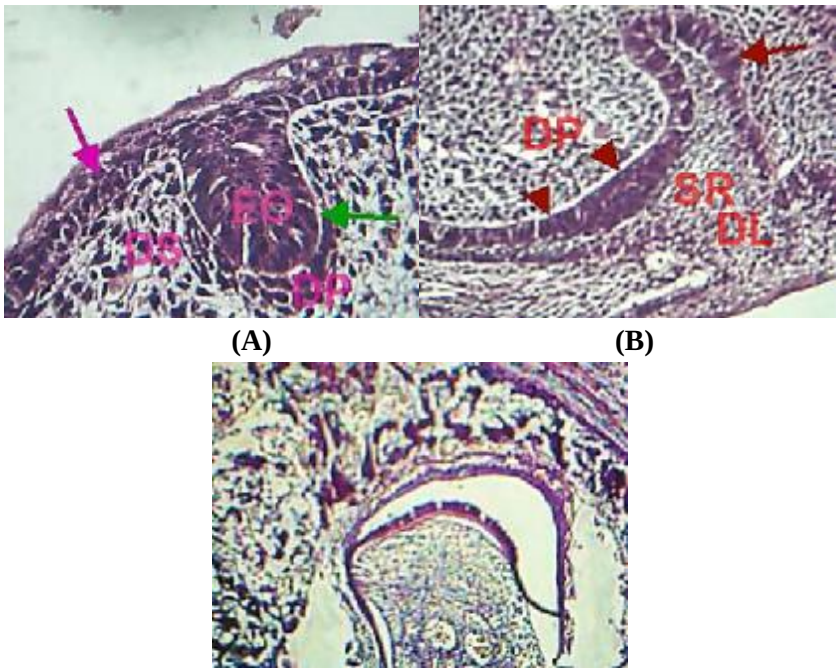
1 day old	Enamel organ	Control	6	15	0.75	0.31	14.4	16.0	14	16	F=44.329 P=0.000 HS
		Exp.	6	17	0.75	0.31	16.4	18.0	16	18	
	Dental papilla	Control	6	14	1.87	0.76	11.5	15.5	11	16	
		Exp.	6	20	1.37	0.56	18.2	21.1	18	22	
	Dental sac	Control	6	18	1.33	0.54	16.4	19.2	16	20	
		Exp.	6	23	1.21	0.49	22.1	24.6	22	25	



(A) **(B)**

(C)

Figure 1: control group (H&E stain). (A) View of upper jaw of rat 16th day I.U.L,tooth germ at bud stage, Oral ectoderm(pink arrow),Basement membrane(green arrow),Enamel organ(EO),Dental papilla(DP),Dental sac(DS) (X20). (B) at 18th day IUL, tooth germ shows Outer enamel epithelium.(arrow),Inner enamel epithelium.(arrow heads),Dental papilla(DP), Stellate reticulum(SR),Dental lamina(DL) (X20).(C) One day postnatal, Tooth germ at bell stage (X10).

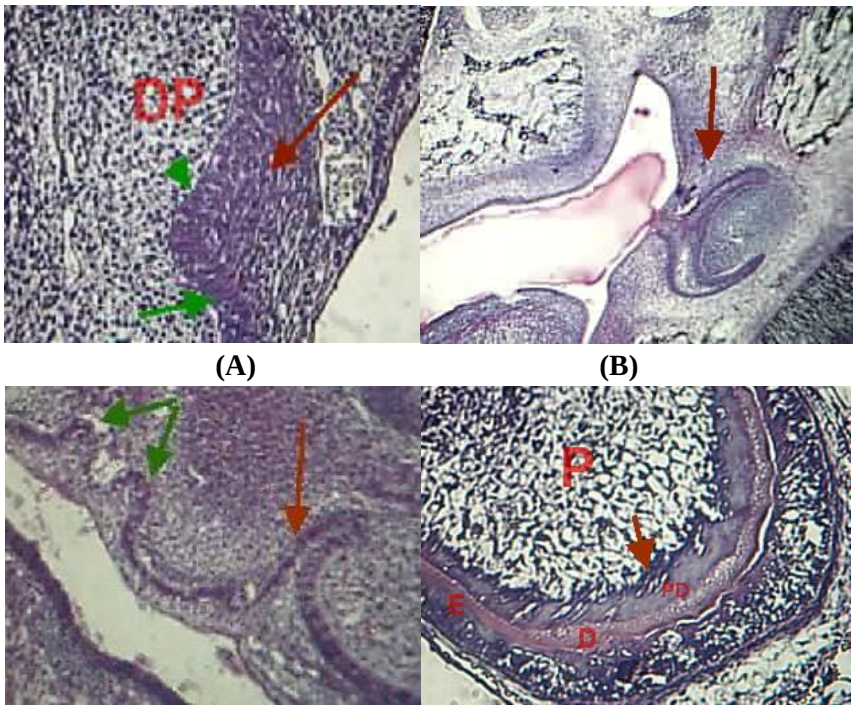


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Figure 2:Experimentalgroup (H&E stain). (A) Upper jaw of rat at 16th day IUL shows. Inner enamel epithelium (green arrow head),outer enamel epithelium(green arrow), proliferating packed cells(red arrow) (X10). (B) At 18th day I.U.L tooth germ at bell stage (arrow) (X4). (C) Multiple tooth germs (X40). (D) One day post natal, tooth germ shows, dentine (D),predentine(PD) pulp(P), enamel(E), odontoblast(OD) (X40).

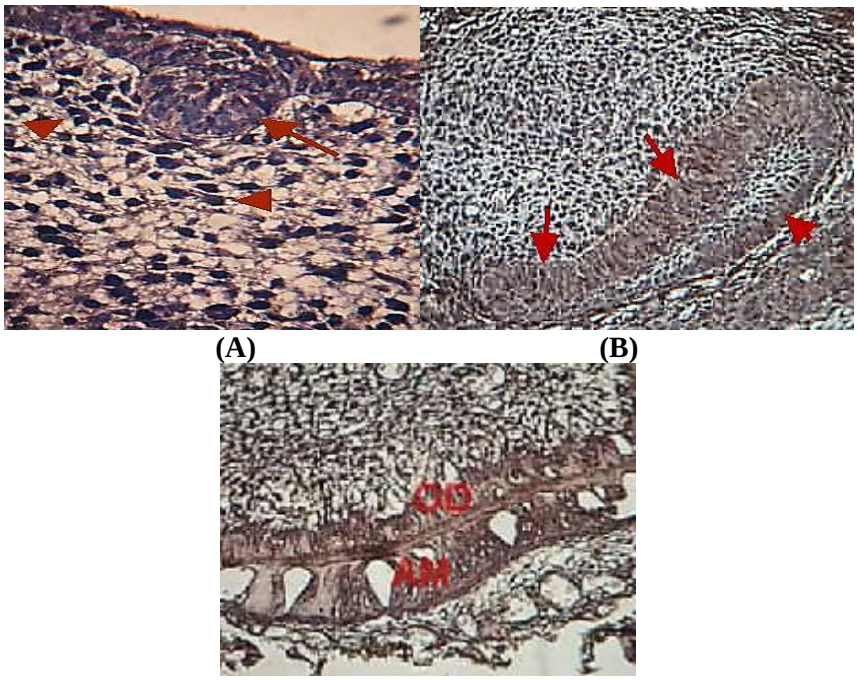

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Figure 3: Control group with immunohistochemical expression of TGF beta1 (A) At 16th day I.U.L upper jaw shows positivity by basal cell of tooth germ (arrow), mesenchymal cell (arrow heads) (X20). (B) At 18th day IUL shows positivity by .Inner enamel epithelial (arrows), outer enamel epithelial (arrow head) in cervical loop (X20).(C) One day old, shows positivity by Odontoblast (OD),ameloblast(AM) (X40).

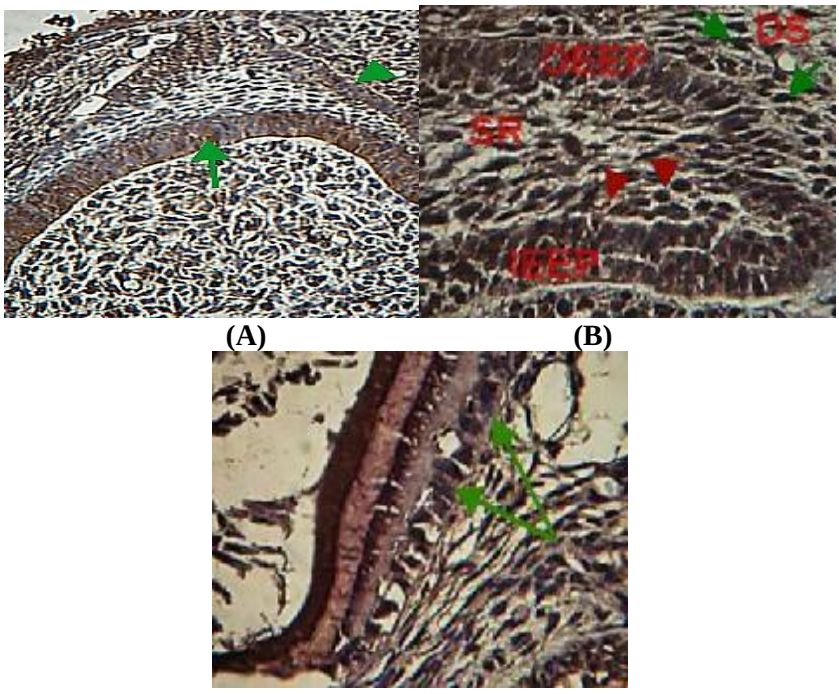

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Figure 4: Experimental group with immunohistochemical expression of TGFβ1 (A). At 16th day IUL shows positivity by.Inner enamel epithelial (arrow), outer enamel epithelial (arrow head) (X20).(B) At 18th day IUL shows positivity by Inner enamel epithelial (IEEP), outer enamel epithelial (OEEP), stellate reticulum (SR),stratum intermedium (red arrow heads), mesenchymal cell(green arrows) in dental sac(DS) (X100). (C) one day old, shows positivity by Ameloblast (arrows) (X100).

DISCUSSION

The present study shows enhancement of tooth germ development, in embryo of 16th day I.U.L of experimental group, tooth germ presents cap stage with proliferating packed cells occupying the whole enamel organ. Dental papilla and dental sac show proliferation of mesenchymal cells Control group shows tooth germ at bud stage with difference in the histological feature in comparisons to experimental group.

1. The employed synthetic exogenous thymosin beta (4) composed of sequence of amino acids from 1to 11 of total 43 of Tβ4 molecule, this part has an important role in the organization of cytoskeleton by binding to and sequesters actin monomer (G-actin) so it inhibits actin polymerization. As actin is an abundant protein present in all cells and it is important in maintaining cell structure and regulating cell motility [5].

2. Laminin-5 is extracellular glycoprotein mediates attachment, migration and organization of cells to tissues during embryonic development, Tβ4 increases the production of laminin5 resulted in promotion of cell migration and cell to cell and cell _ matrix [12].

3. Thymsin β4 may induce stem cell differentiation [13].

Developed tooth germ of experimental group at18th day I.U.L shows bell stage, with a records of multiple tooth germs in two different stages in some specimens .the primitive pulp identifies formation of numerous new blood vessels .At one day post natal embryo a successful apposition of hard dental tissues (dentin and enamel) was detected in experimental group, and can be attributed to the followings:

1. Thymosin beta 4 may promote stem cell migration and differentiation of mesenchymal tissue into odontoblast, osteoblast [14].
2. Thymosin beta 4 has multiple biological activities includes, upregulation endothelial cell differention and stimulate angiogenesis by differentiation and directional migration of endothelial cells [15].

3.Thymosin β4 regulates the expression of Runt-related transcription factor2 Runx2 mRNA that plays important role in production of ameloblastin, amelogenin, dentin matrix protein and dentin sialophosphoprotein all of these are important component of enamel and dentin matrices respectively [16, 17]. Runx2 plays impotant role in development and calcification of tooth germ [18] and it regulates odontogenesis related genes [8].

For TGFB1 expression

1. Thymosin β4 increased expression of many genes associated with angiogenesis, cell proliferation, and migration such as: vascular endothelial growth factor Vegf B, Vegf C, Vegf D, Vegf receptor1, matrix metalloproteinases2(MMP2) and Tgf beta1[19].
2. In secreting ameloblast, it was found that signaling from ameloblast increase autocrine effect of local Tgf β1 on development of enamel organ and provide the basis for synthesis of enamel proteins [20],and in the present study ,it seems that treated with Tβ4enhanced proliferation of ameloblast and then increased expression of Tgf β1.

In conclusions:

- Thymosin beta4 of 50 µl dose injected to pregnant rat at zero time of gestation found to be initiator for the developing tooth germ as the present results showed advanced stages of tooth development in comparisons with control group and as follow:
- Advanced stage for tooth germ in each studied periods.
- Records of multiple tooth germs.
- Acceleration apposition of dental hard tissues enamel and dentin especially (enamel).
- Identification of packed cells in enamel organ.
- Numerous newly blood vessels were seen in mesenchymal tissue for dental papillae and dental sac.
- High expression of TGFB1 by epithelial and mesenchymal stem cells and by specialized cells includeameloblast and odontoblast and osteoblast.

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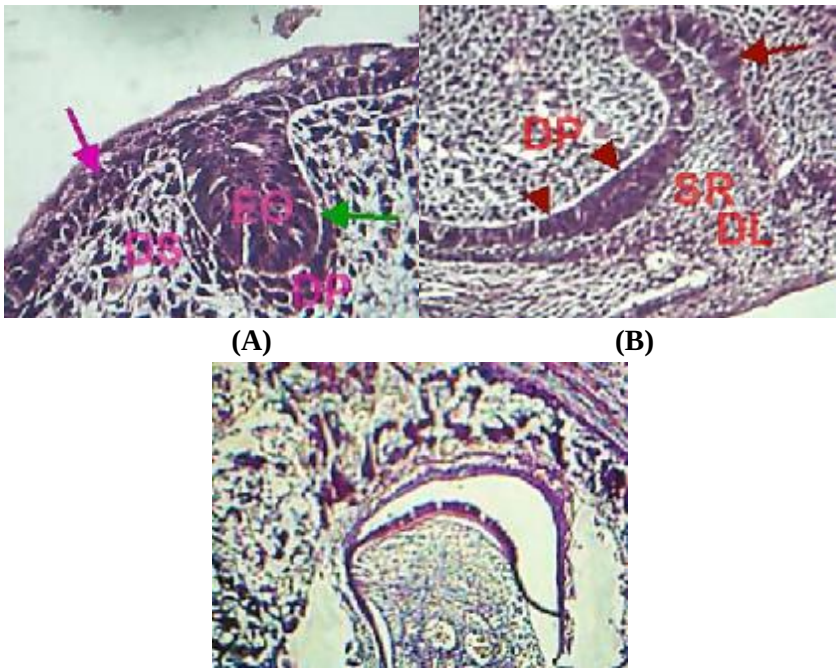

(A) **(B)**

(C)

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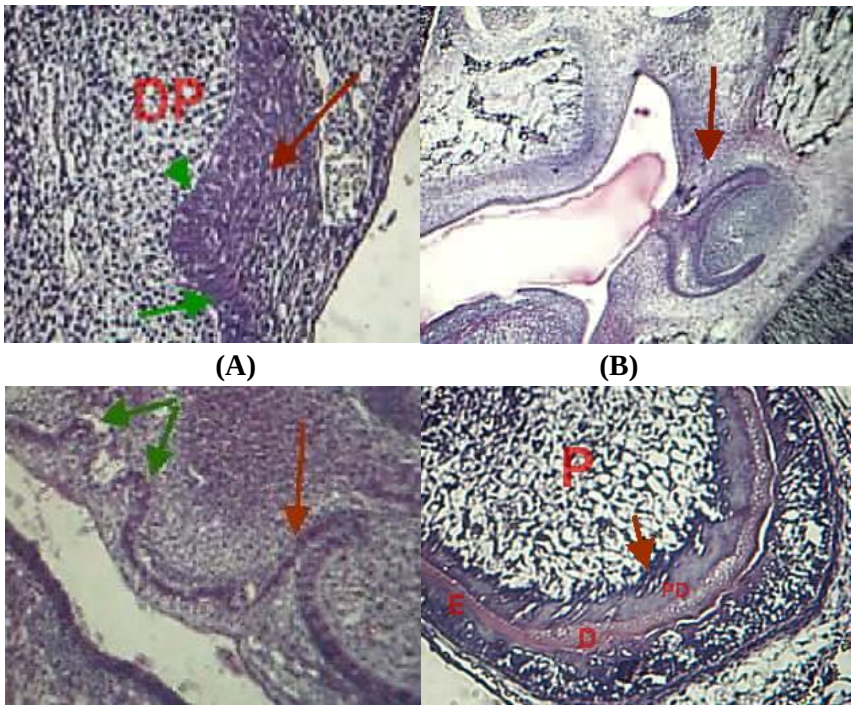
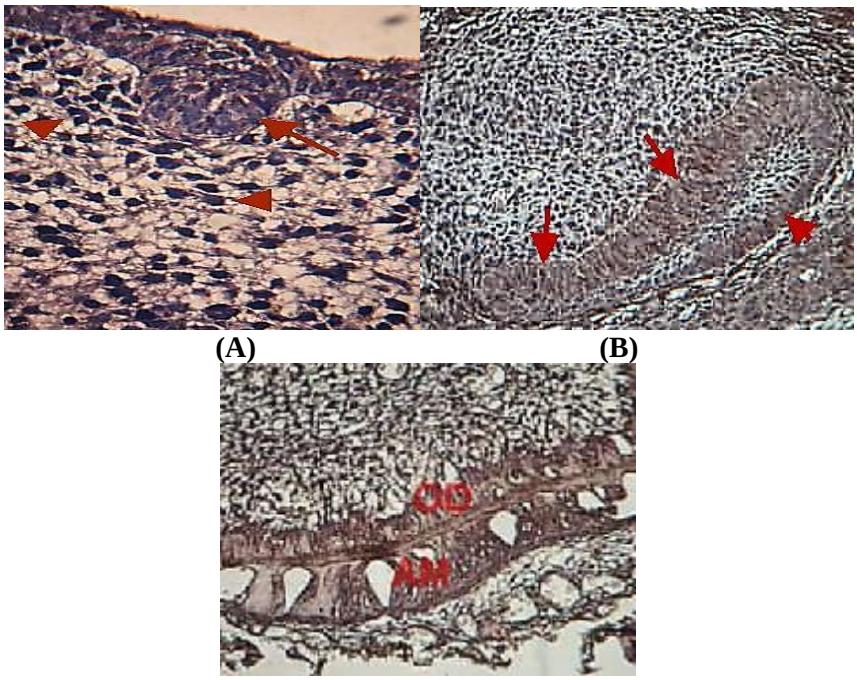


(A) **(B)**

(C) **(D)**

Figure 2:Experimentalgroup (H&E stain). (A) Upper jaw of rat at 16th day IUL shows. Inner enamel epithelium (green arrow head),outer enamel epithelium(green arrow), proliferating packed cells(red arrow) (X10). (B) At 18th day I.U.L tooth germ at bell stage (arrow) (X4). (C) Multiple tooth germs (X40). (D) One day post natal, tooth germ shows, dentine (D),predentine(PD) pulp(P), enamel(E), odontoblast(OD) (X40).



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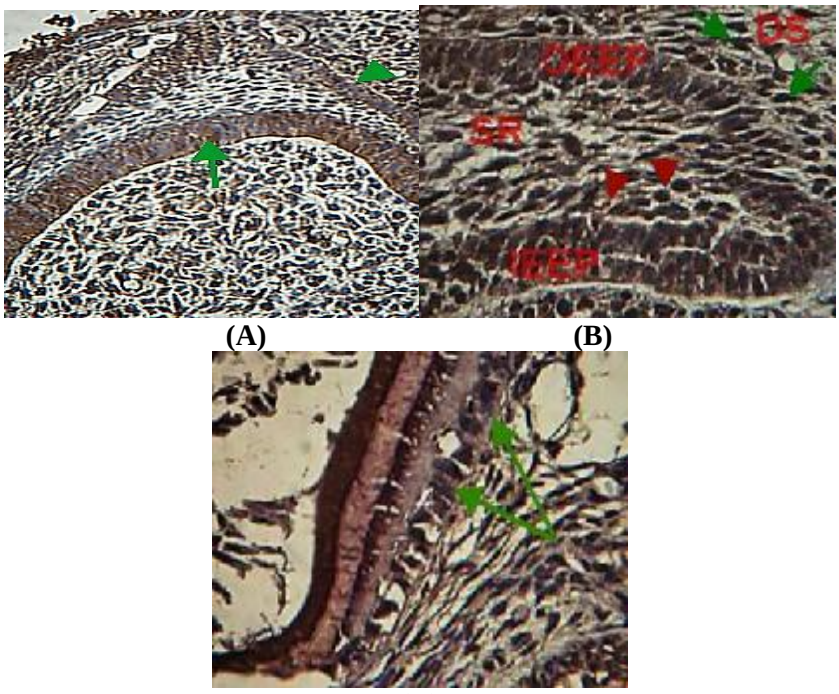

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DISCUSSION

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3. Thymsin β4 may induce stem cell differentiation [13].

Developed tooth germ of experimental group at18th day I.U.L shows bell stage, with a records of multiple tooth germs in two different stages in some specimens .the primitive pulp identifies formation of numerous new blood vessels .At one day post natal embryo a successful apposition of hard dental tissues (dentin and enamel) was detected in experimental group, and can be attributed to the followings:

1. Thymosin beta 4 may promote stem cell migration and differentiation of mesenchymal tissue into odontoblast, osteoblast [14].
2. Thymosin beta 4 has multiple biological activities includes, upregulation endothelial cell differention and stimulate angiogenesis by differentiation and directional migration of endothelial cells [15].

3.Thymosin β4 regulates the expression of Runt-related transcription factor2 Runx2 mRNA that plays important role in production of ameloblastin, amelogenin, dentin matrix protein and dentin sialophosphoprotein all of these are important component of enamel and dentin matrices respectively [16, 17]. Runx2 plays impotant role in development and calcification of tooth germ [18] and it regulates odontogenesis related genes [8].

For TGFB1 expression

1. Thymosin β4 increased expression of many genes associated with angiogenesis, cell proliferation, and migration such as: vascular endothelial growth factor Vegf B, Vegf C, Vegf D, Vegf receptor1, matrix metalloproteinases2(MMP2) and Tgf beta1[19].
2. In secreting ameloblast, it was found that signaling from ameloblast increase autocrine effect of local Tgf β1 on development of enamel organ and provide the basis for synthesis of enamel proteins [20],and in the present study ,it seems that treated with Tβ4enhanced proliferation of ameloblast and then increased expression of Tgf β1.

In conclusions:

- Thymosin beta4 of 50 µl dose injected to pregnant rat at zero time of gestation found to be initiator for the developing tooth germ as the present results showed advanced stages of tooth development in comparisons with control group and as follow:
- Advanced stage for tooth germ in each studied periods.
- Records of multiple tooth germs.
- Acceleration apposition of dental hard tissues enamel and dentin especially (enamel).
- Identification of packed cells in enamel organ.
- Numerous newly blood vessels were seen in mesenchymal tissue for dental papillae and dental sac.
- High expression of TGFB1 by epithelial and mesenchymal stem cells and by specialized cells includeameloblast and odontoblast and osteoblast.

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