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Light Microscopic Investigation of Developing Rat Teeth After Atenolol Drug Administration (Experimental Study)

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الخلاصة

الأهداف: تهدف الدراسة لفحص تأثير دواء الاتينولول على تكوين ونموا اسنان الجرد (نسيجيا وكيميا نسيجيا)**المواد وطرائق العمل** : قسمت جرذان الويستر البيضاء الى مجموعتين رئيستين؛ مجموعة سيطرة و مجموعة معالجة ، كل منها تكونت من (٩) حوامل أعطي للمجموعة الاولى (١ مل) ماء مقطر والمجموعة الثانية (١٠ ملغم) اتينولول معلق في (١ مل) ماء مقطر فمويا ، ولجرعة واحدة منذ اليوم الثاني عشر للحمل ولغاية الثامن عشر من الحمل . بعد الولادة اختير (٣٦)حديث ولاده ليضحى بهم في اعمار (١، ٥، ١٠ بعد الولاده) من كل مجموعتي السيطرة والمعالجة . حضرت النماذج وصبغت بصبغتي الهيماتوكسلين والايوسين والالشن الأزرق وقد بينت **النتائج** إن المجوعة المعالجة بالاتنيولول أظهرت تغيرات نسيجيه واضحة، تأخر في نمو أسنان الجرذ ، زيادة ألاوعيه الدموية ، عدم انتظام خلايا مصورات آلينا ومصورات العاج ، تأخر في صنع(GAG) بشكل ملحوظ **النتيجة النهائية** : إن لدواء الاتنيولول تأثيرا على الأسنان النامية حيث سبب تأخيرا في النمو وتغيرات في عملية تكوين الأسنان وأحبط صنع (GAG).

ABSTRACT

Aims: To investigate the effect of atenolol drug on the formation and development of rat dentition of rat (histologically and histochemically). **Materials and Methods:** Albino Wister rats were divided into two main groups,. The control group and experimental group, each consisted of (9) pregnant rats, given 1 ml distilled water and 10 mg atenolol (powder) suspended in 1ml distilled water , orally , as a single dose, respectively from 12 – 18 day of gestation, after delivery of pregnanent rats, 36 newborn were selected to be scarified at ages 1, 5. and 10 post nataly from each group. The specimens were prepared for processing and stained with H&E and alcian blue stains. **Results:** The treated groups showed obvious histological changes: delay in tooth germ formation, increase blood vascularity, disorganization of ameloblast and odontoblast cells, and delay in glycosaminoglycan synthesis. **Conclusions:** Atenolol drug delay the rat tooth development causing abnormal odontogenic changes and inhibits glycosaminoglycan synthesis.

Key words: Atenolol, teeth, development.

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INTRODUCTION

Atenolol is a cardioselective beta – blocker. It is reported to the lack of intrinsic sympathomimetic activity and membrane – stabilizing properties, and it is very hydrophilic. [1] This preferential effect is not absolute, however, at higher doses, atenolol inhibits beta 2 – adrenoceptors, chiefly in the bronchial and vascular musculature. [2]

Cardiac adrenergic receptors are blocked by cardioselective B – blocker, resulting in decreased heart rate and force contraction, which lower blood pressure. Also, inhibits renin production in the kidney to lower blood pressure. Reduced heart rate and force of the contraction decrease cardiac work load and improve coronary circulation to lessen angina. [3]

Atenolol inhibits epinephrine, xanthine, and B – adrenergic bronchodilators, increases hypotensive effect of calcium channel blockers. [4, 5]

Atenolol can cause fetal harm when administrated to a pregnant woman. It crosses the placental barrier and its concentration in maternal and cord blood is about 1: 1. Adminstration of atenolol, starting in the second trimester of pregnancy, has been associated with the birth of infants that are small for gestational age. No studies have been performed on the use of atenolol in the first trimester and the possibility of fetal injury cannot be excluded. When this drug is used during pregnancy, or in the patient becomes pregnant while taking this drug. [1, 2, and 6]

Atenolol has been shown to produce a

dose – related increase in embryo / fetal resorption in rats at doses equal to or greater than 50 mg/Kg/day or more times the maximum recommended human antihypertensive dose. Although similar effects were not seen in rabbits, the compound was not evaluated in rabbits at doses above 25 mg/Kg/day or 12.5 times the maximum recommended human antihypertensive dose. Atenolol is excreted in human breast milk at concentrations some 3 – 5 times higher than in maternal blood. [7] Caution should be exercised when atenolol is administrated to a nursing woman.

Persistent lesions that cause overall growth retardation or delayed growth of specific organ systems are generally referred to as embryo toxic. For a chemical to be labeled a teratogen, it must significantly increase the occurance of structural or functional abnormalities in offspring after it is administered to either parent before conception, to the female during pregnancy, or directly to the developing organism.[8]

Many teratologists believe that any chemical administered under appropriate conditions of dose and time of development can cause some disturbances in embryonic development in some laboratory species. [9, 10]

For an agent to be classified as a developmental toxicant, it must produce adverse effects on the conceptus at exposure levels that do not induce severe toxicity in mother. [10] Administration of a teratogen on day 10 of rat gestation would result in a high level of brain and eye defects, with intermediate levels of heart and skeletal defects, and a low level of urogenital defects. If the same agent was administered on day 11, a different spectrum of malformations would be anticipated. [11]

Drugs are well known for their effect on developing dental tissue, the most common of these being: antibiotics tetracycline, [12] cyclophosphamide, [13] Daunorubicin,[14] and phenylhydrazin. [15] The literatures have shown there is a lack on the investigated effects of atenolol on the developing dental tissues. So, the present experiments were designed originally to study the expression of atenolol in rat teeth germs at various stages postnatally (during the developing time of newborn teeth) and to correlate it with the standard morphological descriptions and to evaluate the effect of atenolol drug on the formation and development of dentition of the rat histochemically (by alcian blue).

MATERIALS AND METHODS

Female Albino wistar (250± 50 gm) rats were introduced singly in mating cages containing single male rat at 4 p.m. The tray under the cage was checked in the following morning for the presence of vaginal plug which indicate pregnancy. [16] Pregnant rats were separated and housed individually in plastic cages.

- Control group: This group consisted of (9) pregnant rats each was given (1 ml) distilled water from 12 – 18 days of pregnancy, the number of pups ranged from 5 – 11 pups per female, roughly 4 of each 3 mothers (12 newborn rats) were selected randomly to be sacrificed at 1 day old, 5 days old and 10 days old.

The experimental group: This group consisted of (9) pregnant rats, each rat was given 10 mg powder of atenolol (Atenolol (50 mg) beta adrenoceptor blocking drug, packed by Medochemie Ltd, Limassol Cyprus) suspended in 1 ml distilled water, as a single dose, orally, daily from 12 – 18 day of pregnancy.

After delivery of (9) pregnant rats, treated by atenolol drug, the number of pups ranged from 5 – 11 pups per female, roughly 4 of each 3mothers (12 new porn rats) were selected randomly to be sacrificed at 1, 5 and 10 days old. (Table 1) Seventy two newborn rats were examined through this study (36 newborn from each control and treated groups). The specimens (set of serial saggittal sections) were prepared for processing and staining with heamatoxyline and eosin and alcian blue stains then examined under light microscopes. Use of cationic dyes (alcian blue):

These are phthalocyanine dyes, most of these are derivatives of copper phthalocyanin, a blue pigment of high physical and chemical stability. Alcain blue (alcian blue 8GS, 8GX, 8GN: C.174240; Ingrain blue 1), pH 2.5 (for staining of weakly acidic mucopoly saccharides), pH 1.0 (for

Table (1) Experimental design

Groups	1 day old	5 days old	10 days old	
Control group (9) pregnant rats¤	12 *	12 *	12 *	36
Treated group (9) pregnant rats •	12 *	12 *	12 *	36
Total	24	24	24	72

¤ distilled water given. • Atenolol treated * Sacrificed (12 newborn from each 3 mothers)

more selective staining of strongly acidic sulfated mucosubstances. [17]

RESULTS

The general condition of the experimental rats (newborn rats) appeared to be markedly affected by atenolol treatment. This was chiefly reflected in pronounced smaller size than the control group. Also, they were less active than the control rats.

Morphological Profile:

- In comparison to the control groups: At 1 day post natal in the treated group:

There is a decrease in the tooth size and disorganization of pulp cells, abnormal dentin thickness, delay in apposition of dentin layer especially in incisor teeth is observed in serial saggital sections. In atenolol (5 days post natal) treated group; the growth continue but there is some abnormalities like: disorganization of odontoblast and ameloblast cells, increase pulp blood vascularity, abnormal dentin deposition and globular dentin, and cellular inclusion into dentin at tip region more than control group (Figures1and2).

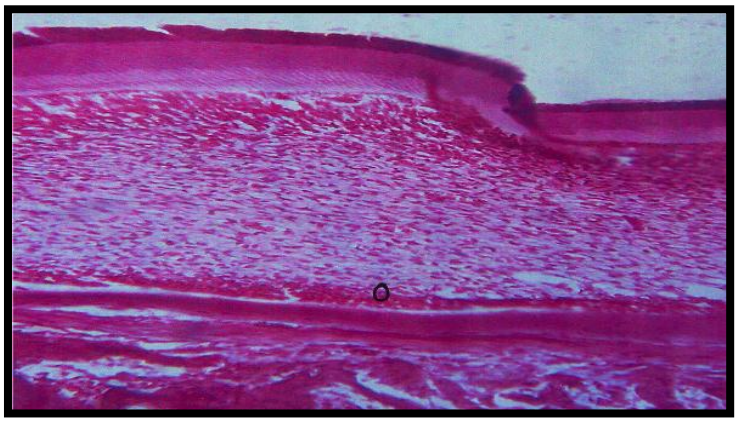

Figure (1) Saggital section of lower incisor, 5 p.n, control group, notice apposition of dentin and well differentiated odontoblast cells. (O). H/E X 10

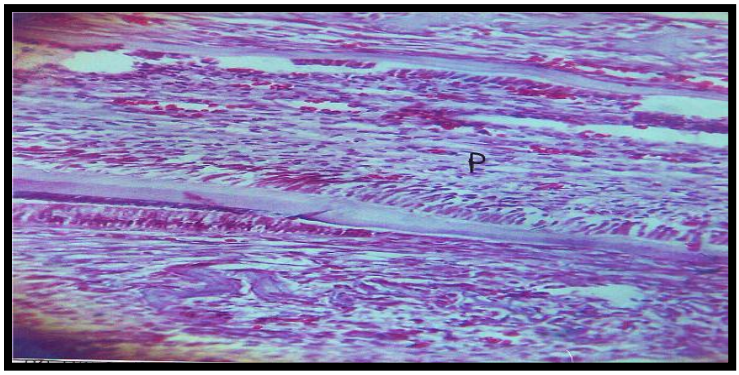

Figure (2) Saggital section of lower incisor, 5 p.n, Atenolot treated group, notice increase in blood vascularity, disorganization of pulp cells. (P). H/E X 10

In atenolol (10 days post natal) treated groups, there is clear abnormal dentin deposition, increase in pulp size, decrease in ameloblast layer and decrease in eruption rate of lower incisor, increase in blood vascularity, decrease in the tooth size and growth (Figures 3, 4 and 5).

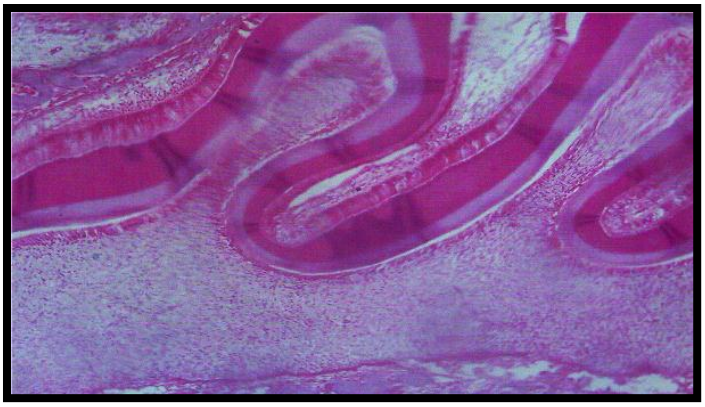

Figure (3) Saggital section of lower molar tooth, 10 p.n, control group, notice dentin thickness. (H/E X 10)

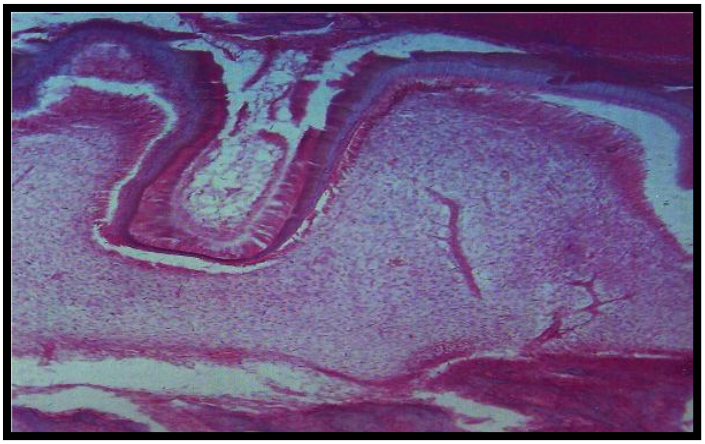

Figure (4) Saggital section of lower molar tooth, 10 p.n, Treated group, notice the dentin thickness in comparison to control group, and increase in blood vascularity. (H/E X 10)

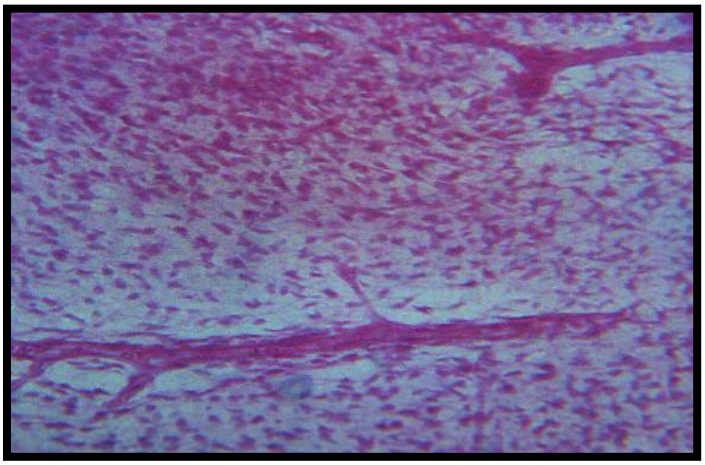

Figure (5) Saggital section of lower molar tooth, 10 p.n, Treated group, notice increase in blood vascularity. (H/E X 40)

Glycosaminoglycans Histochemistry:

In general, the reaction was stronger at pH 2.5 than pH 1, indicating the presence of both sulfated and non sulfated (carboxylated) glycosaminoglycans.

❖ Glycosaminoglycans as revealed by alcian blue pH 2.5.

At 1st day post nataly in the control group: The reaction at dental papilla was weak, it is hardly seen yet at predentin, it is very weak reaction. At 1 day p.n in the treated group: it was very weak reaction at dental papilla, no reaction is seen at predentin area. At 5th day post nataly in the control group; the extracellular reaction was intense at the predentin area (especially at the junction between predentin and dentin on the dentin side), very weak reaction seen at the pulp area, and/or the reaction disappears (Figure 6). At 5th day post nataly in the treated group; the reaction was less intense and weak wavy at predentin area, it remained weak at pulp area (Figure 7)

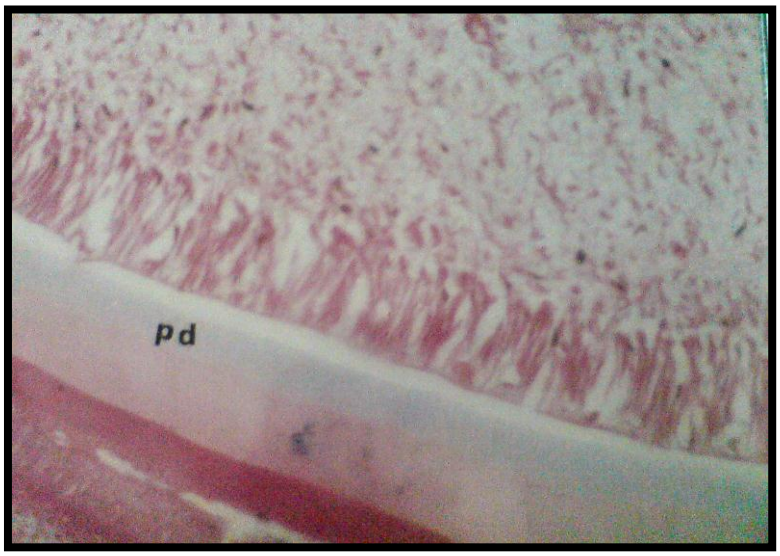

Figure (6) Saggital section of incisor tooth, 5 p.n, control group, Stained with alcian blue pH 2.5, the reaction is intense at predentin area (pd) (X 40)

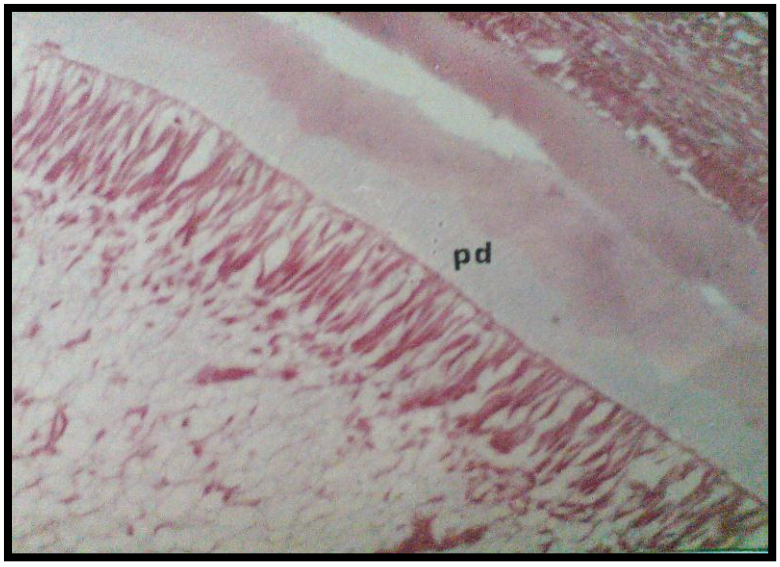

Figure (7) Saggital section of incisor tooth, 5 p.n, Atenolol treated group, Stained with alcian blue pH 2.5, Note the reaction remain weak and wavy at predentin area (pd) (X 40)

At 10th day post natal in the control group: The reaction disappears from the pulp of incisors and molar teeth but it remained intense at predentin area. At 10th post natal treated group: it is easily seen at predentin area more intense than control group.

❖ Glycosaminoglycans as revealed by alcian blue pH 1.

At 1st day post nataly in the control group: The reaction at this age was intense at dental papilla and weak at predentin area, it is weak reaction. But at 1 day p.n in the treated group: it was weaker at dental papilla; no reaction is seen at predentin. At 5th day post nataly in the control group: It was more intense reaction in the predentin area and far from the odontoblast cell layer. At 5th day post nataly in the treated group: It was intense but weaker than the control group and very close to the odontoblast layer. At 10th day post natal in the control group; the extracellular reaction in the predentin area disappear or very very weak (Figure 8).

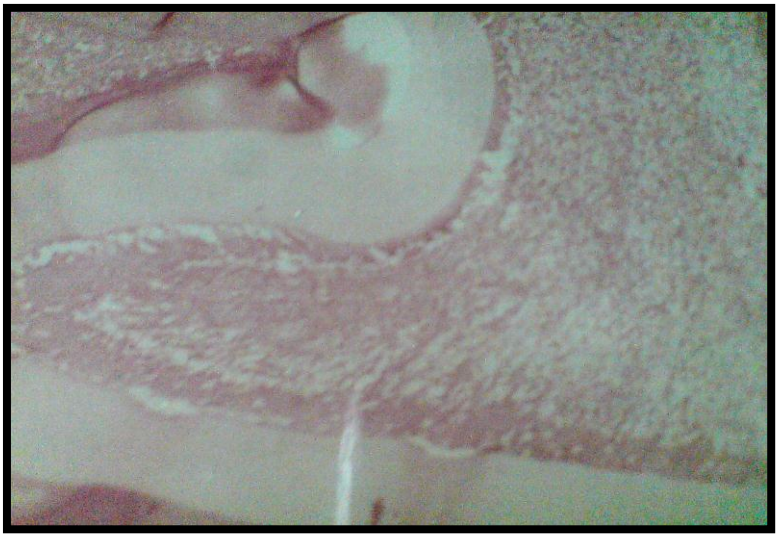

Figure (8) Saggital section of molar tooth, 10 p.n, in the control group, Stained with alcian blue pH 1, Note the reaction disappears or very weak at predentin area (pd) (X 20)

But at 10th post nataly treated group; the extracellular reaction in the predentin is easily seen and was more intense at this area (Figure 9)

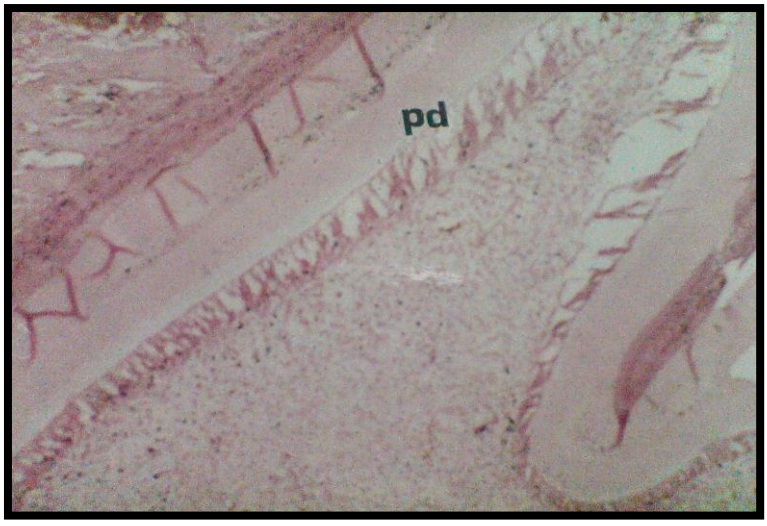

Figure (9) Saggital section of molar tooth, 10 p.n, in the treated group, Stained with alcian blue pH 1, Note the reaction is more intense at predentin area (pd) (X 20)

DISCUSSION

Histomorphological alterations of teeth development and the influence of atenolol:

Many dental researchers have done investigations concentrating on the effects of drugs on teeth and dental tissue and they demonstrated that some drug were capable of causing obvious changes in the dental tissues. [18 - 21]

The current study was designed to evaluate the effect of atenolol on the formation and development dentition of the rat. The control group (1, 5 and 10 post nataly) showed normal histological appearance, but the treated group give obvious histological abnormalities in their developing teeth, indicating alterations in normal developmental processes. It is well established that atenolol, cross the placenta. [22] All body compartments of fetus are affected by atenolol reaching them via fetal blood carrying it from their treated mothers. Atenolol inhibits the growth, causing microcephaly, also inhibits protein and glycoprotein synthesis, decreases amount of proliferating cells accelerates their differentiation and thus delays the development of the tooth germ, similar to effect of hydrocortisone drugs[21] , but atenolol showed higher intrinsic potency to interfere with normal in vitro embryonic development but less than propranolol and metoprolol[23].

In this study, the drug was given to pregnant rats at day 12th of gestation, just before the beginning of the developmental process of the rat dentition. The influence on the embryonic life of the developing tooth germ is on the genetic potential of the tooth germ itself, the immediate local environment around the developing tooth germ, and the general environment of the individual as a whole[24].

Atenolol effects the size or form of the teeth, only if its affects occur during morphodifferentiation which proceeds calcification as cortisol drug [21]. Thus all the atenolol treated animals at (1, 5 and 10 post nataly) were affected by this drug because the administration of drug was given to their mothersin very sensitive stage of tooth germ formation of embryoes (12 – 18 days of gestation age).[25]

In the present study, atenolol treated animals have shown an increased vascularity of the pulp, this result similar to that found by corticosteroid drug effect. [26] One site of the inhibitory action, structural and ultrastructural abnormalities of atenolol on connective tissue development, [22] has been suggested to be the enzyme systems of hyluronidase and sulfatases or may be due to its effect on other enzymes. This indicates the possibility that some of the effects of atenolol on rat incisors and molars may be due to interference with enzymatic processes which, in turn, could affect the normal histodifferentiation of the tooth, the outline alteration was in less magnitude than those shown in laser irradiated teeth [27] and other drug treated rats.[12]

Many factors could affect the dentin thickness in the treated group like, delay in dentinogenesis. This may be due to effect on the work of odontoblast cells, that secrete dentin matrix but at a less amount, or presence of functionless odontoblast or not very well differentiated to secrete enough dentin matrix. Most probably that atenolol affect the metabolic process of dentinogenesis as the effect in hydrocortisone treated rats. [21]

The demonstration of alcian blue binding at pH 2.5 and pH 1 indicated the presence of both carboxylated and sulfated glycoseaminoglycans in the developing dental tissues since both have affinity for cationic dyes.

The major proteoglycans of dental tissue are heparin sulfate, chondroitin sulfate and hyaluronic acid.

The present study suggests that the epithelial cells and mesenchymal cells separated by glycosaminoglycan (GAGs) enriched basement membrane. The area of the tooth germ covered by GAGs generally increases as the newborn rat develops. There is a relationship between the level of GAG in the palatal process and development. [28] The mesenchymal tissue of tooth germ at 1 day p.n was intensely stained with alcian blue suggesting that these glycoconjugates may play an important role in the differentiation of the cells to become odontoblast cells. Lim and Rueda [29] observed the positive staining

occurred mostly in developing mesenchymal tissue surrounding the cochlear duct.

The predentin of the tooth germ at age of 5 post natal in the control group is intensely stained by alcian blue, so this indicate the presence of proteoglycans in the dentin matrix and odontoblast. But when developing continues the staining become less intense at 10 post natal indicating the very little amount of proteoglycans in dentin matrix. Alcian blue staining (pH 2.5) of GAGs was decreased in atenolol treated groups from that in the controls at 1 day post nataly.These results suggested that atenolol drug treatment inhibits GAGs synthesis since the mesenchymal cells don't retain the capability of synthesizing GAGs in normal amount. The results of this study are inagreement with the work of Al Douri. (21)

At ages 10 post natal in atenolol treated groups, there is masking of alcian blue staining especially at predentin area indicating that GAGs presence in higher amount more than control group, there is delay in the formation of proteoglycan contents at predentin, because the reactivity still, these results are in agreement with the results of other researcher, the glycoconjugate accumulate in predentin and are either removed or masked to staining in the dentin. (30)

In control group, after differentiation of the odontoblast cells, GAGs (chondrotin sulfate and heparin sulfate) are present in good amount in predentin area at 1 post nataly, 5 post nataly (in pH 1 stained sections). But when development continues at 10 post nataly n, it disappear or become very weak indicating the less (chondrotin sulfate and heparin sulfate) present at predentin area especially. In atenolol treated group; there was reaction but it indicate delay GAGs synthesis (weak reaction) because the effect of atenolol on metabolism and function of the cells.

At 10 p.n, GAGs are still present in predentin area because the reaction still intense in contrast to the control groups, indicating the delay of GAGs synthesis in treated groups i.e., the synthesis is delayed and decreased because the atenolol drug effect.

CONCLUSIONS

The developing teeth are affected by atenolol drug; delay in tooth growth, decrease in dentin thickness. Atenolol inhibits GAGs synthesis.

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