Original Article

Evaluation of the Effects of Amoxicillin on Tooth Development in Rats by Histological and Histomorphometric Study

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ABSTRACT

Amoxicillin is one of the most commonly prescribed antibiotics in children. As a result, it is prescribed as the first line of defence against cutaneous, gastrointestinal, and respiratory infections. The objective of this study was to evaluate the effects of Amoxicillin on the formation of dentin and enamel during the secretory and early phases of mineralization. Regarding the materials and methods used to perform this study, 16 pregnant adult Wistar rats were equally divided into two groups. The first group did not receive the drug and was prescribed a saline solution (control group), and the other group received 250 mg/kg/day of Amoxicillin (experimental group). From the 13th gestational day until delivery, the treatment was given every day by oral gavage. After birth, the newborns also received the same treatment as their mothers from the first day until 7 or 12 days after birth. The newborns were sacrificed at 7 and 12 days postnatally, the jaws were dissected, the maxilla was taken, the samples were fixed in 10% formaldehyde solution, and the upper first molars were analyzed histologically by H & E stain and histomorphometrically by image J to examine the enamel, dentin, ameloblast and odontoblast mean thickness in both groups and each healing periods. The study's results showed that the mean enamel, as well as ameloblastic and odontoblastic layer thickness, were significantly different in the Amoxicillin 250 mg/kg group, compared to the control group. The result also revealed a non-significant group difference in the dentin thickness in both durations (P-value at day 7=0.147 and the P-value at day 12=0.054). Vacuolization of the ameloblastic and odontoblastic layers was observed in the Amoxicillin-treated group in both durations.

Keywords: Amoxicillin, Enamel, Dentin, Vacuolization, Ameloblast

1. Introduction

The dental lamina represents the earliest sign of tooth formation (1). During the bud, cap, and bell phases, epithelial morphogenesis determines the size and form of the tooth crown. Ameloblasts and odontoblasts, which differentiate by the interaction between the epithelium and the mesenchymal cells, secrete the tooth-specific hard tissue enamel and dentin (2). Dentinogenesis happens both prenatally and postnatally and may be seen to a lesser level throughout one's life when secondary and tertiary dentin is created (3). Dentin is a crystalline substance that is somewhat tougher than bone but less hard than enamel (4). The odontoblasts are specialized cells that generate dentin and have distinct morphological features, such as the extension of cytoplasmic processes into dentinal tubules (5).

Ameloblasts secrete four distinct proteins into the enamel matrix during the secretory stage. While matrix metalloproteinase-20 is a proteinase, enamelin (ENAM), ameloblastin (AMBN), and amelogenin (AMELX) are structural proteins (MMP20, enamelysin). When teeth are forming, they reach their maturation stage. After the opposition stage, when the enamel matrix is only partly mineralized at around 30%, it completes its mineralization process to a full level of 96% (4). According to Hasoon and Al-Ghaban (6), Amelogenin (AMELX) protein controls the hydroxyapatite crystals' beginning and growth during the mineralization of enamel.

Amoxicillin is a bactericidal antibiotic having a wide range of activity against both gram-positive and gram-negative bacteria. In primary care, Amoxicillin is one of the most regularly prescribed antibiotics (7). According to the former FDA categorization system, Amoxicillin is a pregnancy category B medicine, and there have been no studies establishing a definite danger in this regard (8). According to previous studies, Amoxicillin disrupted the early phases of amelogenesis, resulting in a loss of enamel matrix and morphological changes in ameloblasts. Amoxicillin may induce a delay in the development of ameloblasts into secretory cells (9, 10). The current investigation aimed to ascertain the effects of giving Amoxicillin before and after birth on the enamel and dentin structures in Wistar rats.

2. Materials and Methods

In total, 16 female rats (*Rattus norvegicus albinus*), aged 2-3 months and weighted approximately 175-225 gm, were used in this study. The rats were kept under controlled temperature (23±°C), and humidity (55±5 %), with a 12-h/12-h light/dark cycle; food *ad libitum* and water were available. Initially, the Amoxicillin dose was prepared according to the animal weight, followed by animal preparation which was done in the animal house of the Collage of Science, University of Kufa. Then, histological preparation was done in a technical laboratory in Najaf.

A total of 16 pregnant rats were randomly assigned into two groups. The control group (8 pregnant rats) received normal saline twice a week. The experimental group (8 pregnant rats) received 250 mg/kg of Amoxicillin (2 mg/kg) daily from day 13 to day 21 of pregnancy because Amoxicillin can cross the umbilical cord and placenta at this time. It also starts to build the first maxillary molar in rats, which only have a permanent dental system (11). After birth, 48 pups from 16 mother rats, from post-natal day 1 until post-natal day 7 or day 12, received the same treatment as their respective mother. The time selection of post-natal periods (day 7) in this study was related to the scientific base when rats represent the time in which hard tissue, dentin, and enamel were deposited (6). Moreover, 12-day-old rats show the end of the secretory stage in the cervical area and the beginning of the enamel mineralization in the cusp (9).

After the scarification of 7- and 12-day-old neonatal rats in each group, the head of the rats was separated from the body, and all the soft tissue of the head was removed. Then, the head was cut into two parts through the sagittal section, the maxilla was cut only (right and left), and specimens were placed in 10% formalin for 24 hours. After thorough decalcification, tissue samples

were processed in the standard paraffin blocks technique. All the analyzed samples' paraffin-embedded blocks were cut into serial sections of 4 µm thickness and put on glass slides for H&E staining.

2.1. Histomorphometrically Analysis

The following parameters were morphologically analyzed using sections stained with H&E: upper first molar's enamel, dentin, ameloblast and odontoblast thickness in rats aged 7 and 12 days were measured in the control and experimental groups using a light microscope. It was performed by a software program (Image J.exe) to measure enamel and dentin thickness at 40× magnification. In each rat, three sections that presented a central portion of the tooth germs were selected at intervals of at least 60 µm. In each section, three measurements were made from the central portion of the tooth germs. Thus, 36 measurements were obtained per group in each period (12 samples ×3 measurements). The mean enamel thickness in each section was estimated, and the mean value per group was calculated (9).

To measure ameloblast and odontoblast thickness, at 40× magnification, at least 10 measurements per sample were tested for the thickness of the ameloblast and odontoblast. The mean thickness of ameloblast and odontoblast in each rat was estimated, and the mean value per group was calculated. The results were documented after an experienced histologist was blinded to the group allocations, and the results were recorded (12).

2.2. Statistical Analysis

The evaluations of the two groups (experimental and control) with regard to the thickness of the enamel, dentin, as well as ameloblast and odontoblast were performed using two independent Sample T-tests: the parametric test was used to find the statistical difference between the two groups. Shapiro-Wilk test was also used to test the normality distribution of quantitative variables among groups. All of the analyses were performed using Statistical Package for Social Science (SPSS version 21, Chicago In press, USA). The level of significance was $P \leq 0.05$.

3. Results

The histological view of the tooth germ of the upper first molar at post-natal day 7 of the control group can be seen at a late bell stage, with a thick layer of dentin and enamel matrix and a thin layer of predentin close to the odontoblast layer in the crown of the teeth (Figure 1).

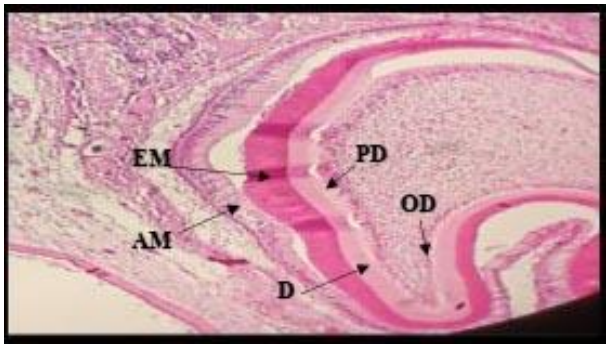

Figure 1. View of the first molar tooth germ of a 7-day control rat was at a late bell stage, with deposition of dentin (D), enamel matrix layers (EM) on the crown and pre dentin (PD), ameloblast (AM) and odontoblast (OD).H&E × 10

In the experimental group of the 7 days, histological examination of the tooth germ of the first molar revealed a group of ameloblasts connected to the dentin and an area of defect in enamel matrix formation (Figure 2A). At higher magnification in the cusp tip of the developing crown, the ameloblasts were reduced in height, exhibited altered cellular polarization, and formed a multicellular layer with no evidence of enamel formation. In addition, some areas of hypomineralization could be observed in the enamel matrix and dentin near DEJ (Figure 2B). Furthermore, vacuole-like structures were noted in the odontoblastic and ameloblastic layers (Figure 3).

The crowns of the upper first molar tooth germ at day 12 in the control group revealed a full deposition of hard tissue enamel and dentin. There was an increase in enamel space around the cusp tip region. The enamel matrix partially filled the enamel void at the cusp,

which indicated the start of enamel maturation (Figure 4). Initial root dentin formation was seen in the crown's cervical area. The tooth germ is encased in a bony crypt of thin bone trabecula (Figure 5).

While in the experimental group, the histological section of a tooth germ at day 12 showed an increased thickness of enamel matrix and dentin and area of enamel space, as well as the start of root production indicated by the presence of a cervical loop. Ameloblasts that surround the enamel matrix exist in many layers, at higher magnification, many vacuoles were detected in the Amoxicillin-treated group at day 12, notably in the odontoblastic and ameloblastic layers (Figures 6 and 7).

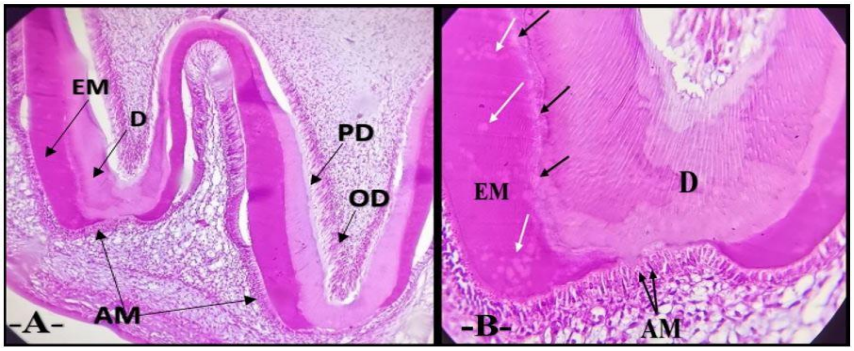

Figure 2. 7-day experimental group of upper molar tooth germs, **(A)** molar tooth germs that reveal enamel matrix (EM), dentin (D), predentin (PD), the pulp (P), odontoblast (OD), ameloblast (AM), H&E×10. **(B)** At Higher magnification shows ameloblast (AM) attachment to the dentin (D) without the creation of an enamel matrix (EM), also shows hypomineralization area in both enamel matrix (EM) (white arrows) and dentin (D) near dentin enamel junction (DEJ) (black arrows). H&E×40

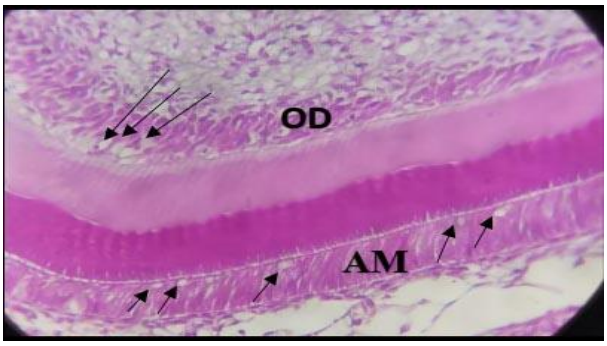

Figure 3. Higher magnification shows the presence of numerous vacuoles in the ameloblastic layer (AM) and the odontoblastic layer (OD) (black arrow). H&E×40

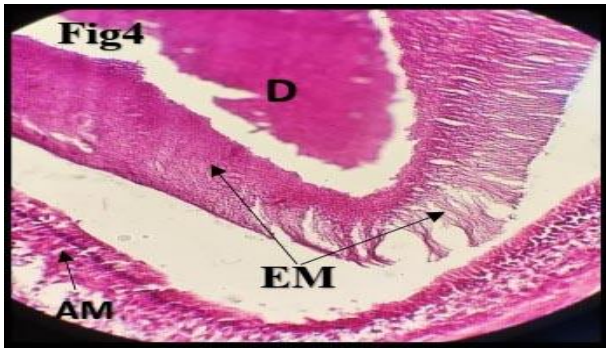

Figure 4. The start of enamel maturation is seen in the first molar tooth germ of a 12-day control group that reveals enamel matrix (EM), dentin (D) and ameloblast (AM). H&E×40

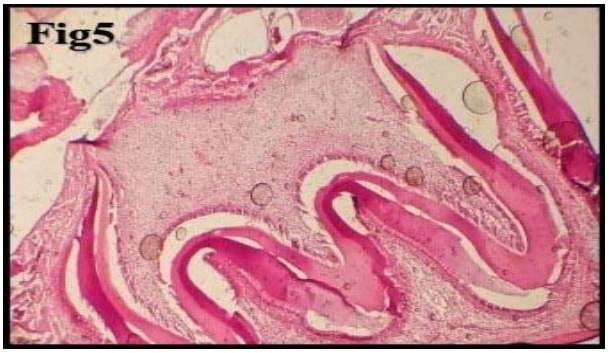

Figure 5. View of the germ of the first molar tooth, the beginnings of root dentin development seen in the 12-day control group. The tooth germ is surrounded by bone trabeculae H&E×4

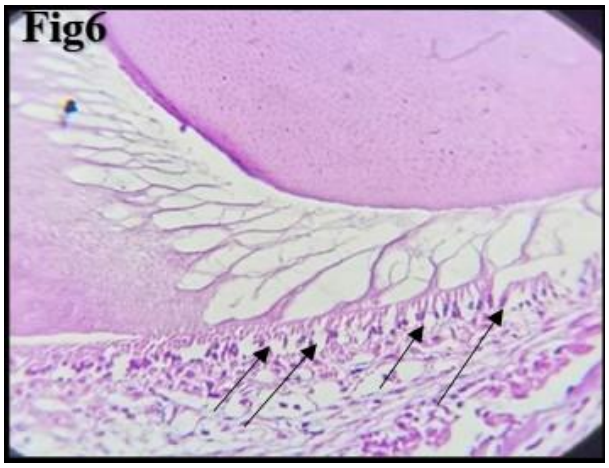

Figure 6. View showing the presence of many vacuoles in the odontoblastic layer (black arrow) (H&E×40)

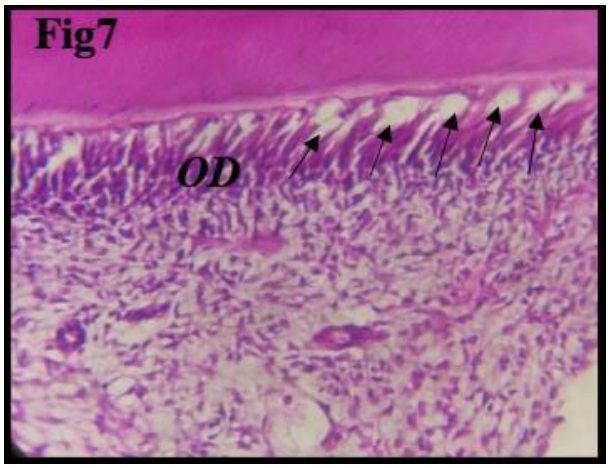

Figure 7. View showing the presence of many vacuoles in the ameloblastic layer (black arrow) (H&E×40)

Statistical analysis showed group comparison differences by t-test for mean enamel and dentin thickness in each duration (7 and 12 days). The results showed a significant duration difference in mean enamel thickness in both groups. Moreover, the results showed a highly significant difference in mean enamel thickness between the control and experimental groups in both durations, as shown in table 1 and figure 8. On the other hand, the result revealed a highly significant difference in mean dentin thickness at day 12 in the control (P=0.002) and the experimental groups (P=0.000068), as well as a non-significant difference in mean dentin thickness between control and study groups in both durations as shown in table 2.

Figure 9 and table 3 showed group comparison differences for ameloblast thickness in each healing duration. The results revealed highly significant duration comparison differences in ameloblast thickness in both control and study groups. Furthermore, it showed a highly significant difference in mean ameloblast thickness between groups in both durations. On the other hand, table 4 revealed non-significant duration comparison differences in the mean of odontoblast thickness for both groups but a highly significant group comparison difference in the mean of odontoblast thickness in both durations.

Table 1. Descriptive and statistical test of enamel thickness among groups and periods

Days		Groups		T-test	*P* value
		Study	Control		
7-days	Min.	0.063	0.140	3.379	0.003 H Sig.
	Max.	0.288	0.787		
	Mean	0.163	0.395		
	±SD	0.065	0.229		
12 -day	Min.	0.148	0.357	10.003	0.000 H Sig.
	Max.	0.369	0.748		
	Mean	0.220	0.549		
	±SD	0.062	0.096		
T-test		2.179	2.150		
P value		0.040 Sig	0.049 Sig.		

NS= non-significant at $P > 0.05$, sig. =significant at $P < 0.05$, highly sig. $P < 0.01$

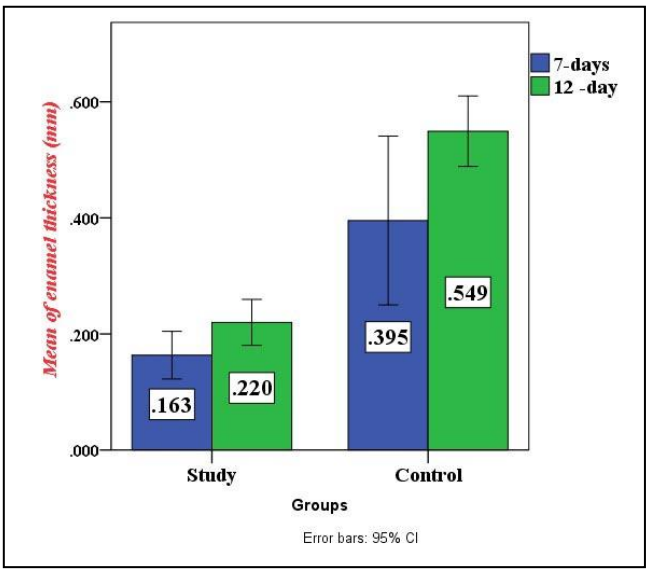

Figure 8. Comparison difference in enamel thickness in both groups in each healing period

Table 2. Descriptive and statistical test of dentine thickness between groups and periods

Days		Groups		T-test	*P* value
		Study	Control		
7-days	Min.	0.048	0.169	1.503	0.147 NS
	Max.	0.497	1.161		
	Mean	0.229	0.363		
	±SD	0.145	0.272		
12 -day	Min.	0.295	0.429	2.040	0.054 NS
	Max.	0.846	1.264		
	Mean	0.548	0.724		
	±SD	0.173	0.243		
T-test		4.892	3.424		
P value		0.000068 Sig.	0.002 Sig.		

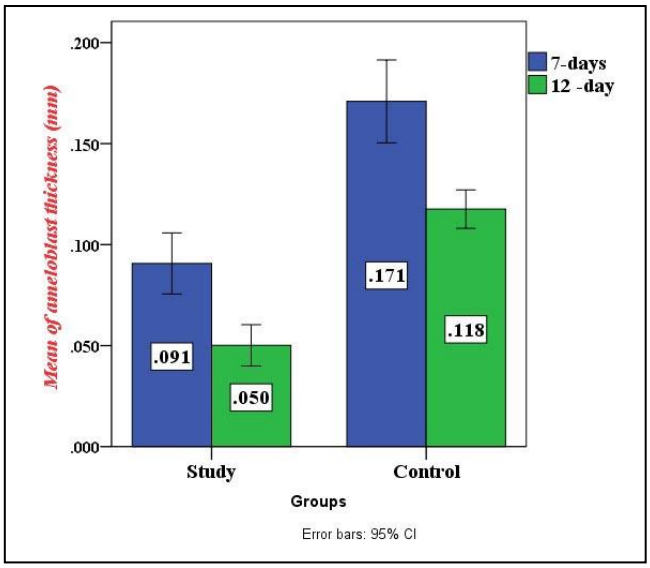

Figure 9. Comparison in group difference in ameloblast thickness in both healing periods

Table 3. Descriptive and statistical test of Ameloblast thickness among groups and periods

Days		Groups		T-test	*P* value
		Study	Control		
7-days	Min.	0.068	0.123	6.925	0.000001HSig.
	Max.	0.157	0.226		
	Mean	0.091	0.171		
	±SD	0.024	0.032		
12 -day	Min.	0.023	0.084	10.629	0.00000 H Sig.
	Max.	0.072	0.135		
	Mean	0.050	0.118		
	±SD	0.016	0.015		
T-test		4.879	5.189		
P value		0.000071 H Sig.	0.000033 H Sig.		

Table 4. Descriptive and statistical test of odontoblast thickness among groups and periods

Days		Groups		T-test	*P* value
		Study	Control		
7-days	Min.	0.157	0.194	3.056	0.006 H Sig.
	Max.	0.317	0.643		
	Mean	0.212	0.321		
	±SD	0.049	0.113		
12 -day	Min.	0.113	0.206	5.691	0.000010 H Sig.
	Max.	0.270	0.378		
	Mean	0.173	0.287		
	±SD	0.045	0.053		
T-test		2.039	0.936		
P value		0.054 NS	0.539 NS		

4. Discussion

The main purpose of this *in vivo* study was to evaluate the effects of Amoxicillin on tooth development in the rat's first molar secretory and maturation stages of tooth formation. Amoxicillin is frequently prescribed to young children to treat acute otitis media or other typical childhood infections. Sahlberg, Pavlic (13) and Souza, Costa-Silva (14) discovered that the majority of children who got Amoxicillin in the first year of life developed Molar-Incisor Hypomineralization. This was based on their study of 903 children aged 6 to 12 years old. A previous investigation found that the same doses of Amoxicillin reduced the thickness of the enamel matrix during the secretory stage (9).

Histological findings of the present study revealed an increase in the thickness of enamel and dentin, followed by a decrease in the thickness of ameloblast and odontoblast from day 7 to 12 but at different rates in both groups. The presence of Tomes' processes in the distal end of ameloblasts signaled the start of the formation of the enamel matrix.

On day 7, it is easy to find the difference between the control and the Amoxicillin-treated groups. In the control group, the main dentin is starting to mature, and the ameloblasts have a good layer of enamel matrix. While the group treated with Amoxicillin exhibited a partial disappearance of the enamel matrix due to ameloblast adhesion to the dentin surface. This enamel-free zone may be due to the inhibition of dentin mineralization that subsequently promotes the failure of enamel protein secretion and deposition by amoxicillin administration. This result is in agreement with the findings of an *in vitro* study by Sahlberg, Pavlic (13), who demonstrated that Amoxicillin and NaF, both separately and together, prevented the production of

enamel in cultured mouse molar tooth germs that were exposed during the presecretory, secretory, and early maturation stages of amelogenesis.

Furthermore, the result of the present study shows vacuole-like formations in the ameloblastic layer that were detected in the experimental group in the secretory stage. These cytoplasmic modifications may be due to the decrease in enamel secretion, which leads to a decrease in enamel thickness. Additionally, vacuole-like formations in the odontoblastic layer were detected in the present study. This finding was in agreement with de Souza, Gramasco (9). They investigated the impact of various Amoxicillin doses on the secretory and maturation stages and observed that pups in all groups which received Amoxicillin had vacuoles in ameloblasts. Moreover, in the amoxicillin-treated group, the ameloblastic layer, odontoblastic layer, enamel, and dentin thicknesses were decreased in the control group in the secretory stage. This occurs because Amoxicillin may inhibit the ameloblast and odontoblast differentiation. This result is in line with the findings of a study by Sahlberg, Pavlic (13) who observed a reduction in enamel thickness and attributed it to Amoxicillin's inhibition of ameloblast development.

The current study showed an area of hypomineralization in some areas of enamel near dentin enamel junction in the Amoxicillin-treated group at 7-day-old in almost all samples. The appearance of hypomineralization regions on the rat offspring molars exposed to Amoxicillin during the prenatal period correlates with findings from several clinical studies that reported children had dental disorders after taking Amoxicillin (15). This coincides with Gottberg, Berné (11), who observed that only 50% of the samples from the group that received Amoxicillin showed enamel changes. Furthermore, the current study reported an area of hypomineralization in some regions of dentin, especially near the dentin enamel junction at day 7 of molar tooth germ in the Amoxicillin-treated group. This agrees with Kumazawa, Sawada (16), who observed this on day 7 following a single amoxicillin treatment.

There were significant regions of hypo-mineralized dentine on their molars, demonstrating that Amoxicillin hampered the mineralization of dentin.

The control group exhibits full-thickness enamel matrix production and the start of enamel maturation at 12 days of age, whereas vacuole structure in the ameloblast and odontoblast layers of the experimental group appeared at 12 days of age. This result coincides with de Souza, Gramasco (9), who showed that the observation of vacuole-like structures in the ameloblastic layer of amoxicillin-treated groups led them to hypothesize that these structures may have developed due to Amoxicillin's interference with the medium for transmitting molecules, which ultimately decreased protein secretion and transmission.

Similar findings were observed by Kameli, Moradi-Kor (12), who showed that the enamel and dentinal layers of the Amoxicillin-treated groups (50 and 100 mg/kg) showed evidence of hypomineralization.

Furthermore, by day 12, the Amoxicillin-treated group had a thinner ameloblastic layer, odontoblastic layer, enamel, and dentin, compared to the control group. This may be due to the effect of Amoxicillin on ameloblast and odontoblast differentiation. This result coincides with Kameli, Moradi-Kor (12), who showed the decrease in ameloblasts and enamel thickness in a group treated with 50 and 100 mg/kg of Amoxicillin, and he explained the cause by the interference of Amoxicillin with the transmitting molecule medium that results in ameloblast dysfunction and a decrease in enamel secretion.

The current study demonstrated how Amoxicillin lowers the thickness of enamel and dentin and interferes with amelogenesis and dentinogenesis. This study implies that Amoxicillin interferes with the early phases of amelogenesis by altering the structure of ameloblasts and decreasing the enamel matrix. Since Amoxicillin may disrupt secretory and early mineralization stages of amelogenesis, pregnant women

and children should exercise caution in its use in the first years of their lives.

Authors' Contribution

Study concept and design: T. S.
Acquisition of data: N. M. H. A.
Analysis and interpretation of data: N. M. H. A.
Drafting of the manuscript: N. M. H. A.
Critical revision of the manuscript for important intellectual content: T. S.
Statistical analysis: T. S.
Administrative, technical, and material support: T. S.

Ethics

All experimental procedures were carried out in accordance with the ethical principles of animal experimentation of the College of Dentistry, University of Baghdad.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Whitlock JA, Richman JM. Biology of tooth replacement in amniotes. Int J Oral Sci. 2013;5(2):66-70.
2. Ahmed GM, Abouauf EA, AbuBakr N, Dörfer CE, El-Sayed KF. Tissue engineering approaches for enamel, dentin, and pulp regeneration: an update. Stem Cells Int. 2020;2020.
3. Matalová E, Lungová V, Sharpe P. Development of tooth and associated structures. Stem cell biology and tissue engineering in dental sciences: Elsevier; 2015. p. 335-46.
4. Fehrenbach MJ, Popowics T. Illustrated Dental Embryology, Histology, and Anatomy E-Book: Elsevier Health Sciences; 2019.
5. Kawashima N, Okiji T. Odontoblasts: Specialized hard-tissue-forming cells in the dentin-pulp complex. Congenit Anom. 2016;56(4):144-53.
6. Hasoon SL, Al-Ghaban NM. The effects of bisphosphonate administration on teeth development and growth of the jaw bones in neonatal rats (histological and immunohistochemical study). J Baghdad Coll Dent. 2013;25(Special Is):77-82.
7. Akhavan BJ, Khanna NR, Vijhani P. Amoxicillin. StatPearls [Internet]: StatPearls Publishing; 2021.
8. Damkier P, Brønniche LM, Korch-Frandsen JF, Broe A. In utero exposure to antibiotics and risk of congenital malformations: a population-based study. Am J Obstet Gynecol. 2019;221(6):648. e1-. e15.
9. de Souza JF, Gramasco M, Jeremias F, Santos-Pinto L, Giovanini AF, Cerri PS, et al. Amoxicillin diminishes the thickness of the enamel matrix that is deposited during the secretory stage in rats. Int J Paediatr Dent. 2016;26(3):199-210.
10. Mostafa HM, Shehata FI, Omar SO, Kawana KY. The effect of amoxicillin on the secretory stage of amelogenesis in rats. Alex Dent J. 2020;45(1):34-8.
11. Gottberg B, Berné J, Quiñónez B, Solórzano E. Prenatal effects by exposing to amoxicillin on dental enamel in Wistar rats. Med Oral Patol Oral Cir Bucal. 2014;19(1):e38.
12. Kameli S, Moradi-Kor N, Tafaroji R, Ghorbani R, Farzadmnesh H, Sameni H. Effects of amoxicillin on the structure and mineralization of dental enamel and dentin in wistar rats. Front Dent. 2019;16(2):130.
13. Sahlberg C, Pavlic A, Ess A, Lukinmaa P-L, Salmela E, Alaluusua S. Combined effect of amoxicillin and sodium fluoride on the structure of developing mouse enamel in vitro. Arch Oral Biol. 2013;58(9):1155-64.
14. Souza J, Costa-Silva C, Jeremias F, Santos-Pinto L, Zuanon ACC, Cordeiro RdC. Molar incisor hypomineralisation: possible aetiological factors in children from urban and rural areas. Eur Arch Paediatr Dent. 2012;13(4):164-70.
15. Serna C, Vicente A, Finke C, Ortiz AJ. Drugs related to the etiology of molar incisor hypomineralization: a systematic review. J Am Dent Assoc. 2016;147(2):120-30.
16. Kumazawa K, Sawada T, Yanagisawa T, Shintani S. Effect of single-dose amoxicillin on rat incisor odontogenesis: a morphological study. Clin Oral Investig. 2012;16(3):835-42.