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Histological Evaluation of the Effect of Local Angiopoietin-1 Administration on Tooth Development in Rats at Postnatal Day 16

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ABSTRACT

Background & Objective: Angiopoietin-1 (Ang-1) is a key regulator of angiogenesis and vascular maturation and may play an important role in tooth development. This histological study aimed to evaluate the effect of the local application of angiopoietin-1 on tooth development in the upper molar region of neonatal rats at postnatal day 16.

Materials & Methods: Twelve neonatal rats weighing 3.5–4.0 g were maintained under controlled environmental conditions with free access to food and water. The animals were randomly allocated to either an experimental group (n = 6) or a control group (n = 6). The experimental group received a local injection of 10 µL of angiopoietin-1 into the upper right molar region, whereas the control group received an equal volume of sterile saline. All animals were sacrificed on postnatal day 16, and histological analyses were performed to evaluate tooth development.

Results: Local administration of angiopoietin-1 significantly accelerated tooth development in all animals of the experimental group. Histological examination demonstrated enhanced deposition of dental hard tissues, characterized by increased dentin and enamel thickness, together with significantly higher numbers of odontoblasts, fibroblasts, and blood vessels compared with the control group. All evaluated histological parameters differed significantly between the two groups ($P < 0.05$).

Conclusion: Local application of angiopoietin-1 promoted and accelerated tooth development in neonatal rats, suggesting that angiopoietin-1 may enhance odontogenesis by stimulating angiogenesis and promoting the formation and maturation of dental tissues.

Keywords: Tooth development, Angiopoietin 1, Angiogenesis, VEGF, Enamel organ

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1. Introduction

Tooth formation is a complex physiological process characterized by bud, cap, and bell stages. This process entails a series of molecular interactions between the odontogenic epithelium and neural crest-derived ectomesenchymal cells (1).

The tooth is formed from the ectoderm and ectomesenchyme. The enamel is derived from the enamel organ, which is differentiated from the primitive oral epithelium lining the stomodeum (primitive oral cavity). Epithelial-mesenchymal interactions take place to determine the shape of the tooth and the differentiation of the formative cells of the tooth and the timing of their secretion (2).

The ectomesenchymal cells that are closer to the inner margins of the enamel organ differentiate into dental papilla and the ectomesenchyme cells closer to the outer margins of the enamel organ become dental follicle. Dentin and pulp are derivatives of the dental papilla while cementum, periodontal ligament, and alveolar bone are all derivatives of the dental follicle (3).

Ectodermal organs such as tooth, hair, mammary gland and feather share common morphological features, which develop from epithelial–mesenchymal interactions during the early stages of morphogenesis (4).

Tooth development by the formation of primary epithelial bands in the place of future upper and lower jaws. These horse-shoe-shaped bands correspond to the future dental arches. These epithelial bands then form two ingrowths called dental lamina (lingually positioned) and vestibular lamina (buccally positioned) (5). These ingrowths extend into the mesenchyme, which is surrounded by the neural crest cells. The vestibular lamina proliferates within the mesenchyme and leads to the formation of the vestibule (between the cheek and tooth-bearing portion of the jaw). The dental lamina gives rise to epithelial outgrowths toward the mesenchyme due to continuous proliferative

activity, which corresponds to the location of forthcoming deciduous teeth (5).

The development of the vascular and nervous systems is regulated by members of various signaling molecular families such as semaphorin and ephrin, netrin, slit, and vascular endothelial growth factor (VEGF), as well as their receptors (6). In the adult tooth, blood vessels and nerves are often located in the vicinity of each other (7).

Epithelial-mesenchymal interactions at the level of individual teeth and VEGF signalling at the level of the teeth as a target are thought to control how the vascular supply of the tooth develops. (8).

Many targets of the growth factors have been identified, and mutations in several genes within the signaling networks cause defective tooth formation in both humans and mice (9).

When producing dentin, the dental pulp of developing apex teeth may show increased cellular activity. This causes the root canal to gradually enlarge until the apex has fully formed. These teeth's dental pulp blood vessels are already capable of keeping normoxia at low levels of angiogenesis (10).

Angiopoietin-1 is a secreted oligomeric glycoprotein belonging to the angiopoietin family of growth factors, which also includes Ang-2 and Ang-3/4. These ligands interact with the endothelial-specific receptor tyrosine kinase Tie2, one of the two members of the Tie receptor family, the other being Tie1. The angiopoietin–Tie signaling pathway plays a pivotal role in the later stages of vascular development and in the maintenance of the adult vasculature by regulating vascular remodeling, maturation, and stabilization. Ang-1 is essential for the proper organization and maturation of newly formed blood vessels and promotes vascular quiescence and structural integrity in mature vessels.

Its critical role during embryonic angiogenesis has been demonstrated in Ang-1-deficient transgenic mice, which die at approximately embryonic day 12.5 because of severe defects in vascular remodeling despite the initial formation of the vascular network (11). Structurally, angiopoietins consist of an amino-terminal superclustering motif, a central coiled-coil domain, and a carboxy-terminal fibrinogen-like domain responsible for receptor binding.

Angiopoietin-1 exerts potent vasculoprotective effects by reducing vascular permeability, suppressing inflammatory responses, and preventing endothelial cell apoptosis. Consequently, preclinical studies have suggested that Ang-1 has therapeutic potential for several pathological conditions, including tissue edema, endotoxemia, ischemic injury, and transplant arteriosclerosis (12). Beyond its vascular functions, Ang-1-mediated angiogenesis is increasingly recognized as an essential component of tissue development and regeneration. Angiogenesis, defined as the formation of new capillaries from pre-existing blood vessels, is a tightly regulated biological process controlled by the balance between pro-angiogenic and anti-angiogenic factors. It occurs under both physiological conditions, such as embryonic development, wound healing, ovulation, and placental growth, and pathological conditions, including cancer, chronic inflammation, and rheumatoid arthritis (13).

Recent evidence suggests that Ang-1 also contributes to craniofacial and dental development. Increased ANGPT1 expression has been associated with complete root development, with significantly higher expression levels observed in teeth exhibiting complete root formation compared with those with incomplete root development (14).

In addition, Ang-1 has consistently demonstrated potent vascular protective effects through the reduction of plasma leakage, attenuation of vascular inflammation, and preservation of endothelial cell viability (15).

The therapeutic potential of Ang-1 has also been demonstrated in experimental disease models. Upregulation of ANGPT1 has been shown to reduce cerebral infarct size in experimental stroke models (16), findings that were subsequently supported by a meta-analysis evaluating animal studies comparing tissue plasminogen activator (tPA) plus Ang-1-related interventions with appropriate control treatments (17). Furthermore, dysregulation of the Angiopoietin-1/Angiopoietin-2 axis has been implicated in pneumonia-induced vascular permeability and inflammation. Elevated circulating Angiopoietin-2 concentrations have been associated with increased mortality and prolonged hospitalization, whereas Angiopoietin-1 has been proposed as a potential therapeutic target for severe pneumonia because of its vascular stabilizing properties (18).

Among angiogenic mediators, vascular endothelial growth factor (VEGF) is the principal endothelial cell-specific mitogen and remains one of the most potent stimulators of angiogenesis. VEGF plays a central role in endothelial cell proliferation, migration, and neovascularization and acts in concert with Ang-1 to regulate vascular development and maturation (19).

2. Materials and Methods

Every experimental method was conducted in accordance with the ethical guidelines of the dentistry college in Baghdad (Ref. number: 463). In this study, twelve neonatal rats weighing (3.5–4) grams were used under controlled temperature, drinking, and food utilization conditions.

The animals subjected to the application of 10µl of angiopoietin 1 in the upper right molar area in the control group (6 rats) and the experimental group (6 rats).

At postnatal day 16, all rats were euthanized, and the heads were separated from the bodies for histological examination. Sagittal sections were prepared through the maxillary molar tooth germ region to evaluate tooth development. Tissue specimens were fixed, processed according to standard histological procedures, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). The stained sections were subsequently examined under a light microscope for histological assessment of tooth germ development.

Statistical analyses were performed using SPSS software (version 26.0; IBM Corp., Armonk, NY, USA). Data are presented as the mean ± standard deviation (SD).

Comparisons between the experimental and control groups were performed using the independent-samples t-test. A two-tailed *P*-value of <0.05 was considered statistically significant, whereas a *P*-value of <0.001 was considered highly statistically significant.

2.1 Histomorphometric Analysis

Dentin thickness (20), enamel thickness (21), and pulpal vascular development (22) were evaluated by histomorphometric analysis using ImageJ software (National Institutes of Health, Bethesda, MD, USA). Measurements were performed on four representative microscopic fields per specimen at ×40 magnification. Histological assessments were conducted using a double-blind protocol to minimize observer bias, and both inter-observer and intra-observer reproducibility were evaluated. All measurements were obtained from specimens collected on postnatal day 16 in both the experimental and control groups.

2.2 Surgical Procedure

All surgical and non-surgical procedures were performed under sterile conditions and in accordance with established animal welfare guidelines. Six neonatal rats assigned to the experimental group received a daily local injection of 10 µL of angiopoietin-1 (Ang-1) into the maxillary right first molar region. The six animals in the control group received an equal volume of sterile normal saline. On postnatal day 16, all animals were humanely euthanized. For local administration, a fine needle was inserted into the elevated gingival bulge corresponding to the underlying first maxillary molar tooth germ at an angle of approximately 45° using gentle manual pressure, as previously described (23).

2.3 Histological Specimen Preparation

Following euthanasia, the maxillary specimens containing the developing tooth germ and surrounding bone were harvested and fixed in freshly prepared 10% neutral buffered formalin for 24 hours. The specimens were subsequently decalcified in 10% formic acid for 1–2 hours. Completion of decalcification was confirmed by gentle needle penetration through the bone tissue.

Following routine histological processing, the specimens were embedded in paraffin wax, and serial sagittal sections of 4–5 µm thickness were prepared using a rotary microtome. The sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for histological and histomorphometric evaluation.

3. Result

In the control group, histological examination of the tooth germ of the upper first molar at post-natal day 16 revealed a layer of dentin close to the odontoblastic layer and an enamel layer approximating the ameloblastic layer in the coronal and cervical areas of the tooth (Figures 1 and Figure 2, Table 1). In the experimental group, the enamel thickness increased at day 16. Ameloblasts were observed adjacent to the enamel. The thickness of the dentin was also increased close to the odontoblastic layer (Figures 3 and Figure 4, Table 1).

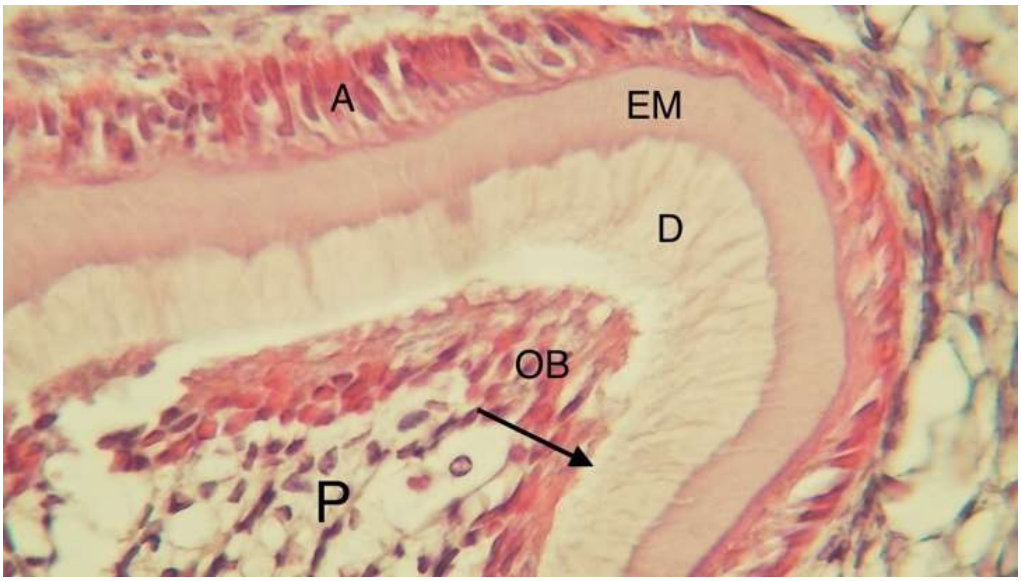

Figure 1. Histological view of the occlusal area of the control group at day 16 showing ameloblasts (A), enamel matrix (EM), dentin (D), and odontoblasts (OB), predentin (Arrow), Pulp (P), Haematoxylin and Eosin ×40. **(Prepared by Authors, 2026)**

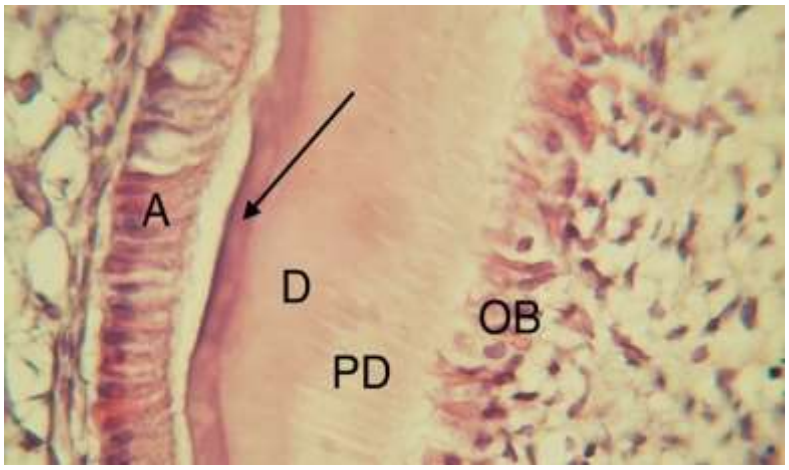

Figure 2. Histological view of the cervical area of the control group at day 16 showing ameloblasts (A), enamel matrix (Arrow), Dentin (D), predentin (PD), and odontoblasts (OB), Haematoxylin and Eosin ×40. (**Prepared by Authors, 2026**)

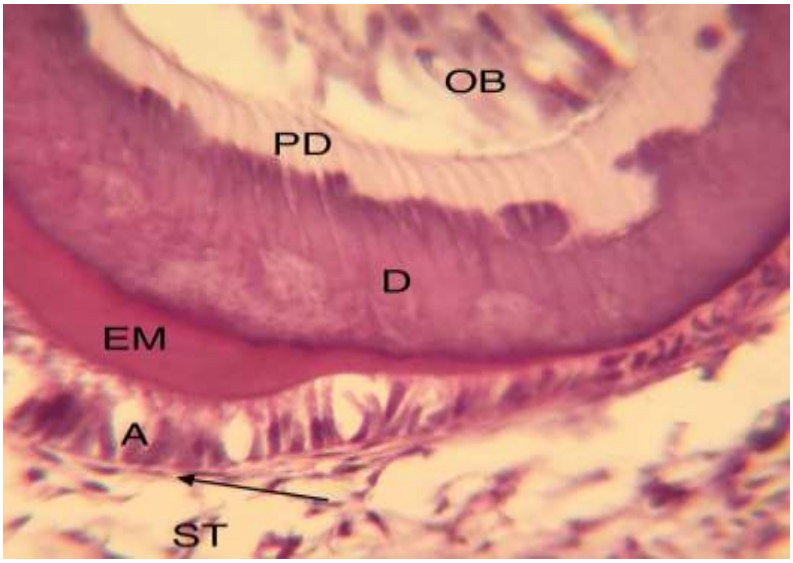

Figure 3. Histological view of the occlusal area of the experimental group at day 16 showing ameloblasts (A), stratum intermedium (Arrow), stellate reticulum (ST), enamel matrix (EM), dentin (D), predentin (PD), and odontoblasts (OB), Haematoxylin and Eosin ×40. (**Prepared by Authors, 2026**)

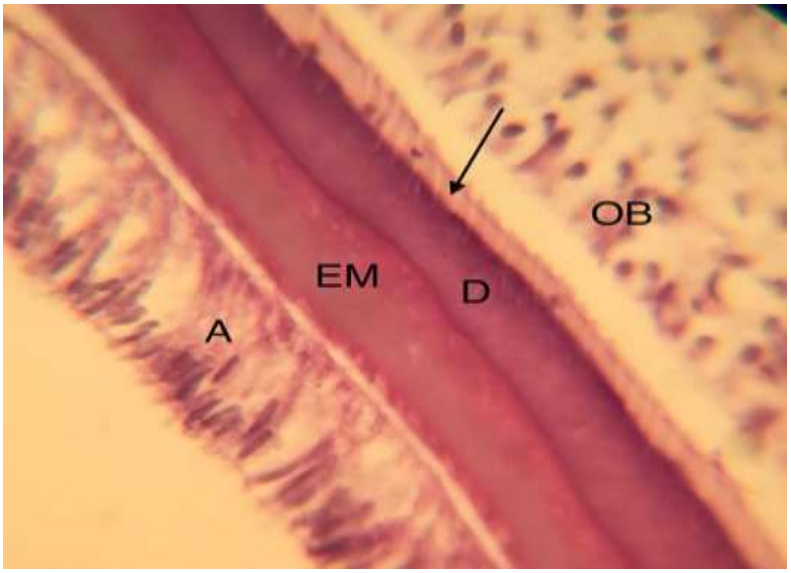

Figure 4. Histological view of the occlusal area of the control group at 16 days showing ameloblasts (A), enamel matrix (EM), predentin (Arrow), dentin (D), and odontoblasts (OB), Haematoxylin and Eosin ×40. (**Prepared by Authors, 2026**)

The cellular components of the pulp tissue and the number of blood vessels were measured histologically and are presented as the minimum, mean, maximum, standard deviation, standard error, and P-value for the two groups. The experimental group exhibited an increase in the number of pulp tissue cells and blood vessels in compared with the control group (Figures 5 and Figure 6, Table 1) and this is in agreement with Fadhil and Alhijazi **(24)** a previous study that focused on bone repair, which observed the formation of osteoid tissue filling the bony defect with newly formed blood vessels.

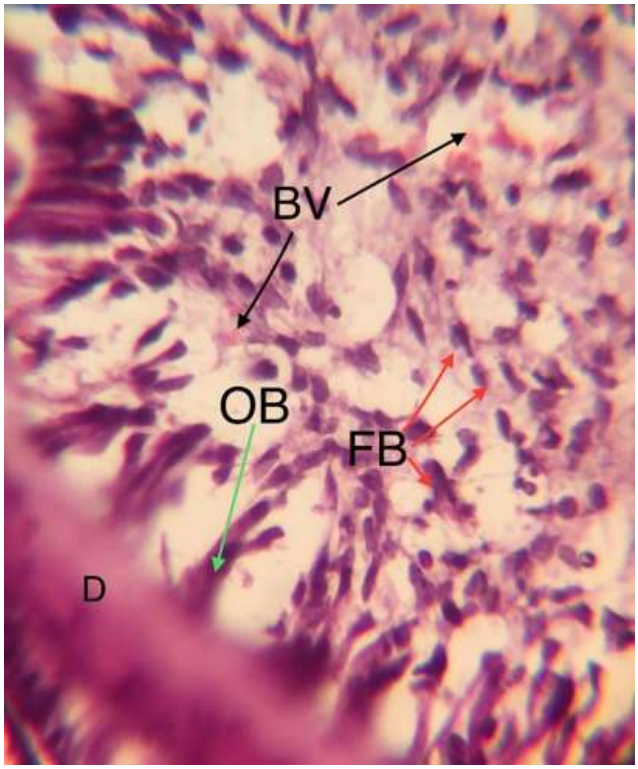

Figure 5. Histological view of the control group at 16 days showing fibroblasts (FB), odontoblasts (OB), and blood vessels (BV). Hematoxylin and Eosin ×40. **(Prepared by Authors, 2026)**

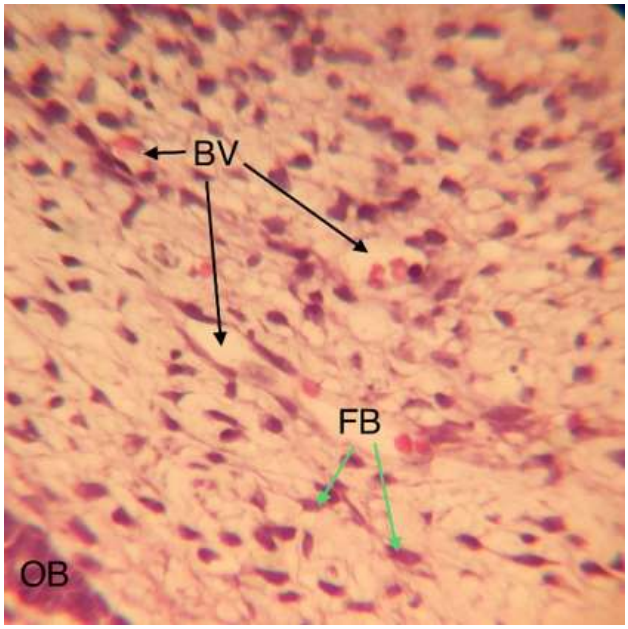

Figure 6. Histological view of the experimental group at day 16 showing fibroblasts (FB), odontoblasts (OB), and blood vessels (BV). Hematoxylin and Eosin ×40. **(Prepared by Authors, 2026)**

Table 1. Descriptive statistics of enamel and dentin thickness (hematoxylin and Eosin) as well as pulp tissue cell in the control and experimental groups at 16 days.

Variables	Group	Descriptive Statistics					Comparison	
		Mean	**S.D.**	**Min.**	**Max.**	**S.E.**	**T- test**	**p-value**
Pulp tissue cell	**Cont.**	241.750	14.881	219.000	261.139	6.075	-16.751	0.000**
	Exp.	412.930	20.128	386.222	431.556	8.217		
Blood vessels	**Cont.**	13.888	1.485	12.000	15.667	0.606	-8.069	0.000**
	Exp.	42.388	8.522	27.000	51.667	3.479		

Note: ***=p<0.05** ****=p<0.001**

4. Discussion

The present study evaluated the effectiveness of local application of Angiopoietin-1 in the upper molar area on tooth development in rats, focusing on histological changes at day 16. Our findings revealed several significant observations, shedding light on the potential role of Ang1 in dental tissue development. The experimental group exhibited increased enamel and dentin thickness compared to the control group, accompanied by the presence of ameloblasts adjacent to the enamel and an increased number of odontoblasts close to the dentin. Additionally, there was a noticeable increase in the number of blood vessels and pulp tissue cells, including odontoblasts, fibroblasts, and endothelial cells.

Tooth development is a complex process that involves intricate interactions between epithelial and mesenchymal tissues. Our study supports the notion that angiogenesis plays a crucial role in dental tissue formation. Ang1, a member of the angiopoietin family, has been shown to have powerful vascular protective effects and is involved in the remodeling and stabilization of vessels. Our findings indicate that the local application of Ang1 in the molar area promoted tooth development, as evidenced by increased enamel and dentin thickness. These effects may be attributed to the angiogenic properties of Ang1, which could facilitate blood vessel formation and enhance the supply of essential nutrients and growth factors to dental tissues.

The results of this study have potential clinical implications for dental tissue regeneration and tooth development. Understanding the role of angiogenic factors, such as Ang1, in dental tissue formation could pave the way for novel therapeutic approaches to enhance tooth development in clinical settings. Local administration of Ang1 or other angiogenic factors may hold promise for promoting tooth regeneration and improving the success of dental tissue engineering procedures.

Despite the promising findings, there are certain limitations to consider in this study. First, the study was conducted on rat models, and caution should be exercised when extrapolating the results to humans. Further studies, including translational research and clinical trials, are necessary to validate these findings in humans. Second, the exact mechanisms underlying the observed effects of Ang1 on tooth development remain unclear. Future investigations should aim to elucidate the molecular pathways and signaling cascades involved in the angiogenic and tissue-remodeling effects of Ang1 in dental tissues.

This study contributes to the existing literature on tooth development by highlighting the potential role of angiogenic factors, specifically Ang1, in dental tissue formation. While previous research has focused on various growth factors and signaling molecules involved in tooth development, the specific effects of Ang1 on dental tissue architecture and angiogenesis have not been extensively explored. Our findings provide valuable insights into the potential application of Ang1 in promoting dental tissue regeneration and may stimulate further research in this area.

Based on the results of this study, several future research directions can be suggested. Firstly, further investigations are needed to elucidate the specific mechanisms through which Ang1 influences dental tissue development. Understanding the downstream signaling pathways and molecular interactions involved will contribute to a more comprehensive understanding of tooth morphogenesis. Additionally, studies should aim to validate these findings in larger animal models and eventually in human clinical trials. This will provide valuable information regarding the safety and efficacy of locally applied Ang1 for tooth regeneration and dental tissue engineering purposes. Moreover, exploring the potential synergistic effects of Ang1 with other growth factors or biomaterials could enhance its therapeutic potential and lead to more effective strategies for tooth regeneration.

5. Conclusion

Local application of the Ang1 protein accelerated the tooth development process compared with the control group by increasing the thickness of dentin and enamel arising from the increase in the number of odontoblasts, fibroblasts, and blood vessels.

6. Declarations

6.1 Acknowledgments

The authors acknowledge the University of Baghdad for providing the experimental facilities.

6.2 Ethical Considerations

All procedures were approved by the College of Dentistry, University of Baghdad (Reference number: 463).

6.3 Authors' Contributions

Conceptualization: Al-Hussein Ali. Methodology: Al-Hussein Ali. Formal analysis: Al-Hussein Ali. Investigation: Al-Hussein Ali. Writing – original draft: all author. Writing – review & editing: Enas Fadhil

6.4 Conflict of Interest

None to declare.

6.5 Fund or Financial Support

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

6.6 Using Artificial Intelligence Tools (AI Tools)

The authors were not utilized AI Tools.

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