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Histological Evaluation of Reparative Dental Pulp Response to Direct Pulp Capping with Mineral Trioxide Aggregate Versus Simvastatin on the Traumatically Exposed Dental Pulp of Rabbits.

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Abstract:

Objective: The goal of the current study was to evaluate the reparative effectiveness of the traumatically exposed dental pulp of New Zealand rabbits after its stimulation either with mineral trioxide aggregate (MTA) or simvastatin (SIM) against the conventional methyl cellulose control substance. **Subjects and methods:** For this study, forty healthy male New Zealand rabbits weighing an average of 1.25 to 1.5 kg at 4 months of age were selected. Four groups of ten rabbits each were randomly selected from among the animals and their traumatically exposed dental pulp was treated as follow: (group 1; normal dental pulp; group 2; methyl cellulose; group 3; MTA; group 4; SIM Pulp exposure was accomplished by creating a class V cavity. in the labial surface of maxillary incisors teeth. After hemostasis, the traumatically exposed dental pulps were capped with either of the tested material, then cavities were filled by resin modified glass ionomer. One week after direct pulp capping, the teeth were extracted, and the animals were sacrificed. fixed, decalcified by formic acid and sodium citrate then microtechnically processed to get tissue sections stained by H&E. Dental pulp of all teeth were comparatively evaluated for the potentiality of the tested material to repair the exposed pulp. The reparative potential of each tested material was then comparatively evaluated in respect to the induced reparative dentin formation and the degree of inflammation. **Results:** histological examination showed the formation of either partial or complete reparative dentine bridge with MTA group while SIM group exhibited significantly thin reparative dentin bridge formation, inflammation and fibrous tissues formation. **Conclusion**: Mineral trioxide aggregate (MTA) had considerable reparative effect as a direct pulp capping agent as manifested by its induced dental pulp tissue repair and reparative dentin formation comparable to SIM.

KEY WORDS: Mineral trioxide aggregate, Methyl cellulose, Simvastatin, Dental pulp capping

Introduction

Preserving the life of all or a portion of the exposed dental pulp is significantly therapeutic importance, either in the traumatic dental pulp or those with incomplete root formation. [(1)] Applying a biocompatible substance to dental pulp tissue that has unintentionally become exposed due to trauma or carious injury is known as "direct

pulp capping." The treatments' goals are to prevent germs from penetrating the exposed tooth pulp, promote the pulp's walling off of the exposed area by starting the construction of a dentine bridge, and preserve wholesome pulp tissue. [2]

Preserving dental pulp life is one of the primary objectives of contemporary restorative dentistry. Therefore, the objective of conservative pulp therapy is to maintain the life of the dental pulp while promoting the restoration of the responsiveness and vitality of the dental-pulp complex by the remaining pulp tissue. Direct pulp capping (DPC) has been proposed as a means of generating reparative dentin and promoting pulp healing. [3] Numerous academics and physicians have conducted studies on the various capping materials. However, choosing the right capping material mimics the secret to a good result when taking into account the particular requirements of each instance. [4,5]

The exposed tooth pulp has been dressed using a variety of materials for pulp capping. Calcium hydroxide was regarded as the benchmark for dental pulp capping in numerous research However, the use of calcium hydroxide as a pulp capping material has been associated with a number of documented drawbacks, such as the production of tunnel flaws in dentin barrier, widespread dentin formation that completely obliterate the pulp chamber, and its high solubility. and bacterial leakage into dental pulp from its poor sealing quality and lack of adhesion with tooth structure. [6,7]

Recently, efforts have been made to create a pulp capping material that is safe, affordable, and capable of biologically activating odontoblasts and promoting the dentinogenesis of the dental pulp. [8] Simvastatin is commonly used to lower cholesterol and treat hyperlipidemia and arteriosclerosis. It has been promoted as a particular competitive inhibitor of glutaryl coenzyme-A reductase (3 hydroxy 2 methyl). [9,10] Due to its low cost and high level of safety, it has been used for a long time all over the world. It is also known to have pleiotropic effects, which include anti-inflammatory qualities and the stimulation of angiogenesis and bone formation. [11]

Mineral trioxide aggregate (MTA) has gained popularity since roughly 1990 as a dependable and efficient dental pulp capping material. [12] It is a kind of Portland cement that has bioactive qualities. It is made up of fine particles that, when combined with sterile water, form a hydrophilic powder that solidifies when moisture is present. Its high alkalinity is attributed to the presence of calcium hydroxide and calcium silicate hydrate, which form a solid, permeable colloidal gel with a pH of 10–12.5. [13,14] Additionally, it has a 4:1 ratio of bismuth dioxide and can show radiopacity. It is sold commercially as Pro Root MTA (Tulsa Dental, Tulsa, OK). [15]

The MTA is made up of 15–25% silicone oxide and 50–70% calcium oxide. It also has trace amounts of magnesium oxide, potassium sulfate, sodium sulfate, and silicone dioxide. [16] MTA is more suitable for clinical therapeutic applications than the original Portland cement due to its lower toxicity and extended setting time, which allows adequate preparation and has smaller particles. [17] For up to 21 days, MTA's compressive strength rises with moisture. [18] The enhancement of material strength during the setting process depends on the hydration process involving tricalcium silicate and dicalcium silicate. [16]

The most prevalent biopolymer found in nature, cellulose is regarded as one of the main constituents of the cell wall in the majority of plants. [19] Since it mimics a key structural element of the primary cell wall of green plants, several algae, and oomycetes, cellulose is the most abundant organic polymer. [20] A chemical compound called methylcellulose (MC), which is derived from cellulose, is added to Portland cement to enhance its performance in damp conditions. It is also used to treat constipation and as an emulsifier and thickener in a variety of food products and cosmetics. Cellulose is neither poisonous nor allergic, nor is it digestible. MTA's moldability and setting time have been shortened by adding 1% MC and 2% calcium chloride (CaCl2), which is comparable to reinforced zinc oxide-eugenol cement with roughly the same compressive strength. [21]

Materials and Methods

Animal models: The animal house at Mansoura University's Faculty of Veterinary Medicine in Egypt served as the study's location. Forty healthy male New Zealand rabbits, each four months old and weighing between 1.25 and 1.50 kg, were sourced from the animal facility at Mansoura University in Egypt. These rabbits were confirmed to be free of dental caries and fractures in all maxillary incisors. The animals were housed in standard cages with sawdust bedding, maintained under controlled environmental conditions, including a temperature of 20 ± 2°C, humidity levels of 30–40%, and a light cycle of 12 hours of illumination followed by 12 hours of darkness. Each rabbit was provided with a standard diet and had unrestricted access to fresh water.

The procedures of the present study were conducted and approved in accordance to guidelines of Medical Research Institute, International guiding principles for biomedical research involving animals, as endorsed by the recommendations of the Ethical Committee regarding the utilization of animals in research. Faculty of Veterinary Medicine, Mansoura University (Egypt). The inclusive dental criteria followed included no pathologic tooth mobility, normal gingiva without periodontal pathosis, including redness, swelling, draining sinus tract, periapical or pathologic root resorption.

Group planning and Experimental Interventions

One week, after animals' adaptation, they were split up into four groups at random, each consisting of ten rabbits.:

Group (I): normal dental pulp

Group (II): the prepared class V cavities of maxillary incisor teeth were capped with methyl cellulose as a control, **(Sigma-Aldrich, St. Louis, MO, USA)**. [22,23]

Group (III): the prepared class V cavities of maxillary incisor teeth were capped with mineral trioxide aggregate (**Dentsply, Tulsa Dental, Tulsa, OK, USA).** [22,23]

Group (IV): the prepared class V cavities of maxillary incisor teeth were capped with simvastatin **(Sigma-Aldrich, St. Louis, MO, USA);** [22,23]

Premedication and general anesthesia

In a sterile surgical environment characterized by partial isolation and aseptic conditions, the procedures were carried out under general anesthesia. The rabbits were premedicated with a 2% injection of xylazine HCl (5 mg/kg) following a 12-hour fast **(Xyla-Ject, ADWIA Company, Egypt)** given intramuscularly. [24] The animals were anaesthetized by general anesthesia induced by 5mg/kg body weight ketamine hydrochloride **(Keiran, Eimc, Egypt)** given intramuscularly. [24]

Preparation of Class V cavity and pulp exposure

During the procedure, a mouth gag was used to keep the rabbits' mouths open while they were fixed on the operating board. 0.1 Chlorhexidine solutions were used to clean each rabbit's mouth. A small sterile round bur size one (Dentsply Maillefer, Tulsa, Oklahoma, USA) was used to prepare standard Class V cavities in the maxillary incisor at 30,000 rpm. The bur was mounted in a contra-angle handpiece (NSK, Tokyo, Japan) and operate the underwater coolant until the floor of the cavity becomes pink, but without exposure. [25] A sharp probe (Dentsply Maillefer, Tulsa, Oklahoma, USA) was used to precisely expose the labial pulp tissue beneath the cavity base in order to standardize the dental pulp exposure size. A sterile cotton pellet was applied to the exposed area, and 2.5% NaOCl (Clorox; Household Cleaning Products of Egypt, Cairo, Egypt) was used to stop the bleeding. After being cleaned with regular saline, the cavity was dried using sterile cotton pellets. [25]

Capping materials of the exposed dental pulp:

a) **Methyl cellulose (MC):** methocel A, (Sigma-Aldrich, St. Louis, MO, USA) cellulose (MC) gel was produced by incorporating the necessary quantity of polymer into heated distilled water, followed by allowing it to cool to room temperature over a period of 24 hours. Subsequently, the gel was transported to the exposure site using a metal carrier. [22]

b) **Simvastatin (SIM):** 1.5mg of SIM powder **(Sigma-Aldrich, St. Louis, MO, USA);** was combined with distilled water to produce a creamy mix, then the mix was carried in a metal carrier to the exposure site. [25]

c) **Mineral trioxide aggregate (MTA)**: As directed by the manufacturer, proRoot MTA calcium silicate-based material (Dentsply, Tulsa Dental, Tulsa, OK, USA) was prepared. The MTA was made right before use and combined in a 3:1 ratio with sterile water. After that, the blend was transported to the site of exposure using a metal carrier. [22]

Restorative material: the GC Fuji IX GP reinforced glass ionomer capsule was used as a labial restorative filling material in the prepared cavity over capping material.

Animals euthanasia: one week after application of the capping material20 ml of a 5% thiopental sodium solution was quickly administered into the cephalic vein to kill the animals by overdosing them with anesthetic solution. The jaw parts that contained the experimental teeth were surgically removed and resected. [26]

Tissue preparation for microscopic investigations

(1)-Fixation and decalcification of tissue specimens: 10% formalin (Gomhorya Company, Cairo, Egypt) was used to fix the experimental incisors and surrounding periodontium for a week. 20% formic acid (Gomhorya Company, Cairo, Egypt) and 25% sodium citrate (Gomhorya Company, Cairo, Egypt) were used for a month to decalcify the incisors and their periodontium. To get rid of any extra fixed and decalcifying agents, the teeth were cleaned under running water. [27]

(2)- Microtechnical processing of tissue specimens: To get rid of the alcohol residue, the tissue samples were dehydrated for a day in increasing grades of ethyl alcohol and methyl benzoate, and then for two hours in paraffin benzol. (28) Melting paraffin wax was used to embed the tissue samples. In the center of the restorative material, a 6 μm section was made parallel to each tooth's primary vertical axis. [28]

(3)- Histopathological examination: Hematoxylin-eosin was used to stain the tissue sections, which were then viewed under a light microscope for histopathological analysis and to gauge the tissue's response to various capping materials in the dentin-pulp complex, such as the pulp cores, the odontogenic zones, and the ostensibly reparative dentin formation, for evaluation of the potentiality of each tested capping material applied in the experiment to repair the exposed site of dentin-pulp complex.

4- Immunohistochemical examination:

a-Transforming growth factor (TGF): The tissue sections underwent deparaffinization using xylene, followed by hydration through a series of increasing concentrations of alcohol. Subsequently, they were briefly rinsed with tap water and phosphate buffered saline (PBS) at a pH of 7.4. The sections were then incubated in a solution of 0.3% hydrogen peroxide in methanol for a duration of 10 minutes. To prevent nonspecific protein binding, normal serum was applied for 10 minutes. For the treatment with primary antibodies, the sections were incubated overnight at 4°C with polyclonal anti-TGF-β1 (TGF-β1 (V) sc-146; Lot #F2306, Santa Cruz Biotechnology, Santa Cruz, CA, USA). The tissue sections that had been previously treated were subsequently washed three times with PBS, each wash lasting 5 minutes. Following this, they were incubated with biotinylated secondary antibodies for 10 minutes, and then with a streptavidin–enzyme conjugate solution for an additional 5 minutes. The antigen, localized by the antibodies, was detected through the peroxidase-mediated activation of 3, 3′-diaminobenzidine for 10 minutes, resulting in a brownish coloration. [29]

b-Vascular endothelial growth factor (VEGV): The detection of VEGF was carried out through the alkaline phosphatase antialkaline phosphatase method (APAAP) utilizing a polyclonal antibody sourced from Santa Cruz, CA. Tissue sections measuring 4 μm were prepared and affixed to slides coated with Poly-L-lysine. The paraffin sections underwent a dewaxing process using xylene, followed by rehydration, and were subsequently washed in Tris-buffer at pH 7.6 for a duration of 10 minutes. Prior to the detection of VEGF, a pre-digestion step with proteinase K was performed using a working solution of 0.4 mg/ml (Dako Corporation, Carpinteria, CA) for 10 minutes at room temperature. The subsequent procedures were enhanced through the use of automatic staining (Dako, TechMate 500): (i) The sections were treated with a primary antibody solution for VEGF, diluted to 1:400, and incubated at room temperature. Following this, the slides were washed with buffer (Buffer Kit, Dako). (ii) The immunoreaction for the tissue sections was finalized using the APAAP kit (Dako). [30]

Results

- **Histology of normal dental pulp tissue (GI):** The areolar connective tissue that fills the pulp cavity makes up dental pulp. Four different zones were visible in its histologic appearance: The odontoblastic zone is located at the edge of the dental pulp and is made up of odontoblast cell bodies. The cell free zone is located beneath the odontoblastic zone and is about 40 microns wide. Macrophages, immunocompetent cells, fibroblasts, undifferentiated mesenchymal cells, and young collagen fibers are all found in the cell rich zone, which is located right next to the cell free zone. (4) Dental pulp proper: made up of loose connective tissue with a lot of cellular components, it also has bigger blood vessels and nerves that radiate outward. (Fig 1).

- **Histology of dental pulp tissue response to methyl cellulose after 7 days (GII):**

a- Direct contact with capping material: the dental pulp tissue was disorganized as reflected by dense reticular fibrous tissue replacing the normal areolar connective tissue, loss of the odontoblastic layer and partial inclusion of pulp tissue in the forming secondary dentin (Fig 2).

b- Indirect contact with capping material the dental pulp tissue at the mid root region showed that in some area disorganized odontoblasts but in another area, odontoblasts are organized and palisading interspersed with newly proliferating blood vessels engorged with RBCs and lymphatic vessels appear dilated (Fig 3).

- **Histology of dental pulp tissue response to simvastatin after 7 days (GIII):**

a- Direct contact with capping material: the dental pulp tissue at the exposure site showed mineralize dentin bridge beneath the parts of capping material, the dental pulp tissues subjacent to partially mineralize thin dentin bridge shows a loss of the odontoblast layer and atubular reparative dentin deeper to capping material. Other specimen showed fibrotic change replacing odontogenic layer with dense hyalinized homogenous collagen fibrous repair of pulp tissue and hyalinized homogenous reparative dentin formation (Figs 4,5).

b- Indirect contact with capping material: the dental pulp tissue at the mid root region showed that; vacuolization in odontoblastic layer where the cell rich zone and pulp core showed dense reticular fibrous tissue island and slightly dilated blood vessels. Other tissue specimens showed that disorganized odontogenic zone where the cell rich zone and pulp core showed follicular fibrous islands dispersed in hyalinized pulp tissue core. (Figs 6,7).

- **Histology of dental pulp tissue response to mineral trioxide aggregate (MTA) after 7 days (GIV):**

a- Direct contact with capping material: the dental pulp tissue at the exposure site showed one large globular dentin formation just beside and two small atubular globular deposition of dentin bridge formation beneath the capping material. The pulp tissue subjacent to mineralized partial dentin bridge showed loss of the odontoblast layer with tissue disorganization, exhibiting tissue granulation replacing pulp tissue with the deposition of tertiary atubular dentin in the wall of the dental pulp tissue that is differs from primary dentin (Figs 8,9).

b-Indirect contact with capping material: the dental pulp tissue at the mid root region showed that; reticular connective tissue and dilated lymph vessels engorged with mineral trioxide aggregate (Fig10). Other specimen showed organized and palisading odontoblasts, newly proliferating blood vessels and dilated blood vessels engorged with RBC (Fig 11).

- **Imunohistochemistry for transforming growth factor (TGFβ1) of dental pulp tissue in positive control (methyl cellulose) after 7 days (GII)**

According to data analysis that looked into the percentage of TGF-β1 in the dentin-pulp complex.: the dental pulp tissue showed negative reactivity (0) for TGFβ in odontoblasts, and marked reactivity (+++) in dentin (Fig 12).

- **Imunohistochemistry for transforming growth factor (TGFβ1) of dental pulp tissue with simvastatin after 7 days (GIII)**

According to data analysis that looked into the percentage of TGF-β1 in the dentin-pulp complex. the dental pulp tissue showed week reactivity (+) for TGFβ in odontoblasts, and in dentin (Fig 13).

- **Imunohistochemistry for transforming growth factor (TGFβ1) of dental pulp tissue with mineral trioxide aggregate (MTA) after 7 days (GIV)**

According to data analysis that looked into the percentage of TGF-β1 in the dentin-pulp complex.: the dental pulp tissue showed week reactivity (+) for TGFβ in odontoblasts, and marked reactivity in dentin (Fig 14).

- **Immunohistochemistry for vascular endothelial growth factor (VEGF) of dental pulp tissue in positive control (methyl cellulose) after 7 days (GII)**

According to data analysis that looked into the percentage of TGF-β1 in the dentin-pulp complex.: the dental pulp tissue showed weak reactivity (+) for VEGF in odontoblasts and in dentin (Fig 15).

[

- **Immunohistochemistry for vascular endothelial growth factor (VEGF) of dental pulp tissue with simvastatin after 7 days (GIII)**

According to data analysis that looked into the percentage of TGF-β1 in the dentin-pulp complex.: the dental pulp tissue showed week (+) for VEGF in odontoblasts, and moderate reactivity (++) in dentin (Fig16).

Immunohistochemistry for vascular endothelial growth factor (VEGF) of dental pulp tissue with mineral trioxide aggregate after 7 days (GIV)

According to data analysis that looked into the percentage of TGF-β1 in the dentin-pulp complex.: the dental pulp tissue showed marked reactivity (+++) for VEGF in odontoblasts and in dentin (Fig17).

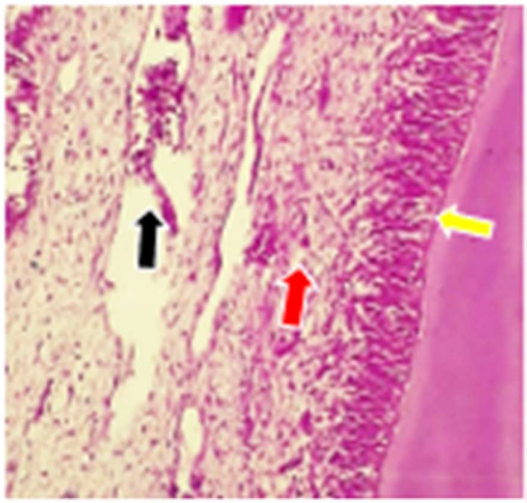

Fig. (1): dentin-pulp complex of normal control group showing pseudostratified odontoblastic layer (yellow arrow), cell-rich zone containing fibroblast, undifferentiated mesenchymal cells, young collagen fibers (red arrow) and richly vascularized pulp core (black arrow). (H &E stain, mag. X 200

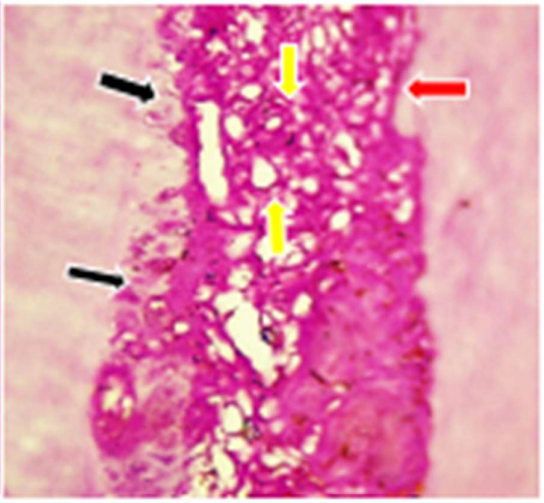

Fig. (2): dentin-pulp complex of positive control with methyl cellulose after 7 days, at the exposure site, showing dense reticular fibrous tissue replacing dental pulp (yellow arrows), loss of the odontoblast layer and reparative dentin deposition including minute pulp tissue (black arrows). (H &E stain, mag. X 200

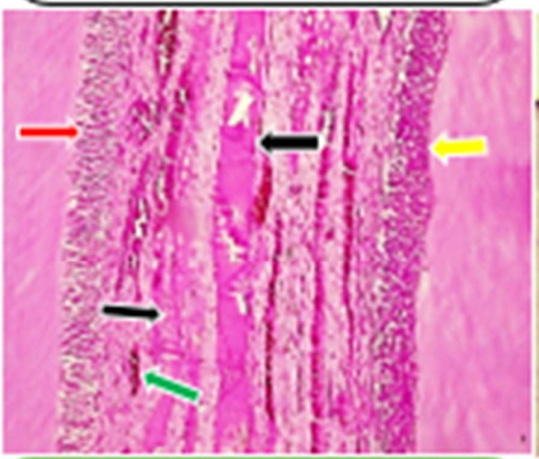

Fig. (3): dentin-pulp complex of methyl cellulose after 7 days at the mid-root region disorganized (yellow arrow) or palisading odontoblasts (red arrow), newly proliferating blood vessels engorged with RBCs (green arrow), and dilated lymphatic (black arrows). (H&E stain, mag. X200).

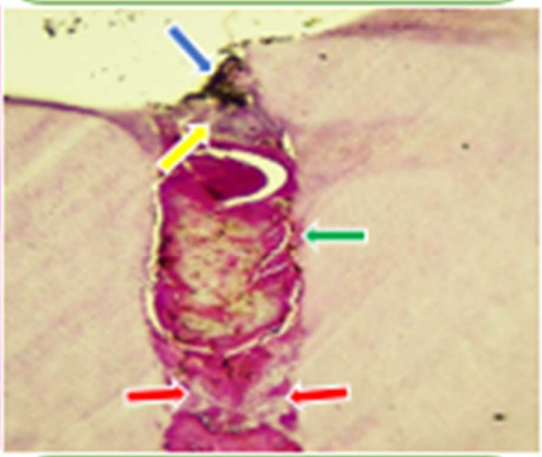

Fig. (4): dentin-pulp complex of simvastatin after 7 days at the exposure site showing pulp capping material (blue arrow), mineralized incomplete dentin bridge (yellow arrow), odontoblasts completely lost (green arrow) and atubular reparative dentin formed deeper to capping material (red arrows). (H&E stain, mag. X200).

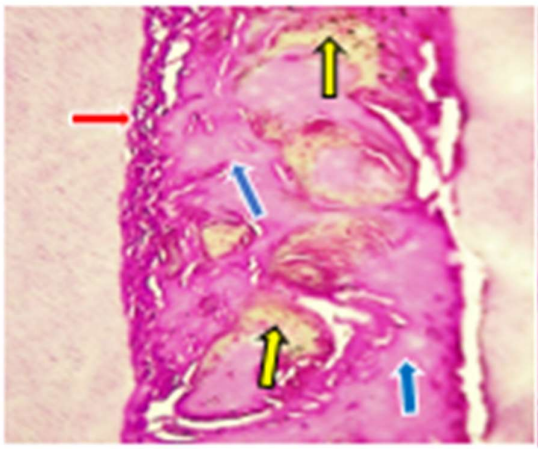

Fig (5): dentin-pulp complex simvastatin after 7 days below the exposure site showing fibrotic change replacing odontogenic layer (red arrow), dense hyalinized fibrous tissue, (green arrow) and mineralized homogenized reparative dentin (blue arrow). (H&E stain, mag. X200).

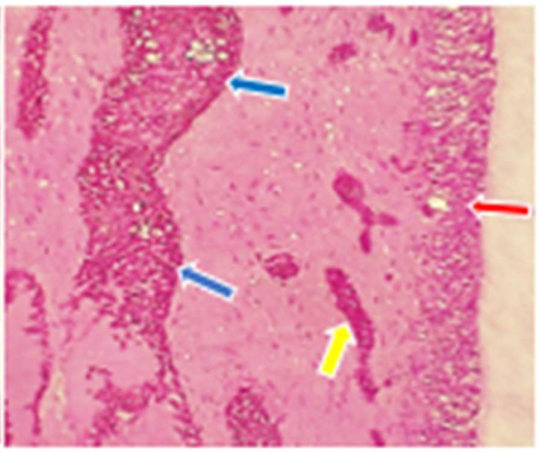

Fig (6): dentin-pulp complex of simvastatin after 7 days at the mid- root region showing vacuolization in odontoblastic layer (red arrow), reticular fibrous islands (blue arrows) and slightly dilated blood vessels (yellow arrow). (H&E stain, mag. X200).

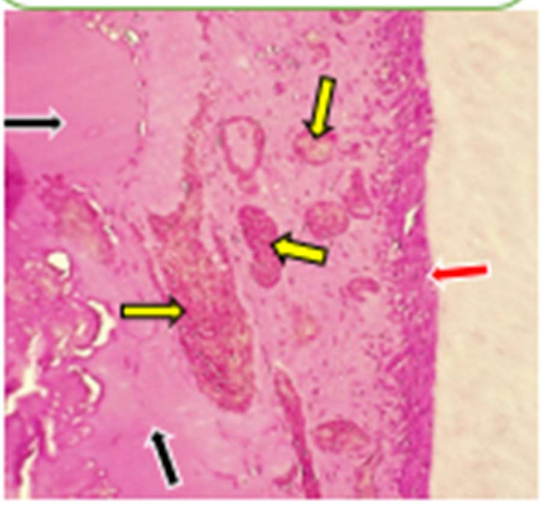

Fig (7): dentin-pulp complex of simvastatin after 7 days at the mid- root region showing disorganized odontoblastic layer (red arrow) and follicular fibrous islands (yellow arrows) dispersed in hyalinized pulp tissue core (black arrows). (H&E stain, mag. X200).

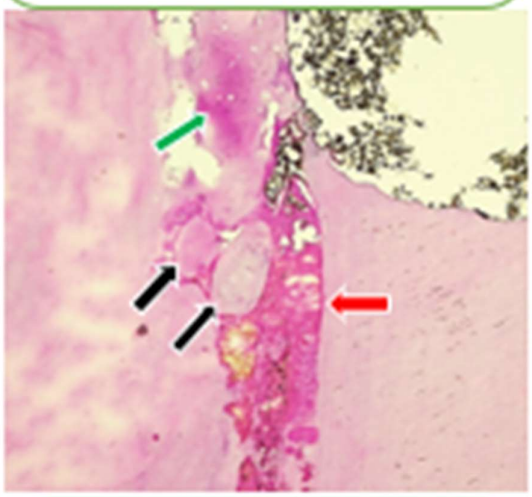

Fig. (8): dentin-pulp complex of mineral trioxide aggregate after 7 days at exposure site showing one large globular dentin bridge formation just beside MTA (green arrow), two small globular dentin (black arrow), dense granulation tissue formation replacing pulp tissue with loss of odontoblastic layer (red arrow). (H&E stain, mag. X200)

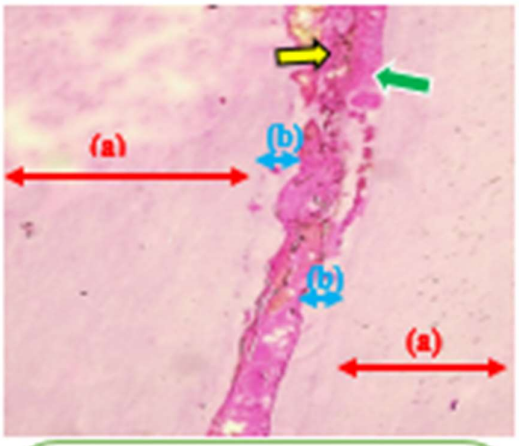

Fig. (9): dentin-pulp complex with mineral trioxide aggregate after 7 days below exposure site showing remnant of capping material (yellow arrow), tubular tertiary dentin deposition (b) differs from primary dentin (a) and granulation tissue replacing dental pulp tissue with loss of odontoblastic layer (green arrow). (H&E stain, mag. X200)

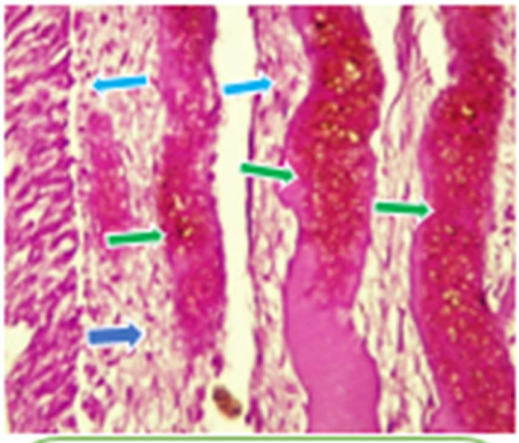

Fig. (10): dentin-pulp complex with mineral trioxide aggregate after 7 days at midroot region showing delicate reticular fibrous tissue (blue arrows), dilated lymph vessels engorged with mineral trioxide granules (green arrows). (H&E stain, mag. X400).

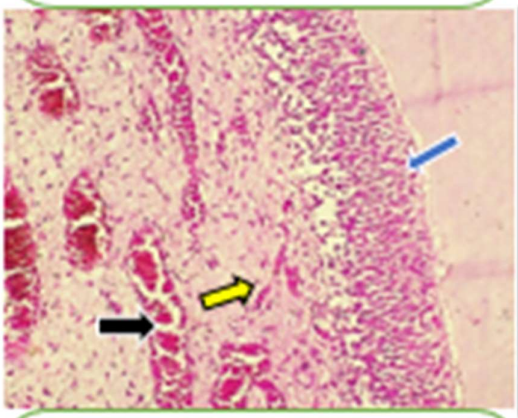

Fig. (11): dentin-pulp complex with mineral trioxide aggregate after 7 days at midroot region showing well organized and palisading odontoblasts (blue arrow), newly proliferating blood vessels (yellow arrow) and dilated blood vessels engorged with RBCs (black arrow). (H&E stain, mag. X400).

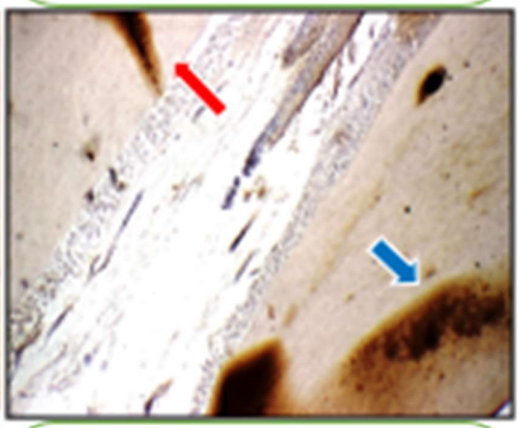

Fig. (12): dentin-pulp complex of positive control with methyl cellulose after 7 days showing negative reactivity (0) for TGFβ in odontoblasts (red arrow), and marked reactivity (+++) in dentin (blue arrow). (TGFβ immunostain mag. x 200).

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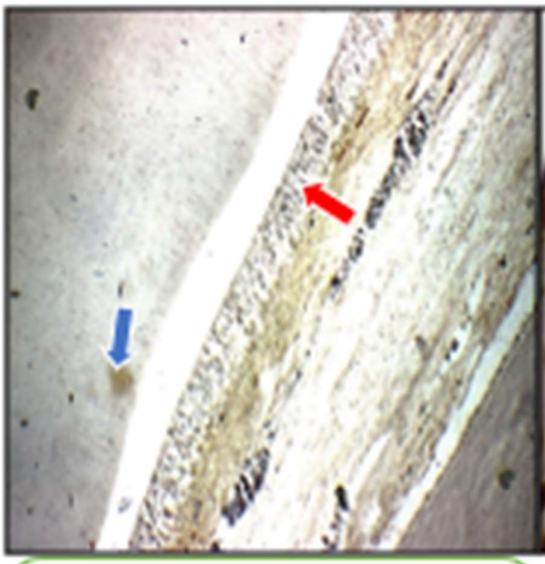

Fig. (13): dentin-pulp complex with simvastatin after 7 days showing week reactivity (+) for TGFβ in odontoblasts (red arrow) and in dentin (blue arrow). (TGFβ immunostain mag. x 200).

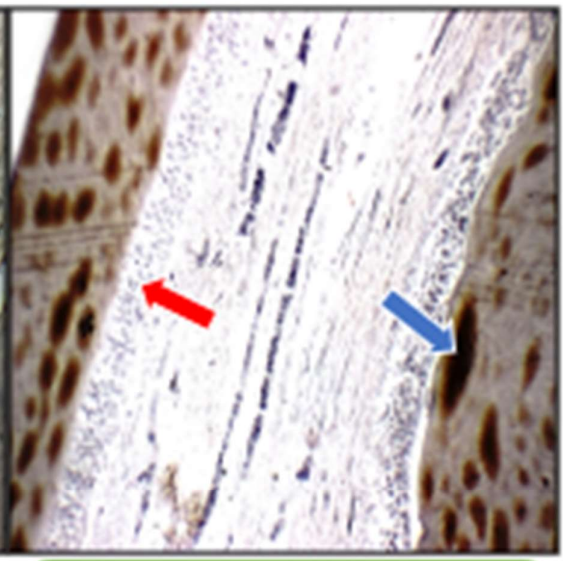

Fig. (14): dentin-pulp complex with mineral trioxide aggregate (MTA) after 7 days showing week reactivity (+) for TGFβ in odontoblasts (red arrow) and marked reactivity (+++) in dentin (blue arrow). (TGFβ immunostain mag. x 200).

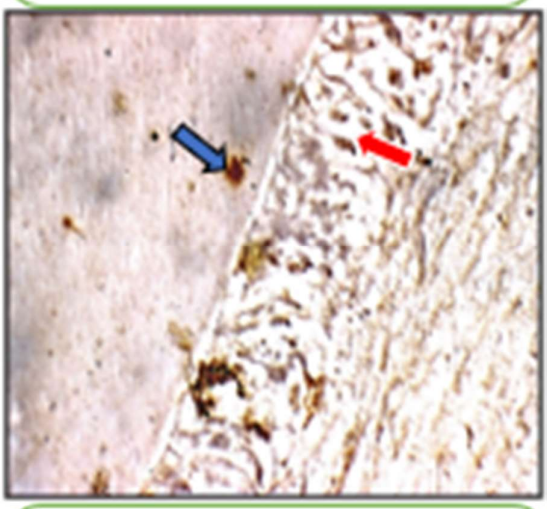

Fig. (15): dentin-pulp complex of positive control with methyl cellulose after 7 days, showing weak reactivity (+) for VEGF in odontoblasts (red arrow) and in dentine (blue arrow). (VEGF immunostain mag. x 200)

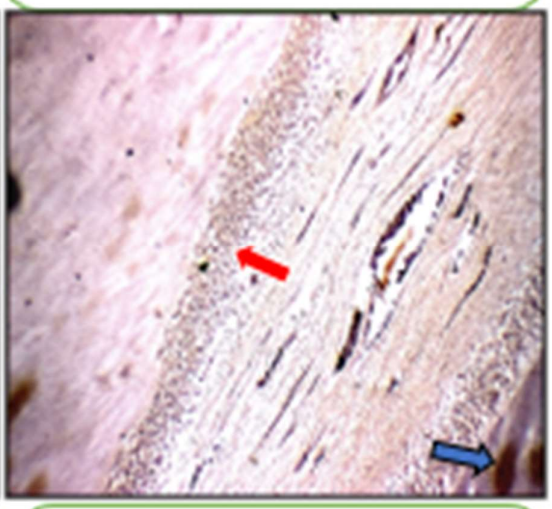

Fig. (16): dentin-pulp complex with simvastatin after 7 days showing weak reactivity (+) for VEGF in odontoblasts (red arrow) and moderate reactivity (++) in dentine (blue arrow). (VEGF immunostain mag. x 200).

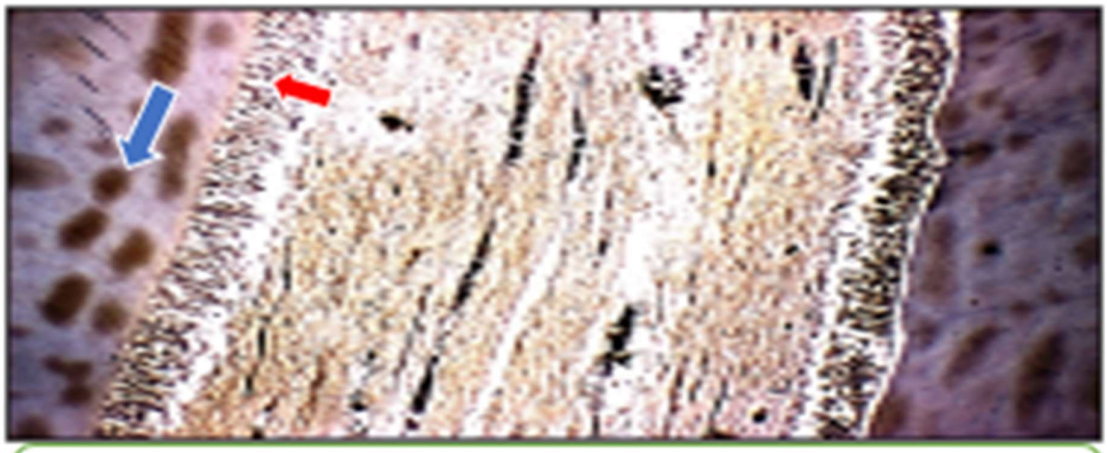

Fig. (17): dentin-pulp complex with mineral trioxide aggregate (MTA) after 7 days showing marked reactivity (+++) for VEGF in odontoblasts (red arrow) and in dentine (blue arrow). (VEGF immunostain mag. x 200).

Statistical Analysis

Calculations were made to compare the amount of reparative dentin deposited at one week between the experimental simvastatin and mineral trioxide aggregate and the positive control methyl cellulose. The statistical package for social science (SPSS) software was used to analyze the data after it had been tabulated in a table.Table (1): statistics of reparative dentin deposition between control, simvastatin and mineral trioxide aggregate groups after one week.

Statistics				
Groups		Reparative dentin deposition in different groups		
		control	sim	MTA
Amount of reparative dentin deposition after one week	N	10	10	10
	Mean	731.85	860.50	1120.50
	SD	57.58	107.79	10.91
	Range	208	374	19
	Minimum	633	689	921
	Maximum	841	1063	1325

Table (2): mean and standard deviation of reparative dentin deposition between control, simvastatin and MTA group after one week. *; significant ($p < 0.05$) ns; non-significant ($p>0.05$)

Variables	Groups			P value
	Control group	**Simvastatin group**	**Mineral trioxide aggregate group**	
	Mean ± SD	Mean ± SD	Mean ± SD	
Reparative dentin	731.85 ± 57.58[a]	860.50 ± 107.79[b]	1120.50 ± 10.91[c]	P < .000*

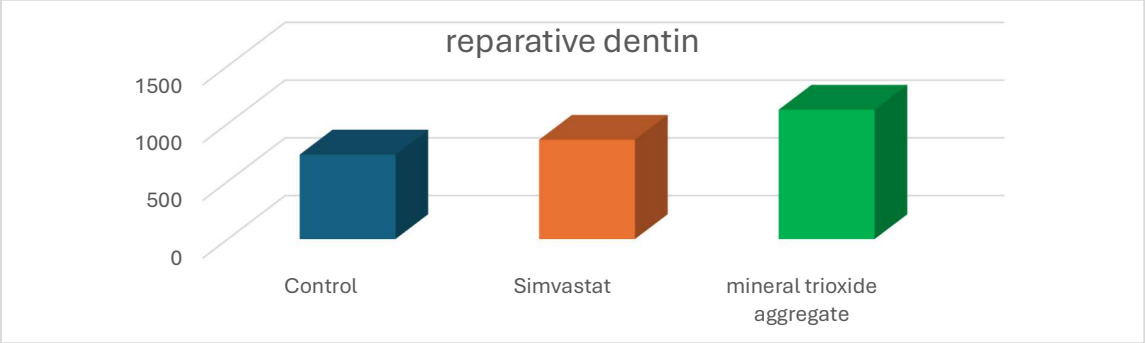

Figure (18): Bar chart show mean values of reparative dentine for control versus simvastatin and MTA group after one week.

Discussion

In the past few decades, scientists have worked hard to find materials that can be used to both caps exposed dental pulp tissue and create a foundation for its healing. The preferred treatment, known as DPC, involves directly covering the revealed pulp with a material that induces pulp potentiality, provides the chance for reparative dentin to form, and ultimately preserves pulp vitality. [31] Maintaining the vitality of dental pulp has long been a fundamental objective in the management of dental caries. In cases where the pulp chamber is inadvertently exposed, it is usually preserved through methods such as direct capping or pulpotomy. These techniques rely on the dentin-pulp complex's ability to promote pulp restore and then create additional layer of dentin protection. [32]

Reducing or eliminating dental pulp inflammation is one of the main goals of pulp capping therapy. Reduced inflammation of the pulp is a sign that pulp-capping materials are highly biocompatible. Dentin bridge formation by itself does not demonstrate the pulp's health, but the incidence of reduced inflammation can be regarded as a sign of effective pulp capping material treatment. [33] Simvastatin or mineral trioxide aggregate was applied to the exposed dental pulp tissue in the current study to assess the histopathological effects of direct pulp capping. The comparative replies of the remaining pulp tissue were also evaluated at; 7 days to examine the reparative potentiality of the two materials have been used in the pulp tissues of the experimental animals.

The rabbit incisor was chosen because it is a legitimate equivalent of the human deciduous or immature tooth due to its large crown size making it appropriate for restorative procedures, its open apex, the fact that it is not affected by age changes, and its histological similarity to human teeth. Additionally, rabbit incisors' high proliferative capacity combined with their biocompatibility and ability to seal with resin-modified glass ionomer (RMGIC) as a final restoration. [34]

In the present study, the pulp capping with methylcellulose for 7 days showed tissue disorganization as reflected by dense reticular fibrous tissue replacing the normal areolar connective tissue, loss of the odontoblastic layer. Also, dental pulp tissue showed deposition of tertiary atubular dentin over the primary dentin that seemed to be partially obliterating the pulp canals. Occasionally, partial inclusion of pulp tissue in the forming secondary dentin. Therefore, methylcellulose (MC) is not ideal control material since it has adverse effect on dental pulp tissue, as it is activities deposition of a tubular tertiary dentin, pulp tissue hyalinization, granulation tissue formation, loss of the odontoblast layer with dentin-pulp disorganization, globular calcification and dilation of lymphatic and blood vessels.

In the present study, the pulp capping with simvastatin for 7 days showed partially mineralize dentin bridge beneath the remaining capping material. The pulp tissue subjacent to partially mineralized thin dentin bridge showed deposition of tertiary a tubular dentin in the wall of the pulp that seems partially obliterated the pulp canal odontoblast layer loss accompanied by tissue disarray, tissue fibrosis with dense hyalinized homogenous collagen fibrous repair of the pulp tissue.

The findings of the present study align with those of Omid Dianat et al. [22] Jung JY et al. [35] and Lee S et al. [36] and demonstrated that simvastatin's positive effects on DPSC differentiation and strong anti-inflammatory properties can improve dental pulpal regeneration. In the present study hard tissue formation with tissue fibrosis, disorganization and hyalinization in simvastatin group may be due to the two-sided effect of statin including: (1 induction of odontogenic gene expression in dental pulp stem cells (DPSCs) and (2) acceleration of DPSCs-mediated mineralized tissue formation while suppressing proliferation and apoptosis of DPSCs. [37]

In the present study, pulp capping with mineral trioxide aggregate (MTA) at the exposure sites showed complete dentin bridge (tubular tertiary dentin) that almost obliterated the pulp chamber with tertiary dentin. However, beneath the parts of capping material, the pulp tissue subjacent to mineralized complete dentin bridge displays tissue granulation, tissue loss of the odontoblast layer, and the deposition of tertiary atubular dentin—which is different from primary dentin—in the wall of the dental pulp tissue.

Our study's discovery of a complete dentin bridge in the MTA group may have resulted from the calcium ions' continuous release during the MTA material's setting time, which can activate a number of signaling molecules, including macrophage colony-stimulating factor (MCSF) and transforming growth factor-β (TGF-β). Calcium ions also can form crystals that attract fibronectin, which controls cellular adhesion and differentiation. [23] In addition, MTA can uncouple and activate growth factors nested in the proximal dentin and also increase the secretion of TGF-β1 from pulp cells. TGF-β1 is the main role in reparative dentinogenesis as stimulate the migration of progenitor cells and accelerate odontoblast cells differentiation. [23]

In the present study, pulp capping with mineral trioxide aggregate (MTA) at the mid-root region showed reticular connective tissue and dilated lymph vessels engorged with mineral trioxide aggregate. High molecular weight solutes may be extracted from the interstitial tissue fluid as a result of dilated lymphatic vessels. This controls the development of tissue edema or interstitial pressure by lowering the interstitial colloidal osmotic pressure. Furthermore, because lymphatic capillaries are semipermeable and passively gather extra tissue fluid and proteins from the intercellular spaces of connective tissue before returning them to the blood vascular system, lymphatic vessels are essential for tissue homeostasis and the body's reaction to injuries. Also, lymph vessels carry to blood streams lymphocytes, fatty acids and immunoglobulin antibodies produced by lymph node. [38]

The pulp capping with mineral trioxide aggregate in the current study demonstrated a significant increase in positive immunoreactivities for the transforming growth factor TGF-β1 in the MTA group, which was consistent with the findings of About, who claimed that MTA significantly increases TGF-β1 secretion. The migration of pulp stem cells was guided by its controlled release. [39] According to reports, this growth factor also plays a role in the odontoblastic differentiation that results in the formation of dentin bridges. Dentin matrix protein 1 (DMP1) and dentin sialophosphoprotein (DSPP), two non-collagenous dentin matrix proteins that are crucial for the appropriate mineralization and maturation of dentin, are expressed in response to TGF-β1. [40]

The current study found that pulp capping with mineral trioxide aggregate significantly increased vascular endothelial growth factor (VEGF) positive immunoreactivities. It is the strongest vasculogenic and angiogenic factor that contributes to the formation of tertiary dentin. It plays a crucial role in controlling the body's reaction to pulp damage, which raises vascular permeability and angiogenesis throughout the healing process. [41]
[

Conclusion

In conclusion, the results of the present study showed that mineral trioxide aggregate had considerable useful effect as a direct pulp capping agent as manifested by complete calcified bridge formation and globular deposition of tertiary atubular dentin that has replaced part of pulp tissue beside calcification of the pulp which have been enclosed in secondary dentin.

Simvastatin had low useful effect when applied as a direct pulp capping agent which were manifested by thin partially mineralized thin dentin bridge with loss of the odontoblast layer and tissue disorganization, exhibiting tissue fibrosis with hyalinized homogenous collagen fibers of pulp tissue.

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