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HISTO-PATHOLOGICAL EVALUATION OF THE EFFECT OF ALOE VERA GEL AS A PULPOTOMY AGENT IN YOUNG PERMANENT TEETH: A PILOT STUDY

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ABSTRACT

Introduction: Preservation of pulp vitality is the principal objective in the management of exposed vital pulp of immature young permanent teeth. It has become challenging to identify the ideal pulp capping agent for each clinical case. Owing to the reported clinical and radiographic success of *Aloe vera*as a pulp capping material in primary teeth, it was tempting to investigate this material as a pulp medicament in young permanent teeth.

Materials & Methods: Eight intact newly erupting immature young premolars, scheduled for extraction for orthodontic purposes, were selected for this pilot study. Two groups were implemented in the study; patients in group I were treated using Aloevera gel as the pulpotomy material while group II received Portland Cement.

Results: Histological sections of both studied groups showed well organized odontoblast-like cells with proximal nuclei and extended odontoblastic processes arranged near the orifices and along the peripheries of the radicular walls, accompanied by predentin layer and dentin with the classic arrangement of dentinal tubules. The gene expressions of IL-1β, TGF-β and DSPP were comparable between the Aloevera and Portland cement group

Conclusion: Based on the results of the current pilot study, Aloevera gel proved comparable results to the control group as a pulp capping material in pulpotomy of young permanent teeth.

Keywords: Aloe Vera, Natural, Medicinal Plant, Young Permanent Teeth, Pulpotomy, Portland Cement, IL-1β, TGF-β, DSPP

Abbreviations: DSPP: Dentin Sialo-Phosphoprotein**;** IL-1β**:**Interlukin-1β, TGF β: Transforming Growth Factor β.

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INTRODUCTION

Preservation of pulp vitality is of utmost importance in pediatric dentistry because a vital functioning pulp initiates reparative pathway protecting the tooth against bacterial invasion. Therefore, the principal objective in the management of exposed vital pulp of immature young permanent teeth is to create an environment to preserve vitality and eventually continue the root development and apical closure, a process referred to as apexogenesis [1].

Since the evolution of dentistry various pulp capping materials have been developed, and with the better understanding of the dentin-pulp complex repair and regeneration process, advances in biomaterials have emerged for maintaining pulp vitality [2]. The pressing demand to find an ideal pulp capping agent has led to several biomaterials being suggested in the literature, such as mineral trioxide aggregate (MTA), formocresol, ferric sulfate, and materials derived from MTA, such as calcium silicate, calcium phosphate and calcium aluminate-based cements [3].

An ideal pulp capping material should adhere to tooth substrate, maintain a sufficient seal, be insoluble in tissue fluids, non-resorbable, nontoxic, non-carcinogenic, radiopaque, and should exhibit biocompatibility.Accordingly, with the wide variety of biomaterials used for vital pulp therapy, it has become challenging to identify the ideal pulp capping agent for each clinical case, taking into consideration that none of the currently available materials are able to satisfy all the requirements of an ideal vital pulp therapy [2].

In a trial to overcome the limitations of the available dental materials, a shift toward herbal compounds has become noteworthy, one of which is the *Aloe vera* compound [4]. Aloe, nativeto Africa, is well known as "lily of the desert, the "plant of immortality," and the "medicinal plant" [5].

As a consequence to its proven antiseptic and anti-inflammatory properties, *Aloe vera*is used in the treatment of gingivitis and periodontitis. It readily reduces the gingival inflammation and pain associated with it. Clinically proven studies have showed that mouth rinses and dentifrices containing Aloe verahave shown a remarkable reduction in gingivitis and plaque accumulation after its use [6].

Indeed, Aloe verais proved to be an effective anti-inflammatory agent with analgesic properties. Studies have shown that the extracts of this gel have inhibitory action on the arachidonic acid pathway via cyclooxygenase enzyme hence hindering the inflammatory process [7].

Acemannan, one of the main bioactive polysaccharides of Aloe vera [8], has proved successful both clinically and radiographically as a direct pulp-capping material in human primary teeth with deep caries when compared to calcium hydroxide after a 6-months follow up period [9].

Similarly, when compared to formocresol, Aloevera showed an equally effective success rate clinically while a higher success rate radiographically, after a 6-months follow up period, when used as a pulpotomy medicament in primary molars [10].

Owing to the reported clinical and radiographic success of Aloe veraas a pulp capping material in primary teeth, it was tempting to investigate this material as a pulp medicament in young permanent teeth. Accordingly, the purpose of the present study was to examine the histopathological effects of Aloe veraas a pulp-dressing agent in young permanent teeth aiming at introducing a natural, cost-effective, alternative to commercially available pulpotomy agents.

MATERIALS AND METHODS

This pilot study comprised healthy participants aged 9–12 years old with intact newly erupting immature young premolars. Eight teeth, scheduled for extraction for orthodontic purposes, were selected for the study. Two groups were implemented in the

study; Patients in group I was received Aloevera gel as the pulpotomy material while group II received Portland Cement.

Aloe Vera gel preparation

The Aloevera gel was prepared in the laboratory of Pharmacology Department, Faculty of Pharmacy, Cairo University, according to ***Ahmed et al., 2018***[11]**.** Briefly, a healthy leaf of the plant *Aloebarbadensis,* belonging to the family *Liliaceae,* selected from the experimental station of medicinal plants-Giza, was cleaned with 70% ethyl alcohol, and stored in distilled water for 1 h, after which, the outer green portion was removed, and a spatula was introduced to collect the *Aloe vera* gel. A 70% *Aloe vera* mucilage was prepared and preserved in sterile containers by mixing the extracted *Aloe vera* gel with both agar (as a thickening agent) and preservatives including sorbitol, potassium sorbateand sodium meta bisulfite.

Portland cement preparation

White PC (Sinai Cement Company, Egypt) was refined using silk refiner and sterilized using dry heat sterilization according to ***Salama et al***.[12] and***Simon et al***.[13].

Clinical study

This pilot study was conducted in the Pediatric Dentistry Department, Faculty of Dentistry, Cairo University. The research protocol was reviewed and approved by the Research Ethics Committeeprior to the beginning of this study. Parents/legal guardians of the selected participants signed an informed consent form after receiving a detailed information about the study and materials being used including their advantages and disadvantages. The patients or their guardians had the right to decline enrollment and to leave the study at any time.

Study population

Inclusion criteria: Pediatric patients of both genders in an age range of 9-12 years with any immature, clinically and radiographically free, young premolars with two third or more of root completion but with open apex assessed by periapical radiograph, assigned for orthodontic extraction.

Exclusion Criteria: Teeth with traumatic or carious pulp exposure clinically presenting with reversible pulpitis, teeth exhibiting signs and symptoms of irreversible pulpitis or necrosis, along with patients with special needs, medical problems or uncooperative patients who will refuse or hamper the proceedings of the operative procedure.

Pulpotomy Procedure

All clinical procedures were performed by the pediatric dentist (Principal Investigator). Under local anesthesia and rubber dam isolation (split-dam technique), coronal pulp tissue was removed to the level of pulp canal orifice with a sterile diamond round bur in high-speed handpiece followed by copious saline irrigation (**Figure 1a).** Hemorrhage was controlled by direct pressure of moist cotton pellets for 2-3 min [9].

A layer of either Portland Cement (PC) or *Aloevera* at least 3 mm thick was placed over the exposed pulp tissue. The cavity was sealed with glass ionomer resin restoration [12]**(Figures 1b,c).**

Clinical follow-up was carried out at 1 week, 2, 4, 8 and 12 weeks for the presence or absence of pain, tenderness to percussion, gingival inflammation, draining sinus, or mobility. Finally, teeth were extracted after 12 weeks for orthodontic purposes, then decalcified and prepared for histological examination as well as for quantitative analysis of gene expression of Interlukin-1β (IL-1β), Transforming Growth Factor β (TGF β) and Dentin Sialo-Phosphoprotein (DSPP) by real time PCR.

Histo-pathological examination

After extraction teeth were fixed in 10% buffered formaldehyde for 24 hours. The teeth

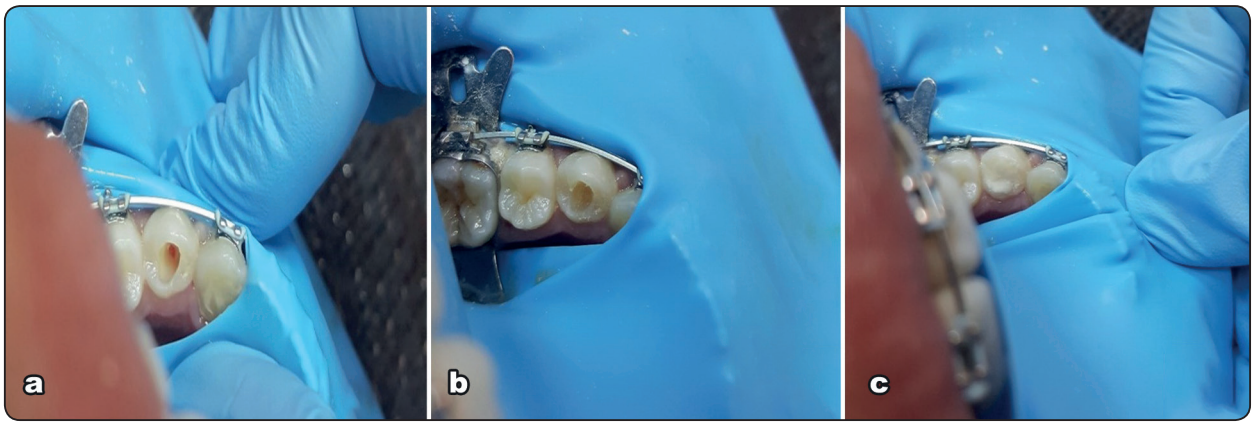

Fig. (1) (a): Removal of coronal pulp tissue (b): Hemorrhage control (c): Application of Portland cement/ Aloe vera gel.

were decalcified using a solution containing 12% Ethylene Diamine Tetra-acetic Acid (EDTA) [14] buffered in pH7.2 PBS for 30 days at 4°C. After complete decalcification, teeth were divided bucco-lingually and were processed, infiltrated in paraffin wax and embedded in the center of wax blocks. The embedded specimens were cut into 5 microns thick sections. Specimens were sectioned and were stained by ***Hematoxylin and Eosin (H&E) stain.*** The sections were examined by light microscope (Trinocular microscope Olympus, BX46).

Gene expression examination

Total RNA extraction:

Teeth from both groups were homogenized where total RNA was extracted with Direct-zol RNA Mini prep Plus (Cat# R2072, ZYMO RESEARCH CORP. USA) and quantity/quality were assessed by Beckman dual spectrophotometer (USA).

Complementary DNA (cDNA) synthesis

Super Script IV One-Step RT-PCR kit (Cat# 12594100, Thermo Fisher Scientific, Waltham, MA USA) was utilized for reverse transcription of extracted RNA followed by PCR. 48-well plate Step One instrument (Applied Biosystem, USA) was used in a thermal profile as follows: 10 minutes at 45ºC for reverse transcription, 2 minutes at 98 ºC for RT inactivation and initial denaturation by 40 cycles of 10 seconds at 98ºC, 10 seconds at 55ºC and 30 second at 72 ºC for the amplification step.

Real-time quantitative PCR (RT-PCR)

After the RT-PCR run the data were expressed in Cycle threshold (Ct) for the target genes and housekeeping gene. Normalization for variation in the expression of each target gene; p21 and p16 was performed referring to the mean critical threshold (CT) expression value of β-actin housekeeping geneby the ΔΔCt method. The relative quantitation (RQ) of each target gene is quantified according to the calculation of $2^{-\Delta\Delta Ct}$ method.

Table (1): Showing the primer sequence of the studied genes

	Primer sequence
IL-1β gene	forward 5′- CCTCGTCCTAAGTCACTCGC-3′ reverse 5′-GCAGAGTCTTTTTGACCCTCCT-3′
TGF-β gene	forward 5′-CCT GGA AAG GGGC TCA ACA-3′ reverse 5′-CCT GGA AAG GGGC TCA ACA-3′
DSPP gene	forward 5′-ATATTGAGGGCTGGAATGGGGA-3′ reverse 5′- TTTGTGGCTCCAGCATTGTCA-3′
β-actin housekeeping gene	forward 5’-CTAAGGCCAACCGTGAAAAG-3’ reverse 5’-GCCTGGATGGCTACGTACA-3’

Statistical analysis

Statistical analysis was performed via Microsoft excel statistical analyzer, AVERAGE, STDEV.P and

P valuecalculated by T-test to compare variables between the two groups. Statistical significance was set at $p \leq 0.05$.

RESULTS

At the end of 12-weeks follow-up, no clinical difference was detected between the studied groups. Both the Aloe Vera and Portland Cement groups showed absence of pain and tenderness to percussion. There were also no signs of gingival inflammation, draining sinus, or mobility.

Histopathological Results

After 12 weeks following the pulpotomy procedure with either Portland cement or Aloevera gel, both materials resulted in comparable histological reactions when premolars were examined using the light microscope.

Aloe vera group

Histological sections of teeth treated with the Aloevera gel showed well organized odontoblast-like cells with proximal nuclei and extended odontoblastic processes arranged near the orifices and along the peripheries of the radicular walls, accompanied by predentin layer and dentin with the classic arrangement of dentinal tubules.

Localized areas of irregular atubular dentin surrounded by odontoblast like cells, some of them were entrapped within the matrix (osteodentin). These islands were found near the orifices of the canal, also remnants of the Aloevera gel were noticed around them.

Moreover, central core of the pulp canal tissues showed spindle form fibroblasts and mild inflammatory cells infiltration, no signs of necrosis were revealed. Around the apical portion of the roots, dilated blood vessels, fibroblasts and undifferentiated mesenchymal cells were observed, encircled by collagen fibers and some inflammatory cells.

Control group

Similarly, specimens of the Portland cement treated teeth showed well-arranged odontoblastic-like cells near the orifices while those along the peripheries to the apical area appeared scattered and less organized. Dentinal tubules appeared with their usual spiral track, atubular reparative dentin layer inbetween them and predentin.

Very small, mineralized dentin islets were shown near the orifices and in the central core along with mild inflammatory cells and scattered fibrosis with no signs of necrosis. Some specimens showed remnants of the material especially near the orifices.

Gene expression Results

The quantitative analysis of IL-1β gene expression of the Aloevera group was 0.889 ± 0.433 compared to 0.784 ± 0.137 for the control group. The calculated P-value between both groups showed no statistically significant difference ($p=0.598$). On the other hand, analysis of the TGF- β showed an average of $1.805 \pm 0.195$for the intervention group which was found higher than the control (average = 0.593 ± 0.182) albeitthat difference was still statistically insignificant (P value = 1.633). The results of the DSPP showed an average of 3.179 ± 0.328 for the Aloe vera samples which were markedly higher than the control specimens averaging 1.689 ± 0.090, with also a statistically insignificant difference in the specimens of both groups according to the calculated P value of 7.534.

TABLE (2): The average gene expressions of IL-1β, TGF-β and DSPP of studied groups.

	IL-1β	TGF-β	DSPP
Control group	0.784 ± 0.137	0.593 ± 0.182	1.689 ± 0.090
Intervention group	0.889 ± 0.433	1.805 ± 0.195	3.179 ± 0.328
P-value	0.598	1.633	7.534

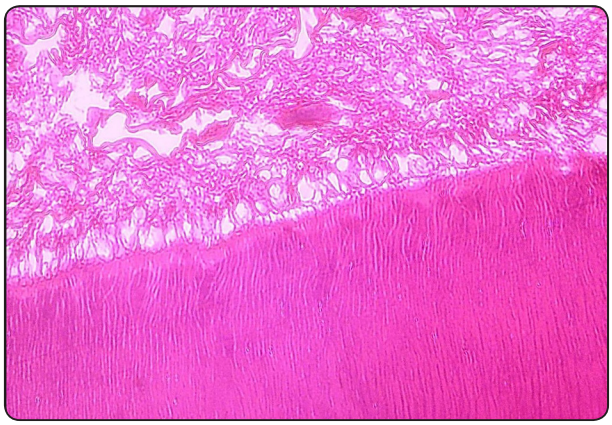

Fig. (2): Photomicrograph of specimens treated with the Aloevera gel showing well organized odontoblast like cells, with proximal nucleiand extended odontoblastic process (black arrows), accompanied by predentin layer (white arrows) and dentinal tubules(stars). H&E x200

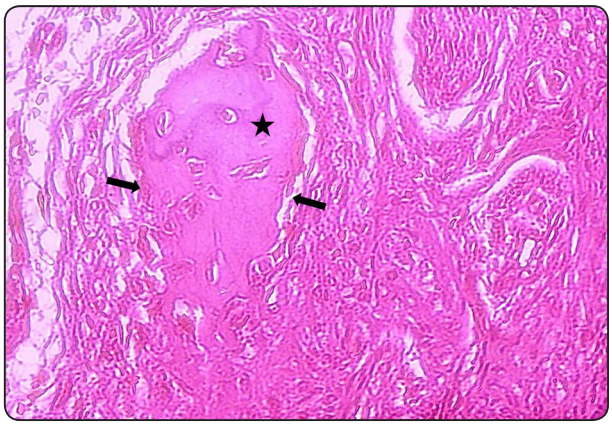

Fig. (3): Photomicrograph of specimens treated with the Aloevera gel showing Osteodentin (star) surrounded by odontoblast like cells(black arrows). H&E x200.

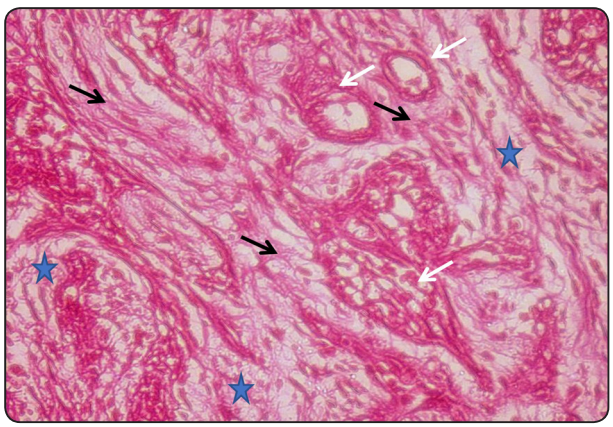

Fig. (4): Photomicrograph of the Aloevera group showing dilated blood vessels (white arrows), fibroblasts and undifferentiated mesenchymal cells, as well as some inflammatory cells (stars) and collagen fibers (black arrows).H&E X400.

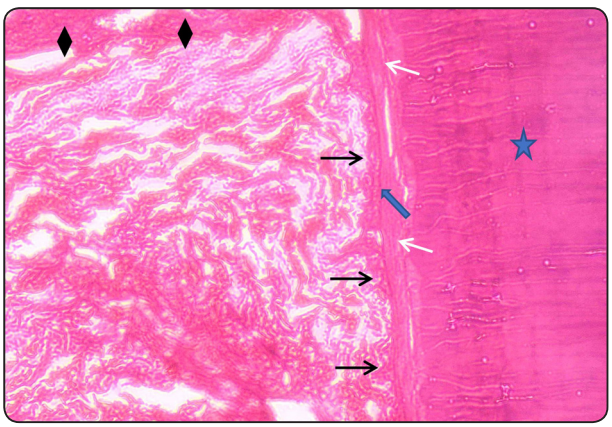

Fig. (5): Photomicrograph of the Portland cement specimens showing scattered and less organized odontoblastic-like cells along the peripheries and the apical area (black arrows), dentinal tubules appeared with their usual spiral track (star), a tubular reparative dentin layer(white arrows) in between them and predentin(blue arrow). Small, mineralized dentin islets were also shown near the orifices and in the central core (diamonds). H&E x200.

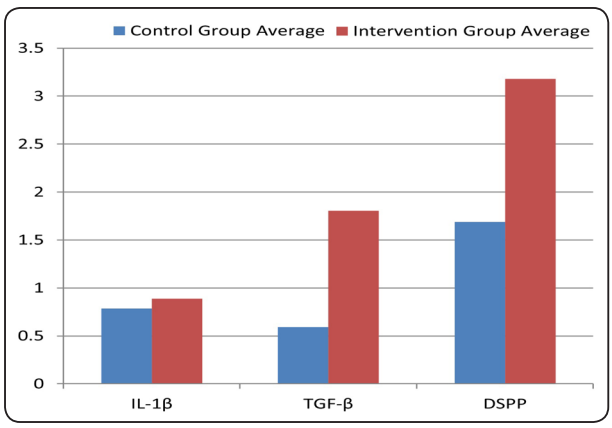

Fig. (6): A bar chart showing quantitative averages of IL-1β, TGF-β and DSPP of boththe Intervention group (Aloevera) compared to the control group (Portland Cement).

DISCUSSION

The main objective of pulp therapy of young permanent teeth with immature roots is to conserve the integrity and health of the teeth and their supporting tissues while maintaining the vitality of the pulp of the affected tooth. In order to keep this permanent tooth for long period of time, it requires a root with a favorable crown/root ratio and dentinal walls that are thick enough to withstand normal function. Therefore, pulp preservation is a primary goal for treatment of the young permanent dentition [15].

The usage of biocompatible medicinal plant has become a major concern in modern dentistry, especially when it comes in direct contact with the vital dental tissues. Aloe vera is one of the herbal natural materials; it possesses anti-inflammatory, antibacterial, antifungal, antiviral, moisturizing and pain-relieving properties. Acemannan is a major bioactive polysaccharide of Aloe vera gel, it has been recognized for its compatibility and healing potential [16], [17].

Aloe vera gel was found to promote new dentine formation by stimulating dental pulp cell proliferation, differentiation, extracellular matrix formation and mineralization in rats [18]. Ashistopathological examination has long been recommended as the best method to evaluate the effectiveness of a biomaterial at the cellular levels [19], the purpose of this study was to investigate the effect of Aloe vera gel as pulp dressing material compared with the commonly used Portland cement on dentin-pulp complex of young permanent teeth histologically and with quantitative analysis of IL-1β, TGF-β and DSPP gene expressions.

In the current study, the histological results revealed well-arranged odontoblast like cells near the orifices of both groups, cells along the peripheries of the radicular walls and the apical part appeared more organized in the Aloe vera group compared to the control group. Localized areas of irregular atubular dentin (osteodentin) were observed in most specimens of the aloe vera group, while small, mineralized islets were shown in the control group. Inflammatory cells infiltrations and dilated blood cells were evident in the studied groups, fibroblasts, collagen fibers and undifferentiated mesenchymal cells as well, although slightly higher in the Aloe vera group. Remnants of both materials were noticed in specimens and no signs of necrosis were detected in the studied groups.

The clinical and radiographic success of Aloe vera as a direct pulp capping or as a pulpotomy material on primary teeth have been stated by many studies [18],[19]. Also the histological studies of the Aloe vera in primary teeth or in animal models showed proliferation of pulp cells, odontoblastic like cells differentiation and organization, dentin formation and mineralization [18],[20]. In accordance with previous studies on primary teeth, the present work, on young permanent teeth, showed that aloe vera promoted pulp cell differentiation, proliferation and enhanced new dentin matrix formation and mineralization. The vitality of the pulpal tissues and mild inflammatory cells infiltration indicates the biocompatibility and the anti-inflammatory properties of the Aloe vera gel.

IL-1β is a proinflammatory cytokine that can be produced and released by many different cell types making it one of the most important interleukins in the study of pulp inflammatory process [21]. The quantitative analysis of IL-1β gene expression in the present study was found to be 0.889 ± 0.433 in the Aloe vera group while the average of the control group was 0.784 ± 0.137 and the calculated P-value was 0.598 which denoted statistically non-significant difference between the Aloe vera and control groups. Therefore, it could be postulated from the current research that Aloe vera resulted in negligible elevation in the level of IL-1β, thus confirming the high biocompatibility of the material.

In the current study, the average TGF-β expression of Aloe vera group was found to be

almost double the average of the control group, albeit the difference is statistically insignificant. These resultssuggestan anti-inflammatory effect of the Aloe vera material as well as a tendency for cell proliferation regulation and human dental pulp repair. Indeed,it has been stated that TGF-β is a potent regulator of pro-inflammatory responses and defensive reactions in dentin–pulp complex, in additionit is involved in regulating odontoblast-like cell migration, proliferation and extracellular matrix production, subsequently enhancing tertiary dentinogenesis [22],[23].

Dentin sialophospho protein (DSPP) is the most abundant non-collagenous protein in the dentine, it acts as an indicator of odontoblastic differentiation as it is expressed by odontoblast-like cells underlying the reparative dentine, furthermore, it has a crucial role in hard tissue mineralization [24],[25]. Even though DSPP expression between groups of the current study was statistically non-significant, which could be attributed to the small sample size, but its expressionin the Aloe vera samples was markedly higher than the control specimens. This could explain the slight increase in the number of well-organized odontoblastic-like cells in the Aloe vera specimens and consequently the relatively higher amount of dentin matrix formation and mineralization.

CONCLUSIONS

Based on the results of the current pilot study, Aloevera gel proved comparable to the control group as a pulp capping materialin pulpotomy of young permanent teeth. However, randomized controlled trials using the material with appropriate sample size and longer follow-up duration is further needed to ensure the efficacy of Aloevera gel.

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Conflict of Interest

The authors declare no conflict of interest.

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