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Original Article

A histopathological comparison of formocresol, propolis, and growth factor as pulpotomy medicaments in primary teeth: An *in vivo* study

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ABSTRACT

Background: Pulpotomy is the most common pulp treatment of primary molars, where surgical amputation of infected coronal pulp results in preserving the vitality and function of radicular pulp. With introduction of newer materials, the emphasis has shifted towards regeneration, in this scenario; novel materials such as platelet derived growth factor (PDGF) and propolis (PS) have been considered.

Materials and Methods: This was a single-blind *in vivo* study; ninety human primary teeth from children aged between 5 and 10 years were divided into three equal groups in whom pulpotomy procedure was performed and they were recalled after 3- and 6-month interval for histological evaluation. Observations were subjected to statistical analysis using Pearson's Chi-square test.

Results: No statistically significant difference was found between the three materials with respect to inflammatory response, soft-tissue organization, and dentin bridge formation ($P > 0.05$). Majority of the samples in both growth factor and propolis exhibited dentin bridges at the interface of the exposed pulp, bringing or attempting to bridge the site exposed to the pulpotomy material. The ability of the material to evoke a foreign and inflammatory cell response in the pulpal tissue was not significant. The samples of both formocresol and growth factor group showed signs of pulpal necrosis which revealed the presence of a mild necrotic zone in one specimen at 3 months. One specimen from the propolis group showed mild areas of necrosis at the end of 6 months, where none of the specimens in the growth factor group showed areas of necrosis at the end of 6 months.

Conclusion: The results of the present study showed a positive outcome for growth factor and propolis groups. Further clinical trials with a larger sample size and long-term review have to be conducted for the material to be used widely.

Key Words: Formocresol, growth factor, primary molars, propolis, pulpotomy

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INTRODUCTION

Pulpotomies in primary teeth continue to be one of the most common treatments in pediatric dentistry. The main objective of pulpotomy is to maintain radicular pulp asymptomatic without adverse clinical signs or symptoms such as sensitivity, pain, or swelling.[1] Only in this way could early root resorption be preserved and teeth enter into

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exfoliative process at the appropriate time.[2] Hence, the ideal requisites of any pulpotomy material should be bactericidal, be harmless to pulp and surrounding structures, promote healing of remaining radicular pulp without interfering with the physiologic root resorption, and should not possess any toxicity.[3]

Conventionally, formocresol is the material of choice for pulpotomy procedure because of its ease in usage and proven clinical excellence. Buckley's formocresol was first introduced as a medicament in 1904 which consists of 19% formaldehyde, 35% cresol, in a vehicle of 15% glycerin, and water.[4,5] It is regarded as the "gold standard" and is being used for the past 80 years. However, the use of formocresol has been challenged because of its deleterious effects, potential carcinogen in action, immune sensitization, mutagenicity, and cytotoxicity.[3,6,7]

Many medicaments have been introduced as an alternative to formocresol for pulpotomy which include glutaraldehyde, ferric sulfate, calcium hydroxide, mineral trioxide aggregate, bone morphogenic proteins, enamel matrix derivatives, freeze dried bone, growth factors, and various techniques such as electrosurgery and lasers have been tried with variable clinical, radiological, and histological success.[2,8] Henceforth, to maintain more vital radicular tissue in primary teeth, natural products are in their way to overcome the disadvantages of the available pulpotomy medicaments. With this regard, newer biological products such as propolis and aloe vera are being considered.[9,10]

Exogenous application of growth factor into the injured pulp may reduce the inflammatory response, hasten tissue regeneration, and lead to the deposition of mineralized dentin of physical quality.[11]

The aim of the study was to assess the histological success of formocresol, propolis, and growth factor as pulpotomy medicaments in primary teeth.

MATERIALS AND METHODS

Study population

Pulpotomy was done in 90 primary molars in 75 children who were randomized into groups As follows: FC group (n = 30), propolis (n = 30), and growth factor (n = 30). After applying the inclusion and exclusion criteria, the teeth that were excluded from the study were those with tenderness to percussion, mobility, fistulation or any signs and symptoms indicating extensive, pulpal, and periapical involvement, and medically compromised children. Pulpotomy was done in 90 primary molars in 75 children aged between 5 and 10 years old participated in the study. They were selected from the outpatients of a pediatric dental clinic. The collection of sample started from May 2015 and the follow-up period ended in May 2018. A minimum sample size of 90 (30 in each group) was determined using Epicale program version 1.02 (1979 Milkyway, Verona, Wisconsin). Assuming a power of 80% and alpha = 0.005 and was based on the number of teeth with possible radiographic success failure.[12,13]

Patient selection criteria

1. Absence of systemic diseases such as bacterial endocarditits, kidney disease, leukemia, diabetes, neutropenia, and bleeding problems
2. Absence of any type of medical treatment or continues use of any medication
3. Absence of drug allergies, anesthetics, and environmental allergies[12,13]
4. Patients' compliance with the treatment.

Tooth selection criterion[14]

Teeth with no clinical or radiographic pulpal degeneration.

Clinical selection criteria

1. Teeth with deep decay lesions and no symptoms
2. Teeth with vital pulp exposed by decay; no spontaneous pain; and absence of edema, pain, and fistula[12,13]
3. Absence of sensitivity on percussion
4. Teeth with manageable pulpal hemorrhage.

Radiographic criteria

1. Teeth with Code 3 decay (radiographic spread to 1/3rd of the dentine) and Code 4 decay (radiolucency spread to 1/3rd of the pulp) according to the Codes for Decay Lesion Grade and Severity used by Ekstrand *et al.*[15]
2. Teeth with no pathological root resorption
3. Teeth with no periradicular or furcal radiolucency
4. Teeth with <1/3rd physiological root resorption (no resorption or ¼ resorption of the root).[16]

The randomized clinical trial was reviewed and approved by the Institutional Human Ethical Committee. The procedure and the possible discomforts or risks versus benefits were explained to the subjects and their parents/guardians, and informed consent was obtained prior to the initiation of the CONSORT 2010 Statement: Updated guidelines

for reporting parallel group randomized trials. Only parents and patients who signed the consent and accent form respectively were included in the study.[17]

Materials

The following three pulpotomy medicaments were used in this study: formocresol (Sultan Health Care, Inc., 1301 Smile Way, York, PA 17404-087, USA), propolis (Eedorian Rainforest LLC, USA), and growth factor (PLERMIN-Reddy labs, Hyderabad). The pulpotomy procedure was done on the lower right and left first molar and second molar for accessibility and accuracy. The tooth was anesthetized with 2% lidocaine (Lignox) 1: 80,000 adrenaline, and rubber dam (Instadam, USA) isolation was obtained. Soft debris, caries, and unsupported enamel and dentin were removed with a spoon excavator before opening the pulp chamber. Caries removal and coronal access was made using a ≠ 245 bur (DENTSPLY, USA) using a high-speed hand piece. The coronal pulp was removed with a small sharp spoon excavator.

The pulp chamber was then irrigated with saline to remove all debris. Hemostasis was obtained with a sterilized moistened cotton pellet gently pressed against the amputated pulp stumps in all the three groups. If hemostatis could not be achieved, a pulpotomy or extraction was performed, and the tooth was excluded from the study.

All radiographs were taken with size 0 ultra-speed dental film (Eastman Kodak Co, Rochester, NY, USA) using a planmeca prostyle intra X-ray unit (Helsinki, Finland) set at 70 KV, 8 Ma with an exposure time of 0.32 s; patients were fitted with a lead apron and thyroid collar film was positioned intraorally with a Rinn Snap-A-Ray (DENSPLY Rinn snap, IL, USA). An immediate postoperative intra-oral periapical radiograph was taken after the completion of the procedure in the three groups.

In the formocresol group (control group) (Sulthan Health Care, Inc., 1301 Smile Way, York, PA 17404-0807, USA) (after achieving the complete hemostasis, the formocresol cotton pellet was squeezed and dried and 1/5th dilution of Buckley's formocresol was placed on the amputated pulp stumps for 1 min. After removing the cotton pellet, the remaining pulp chamber was filled with intermediate restorative material (IRM) (L. D caulk) thick paste.

In the propolis group (Ecudorian RainForest LLC, USA), after achieving the complete hemostasis, propolis powder was mixed with titanium dioxide powder in the ratio of 2:1[18] to achieve slight radio opacity to the material which can be appreciated on the radiographs. The powder was mixed with 70% ethyl alcohol to a thick consistency on a paper pad with the aid of a cement spatula which was carried directly to the pulp chamber with a plastic filling instrument and placed onto the pulp stumps. The remaining pulp chamber was filled with IRM thick paste.

In the growth factor group, after achieving complete hemostasis, the growth factor gel was mixed with dry collagen powder[19] to obtain a thick consistency on a glass slab with the aid of a plastic spatula which was then carried directly to the pulp chamber with a plastic filling instrument and placed onto the pulp stumps. Over this, a thin dry resorbable collagen membrane was placed to act as a barrier from the coronal restoration. The remaining pulp chamber was filled with IRM for proper strength.

Only teeth undergoing serial extraction for the interceptive orthodontics purpose were taken for histological assessment.

Tissue processing

Dehydration was done in ascending grades of alcohol – 50%, 70%, 90%, and 100% for 1 h in each solution and cleared with xylene solution for 30 min. After processing, the tissue was embedded in paraffin wax solution for 30 min. After processing, the tissue was embedded in paraffin wax and sections 4–5 μ were obtained using soft-tissue microtome.

Outcome of histopathological assessment

The photomicrographic sections were evaluated by a histopathologist (H. *P* No. GF5/14, Dental College) using the following criteria given by Cox *et al*.[20] The final sample were reevaluated by an examiner (unware of the study design) to avoid any bias. The histological features evaluated were odontoblastic integrity, pulp inflammation, pulp calcification, dentin bridge formation, and presence of pulp stone.

Statistical analysis

Statistical analysis of the results for the histological evaluation between the three groups was made using Pearson's Chi-square test and by using Microsoft Excel software (IBM company) (SPSS-17.0) (SPPSS-17.0) (SPSS Inc,Chicago IL,USA).

RESULTS

Table 1 represents samples available for follow-up, giving a total sum of 25 (FC) and 26 (each in propolis

Table 1: Sample available for follow up

Medicament	3 months		6 months	
Formocresol	30	26	30	25
Propolis	30	27	30	26
Growth factor	30	27	30	26

Table 2: Percentage of sample loss

Medicament	3 months	6 months
Formocresol	4 (13.3%)	5 (16.67%)
Propolis	3 (10%)	4 (13.3%)
Growth factor	3 (10%)	4 (13.3%)

and growth factor groups) subjects at 6-month interval.

The percentage of sample loss was 16.67 for formocresol, 13.3 in propolis, and 1.3% in growth factor group at the end of 6-month interval as shown in Table 2.

The results of histological evaluation of samples obtained are listed in Table 3. A total of 15 teeth were subjected to histological evaluation, with 5 teeth in each group. The parameters and scoring criteria were according to the system proposed by Cox *et al* 1996[20] [Table 4].

The histological evaluation demonstrated formation of dentin bridge in the growth factor and propolis groups, which was continuous and thick with odontoblastic layer, at both 3- and 6-month intervals [Figures 1b, 2a,b and 3a,d], while the formocresol group did not reveal formation of dentin bridge at both time periods [Figure 3c].

Regarding the inflammatory cell infiltrate, all the three groups [Figures 2a, b and 3b] showed chronic, diffuse, minimal chronic inflammatory cell infiltrate predominantly of lymphocytes (Grade 2) in specimens extracted after 3 months. After 6 months, specimens in the formocresol group continued to showed chronic diffuse, minimal inflammatory cell infiltrate [Figure 1e], whereas specimens of propolis and growth factor groups [Figures 2b and 3d] showed normal pulp tissue with the presence of abundant collagen bundles and fibroblasts adjacent to the odontoblast layer [Figures 2a and 3a].

One of the specimens each from the formocresol and propolis groups which were extracted after 6 months showed single pulpal calcification at the center of the radicular pulp [Figures 1d and 2c].

These findings with respect to pulpal necrosis revealed the presence of a mild necrotic zone at the coronal third of the radicular pulp in one specimen at 3-month interval from the formocresol and growth factor groups [Figure 1a and 3c]. One specimen from the propolis group showed mild areas of necrosis at the end of 6 months [Figure 2d], whereas none of the specimens in the growth factor group showed areas of necrosis at the end of 6 months.

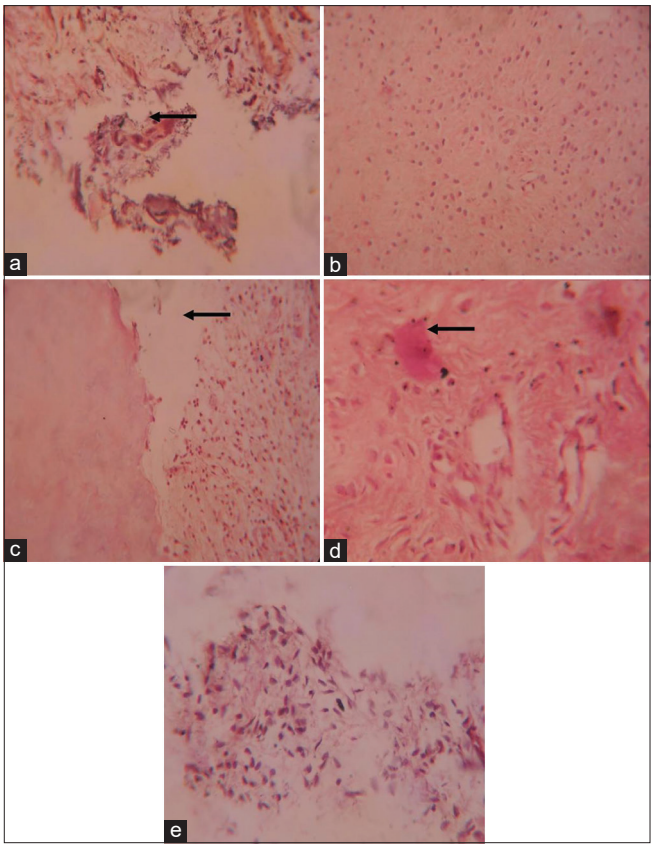

Figure 1: Histological photomicrograph of formocresol group. (a) Photomicrograph ×40– Areas of mild necrosis in radicular pulp at 3 months. (b) Photomicrograph ×40– area of mild chronic inflamatory cell infiltrate predominantly composed of lymphocytes at 3 months. (c) Photomicrograph at ×40 – no evidence of dentin bridge formation at 6 months. (d) Photomicrograph at ×40. Pulp calcifications are found as isolated discrete masses in radicular pulp at 6 months. (e) Photomicrograph at ×40 showing chronic, minimal inflamatory cell infiltrate composed predominantly of lymphocytes at 6 months.

DISCUSSION

A recent study done on platelet-derived growth factor (PDGF-BB) on its dentin pulp tissue regeneration ability proved its successful enhancement in neoangiogenesis, dentin production, and enhancement of human dental pulp stem cell proliferation and odontoblastic differentiation.[21]

Table 3: Results of Histological evaluation of samples

Material	Time Interval	Total teeth	Inflammatory response (codes)				Dentin Bridge formation (scores)			Pulp necrosis		Pulp calcification	
			1	2	3	4	1	2	3	Present	Absent	Present	Absent
Formocresol	3 months	3	2	1	0	0	0	0	3	3	0	2	1
	6 months	2	0	2	0	0	0	0	3	0	2	0	2
Propalis	3 months	3	1	2	0	0	2	1	0	0	3	0	3
	6 months	2	2	0	0	0	2	0	0	1	1	1	1
Growth factor	3 months	3	2	1	0	0	2	1	0	0	3	0	3
	6 months	2	2	0	0	0	2	0	0	0	2	0	2

*P**>0.05(not significant).No statistically significant difference was found between the study groups respect to inflammatory response , dentin bridge formation, pulp necrosis, and pulp calcification

Table 4: Histological Criteria Cox *et al*.[20]

Scores	Inflammatory Response
1.	None or a few scattered inflammatory cells present in the pulp beneath the exposure site.
2.	Polymorpho nuclear leukocytes (acute) or mononuclear lymphocytes (chronic) in an inflammatory lesion.
3.	Severe inflammatory lesion appearing as an abscess or dense infiltrate involving one third or more than the coronal pulp.
4	Completely necrotic.
	Dentinal Bridge Formation
1	New barrier tissue directly adjacent to some portion of the restorative material.
2	New dentin bridge formation some distance from the material interface
3	No evidence of any dentin tissue formation in any of the tissue sections

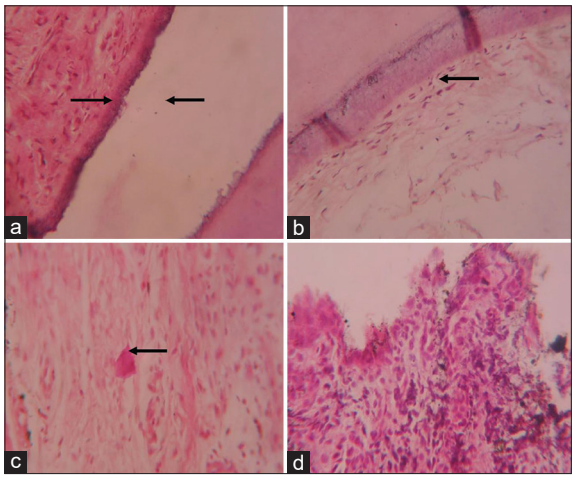

Figure 2: Histological photomicrograph of propolis group. (a) Photomicrograph ×40– dentinal bridge formation between propolis and pulp chamber with intact odontoblastic layer, abundant fibroblasts, and collgen bundles adjacent to odontoblastic layer with minimal inflamatory infiltrate at 6 months. (b) Photomicrograph ×40 – dentinal bridge formation between propolis and pulp chamber with intact odontoblastic layer and no inflamatory cells at 6 months (c) Photomicrograph-×40 – pulp calcifications are found as isolated discrete masses in radicular pulp at 6 months. (d) Photomicrograph ×40 – areas of mild necrosis at the radicular pulp at 6 months.

In a study which evaluated the propolis effect on the formation and activation of osteoclasts cells, it was shown that propolis effectively reduces bone loss. Propolis decreases the number of giant cells, positive TRAP (Tartrate Resistant Acid Phosphatase), and has an inhibitory effect on the initial phase of osteoclastogenesis. This inhibitory effect is dose dependent.[22] Propolis increases osteoprotegerin expression and decreases the number of osteoclasts, therefore inhibiting osteoclastogenesis.[23]

To our knowledge, the present study may be the first study comparing formocresol with propolis and growth factor as pulpotomy agents in primary teeth. Previous studies have employed several biocompatible materials such as bone morphogenic protein, emdogain gel, platelet-rich fibrin, and platelet-rich plasma, which releases growth factors after certain period of time, but direct application of PDGF in deciduous teeth was done in the present study yielding satisfactory results histopathlogically.

With respect to the histopathological evaluation, the samples in formocresol group revealed mild areas of necrosis of radicular pulp at 3 months, which is in support to Berger (1965)[24] who reported complete loss of vitality with fibrous granulation tissue in the apical part of the radicular pulp. None of the samples were observed for the formation of dentin bridge as reported by Fuks *et al*.[25] Sarkar *et al*.[26] and after 2, 6, and 9 months. In accordance to the study conducted by Srinivasan and Jayanthi,[27] we could notice the presence of single, isolated pulpal calcifications at the center of the pulp in one sample of formocresol group, which is due to excessive odontoblastic activity. Mild-to-moderate inflammation (Grade 2) was observed in almost all the samples, which is in accordance to previous studies.[25-27]

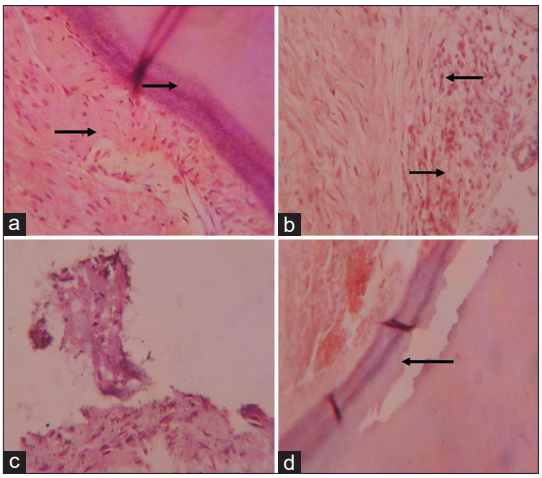

Figure 3: Histological photomicrograph of growth factor group. (a) Photomicrograph at ×40 showing dentinal bridge formation between growth factor and pulp chamber with intact odontoblastic layer, abundant fibroblasts, and collagen bundles adjacent to odontoblastic layer and minimal inflamatory infiltrate composed of lymphocytes at 3 months. (b) Photomicrograph ×40 showing area of mild chronic inflamatory cell infiltrate predominantly composed of lymphocytes and with numerous dilated blood vessels at 3 months. (c) Photomicrograph ×40 – areas of mild necrosis in radicular pulp at 3 months. (d) Photomicrograph ×10 – dentinal bridge formation between growth factor and pulp chamber with intact odontoblastic layer and no inflamatory cells at 6 months.

Propolis samples revealed a very few and scattered inflammatory cells in specimens at the end of 3 months, which is in accordance with the study done by Abhishek *et al*.,[28] who reported few scattered inflammatory cell infiltrate in 33% of the samples after 45 days. The flavonoids and caffeic acid present in propolis are known to play an important role in reducing the inflammatory response by inhibiting the lipoxygenase pathway of arachidonic acid suppression of immune cell activation, macrophage-derived nitric oxide, and cytokine production and neutrophil activation. Alternatively, flavonoids inhibit the bacterial growth in pulp chamber, thereby reducing the host response to bacterial antigens[29] in contrary to previous studies.[28,30]

Two samples in the present study showed mild areas of necrosis and isolated pulpal calcifications at 6-month interval. The previous histological finding suggests that flavonoids from propolis stimulate reparative dentinogenesis. Dentin formation is known to involve differentiation of odontoblast-like cells that form reparative dentin and biosynthetic activity surrounding primary odontoblasts. Ansorge (2003) reported that propolis is capable of stimulating the production of *transforming growth factor*–β1, known to be important for odontoblast-like cell differentiation.[31] Ahangari *et al*. documented that propolis plays crucial role in the stimulation of dental pulp stem cells resulting in dentin regeneration.[32] All these mechanisms might have contributed in the formation of continuous and thicker dentin bridge in the present study, at the end of 3 and 6 months.

These observations were similar to Sabir *et al*.[29] Abhishek *et al*.,[28] Lima *et al*.,[33] and Ozorio *et al*.,[34] who also reported the formation of dentin bridge at the end of 1 month.

The stimulation of various enzyme systems, cell metabolism, circulation, and collagen formation could have also contributed to the hard tissue bridge formation by propolis. These effects shown were thought to be as a result of the presence of arginine, Vitamin C, provitamin A, B complex, and trace minerals such as copper, iron, zinc, as well as bioflavonoids. All these factors assist in faster healing of the wound.[28]

Histological observations of growth factor group confirmed the presence of intact odontoblastic layer in all samples as PDGF is a potent mitogen during wound repair and promotes cell aggregation by chemotaxis. These properties may have encouraged fibrous tissue repair over a calcified barrier, and also it stimulates pulp to differentiate into odontoblast to deposit a layer of dentin.[32] Minimal inflammatory cell infiltrate (Grade 2) was noticed in 3-month sample along with hyperemic pulp, few scattered chronic inflammatory cell infiltrates predominantly lymphocytes, and numerous dilated blood vessels, suggesting angiogenesis, which is in support with the findings of Hu *et al*.[11] and Denhalam *et al*.,[35] who reported localized vascular dilation, neoangiogenesis inflammatory cell infiltration, and uniform dentin bridge barrier in their study.[35,36] Sheridan *et al*.[36] Peters *et al*.,[37] Sun *et al*.,[38] and Stiver *et al*.[39] also considered the use of angiogenic factors such as PDGF and vascular endothelial growth factor, which enhances and accelerates the pulp angiogenesis. Interestingly, none of the samples showed inflammation or necrosis at the end of 6 months, therefore proving this material to be biocompatible.

CONCLUSION

Comparing the three medicaments used in the present

study, the histological success rates obtained were more favorable for both growth factor and propolis groups, whereas the formocresol group showed more failure rate. When assessed individually, the results obtained are evident to prove growth factor and propolis as better suitable pulpotomy agents in primary teeth. Due to the devitalization nature of formocresol, more number of cases resulted in failure.

Further, there was a relative superiority of growth factor group regarding the overall success rate; however, the results showed no significance statistically when compared with propolis.

Limitations

As the present study observed only ninety carious teeth after 3 and 6 months, more experimental data and further human research with larger sample size and longer follow-up period and studies on caries exposed teeth are recommended to conclusively prove the efficacy of propolis and growth factor including a favorable pulpal response.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real, or perceived, financial or non-financial in this article.

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