

Histological Evaluation of Pulpotec Effect on Pulpotomized Primary Teeth in Puppies

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Abstract

Aim: The present study was conducted to histologically evaluate the inflammatory response of dental pulp to Pulpotec versus Formocresol in primary teeth of puppies.

Methods: The study was conducted on 24 primary molars of three Mongrel puppies between the ages of six to ten weeks. The mouth of each puppy was divided into two halves; the teeth on the left half were treated using the Pulpotec while those on the right half were treated with the Formocresol. Puppies one, two and three were sacrificed performing the pulpotomies at two, four and six weeks, respectively. The portions of the jaws including pulpotomized teeth were carefully sectioned, stained with hematoxylin and eosin (H & E) and examined histologically by light microscope.

Results: The results of the two groups showed pulp inflammation that varied from mild, moderate to severe, hydropic degeneration, increased fibrosis, capillary dilatation, disruption of odontoblastic layer and internal root resorption that started earlier in the Formocresol group.

Conclusion: It has been concluded that both groups showed comparable unfavourable histological response.

Key Words: *Pulpotec, Formocresol, Pulpotomy, Histological Evaluation*

Introduction

The aim of vital pulp therapy is to treat reversible pulpal injuries in both permanent and primary teeth maintaining pulpal vitality and function. In addition, in primary teeth it is important to preserve the tooth until its natural exfoliation time, thus preserving arch integrity [1]. Pulpotomy is still the most common treatment for carious exposed pulp in symptom free primary molars [2]. Formocresol (Fc) has been a popular pulpotomy medicament in the primary dentition for the past 70 years since its introduction by Sweet [3]. It is still considered the most universally taught and preferred pulp treatment for primary teeth [4-7]. Concerns have been raised over the use of Fc in humans mainly as a result of its toxicity and potential carcinogenicity [8,9]. Thus, it is no longer used in some countries as result of safety concerns [2]. The international agency for research on cancer classified formaldehyde as carcinogenic for humans in June 2004 leaving the profession to look for other alternatives to Fc [10]. On the other hand, several studies found no correlation between Fc and human carcinogenicity. The human body is physiologically equipped to handle Formaldehyde through multiple conversion pathways. Milnes [11] in his review aimed to examine more recent research about formaldehyde metabolism, pharmacokinetics, and carcinogenicity. These results indicated that formaldehyde is probably not a potent human carcinogen under low exposure conditions. Extrapolation of these research results to pediatric dentistry suggests an inconsequential risk associated with formaldehyde use in pediatric pulp therapy. Nevertheless, several studies have reported that the clinical success of Fc Pulpotomy decreases with time, and the histological response of the primary pulp is "capricious" ranging from chronic

inflammation to necrosis [12]. Presently Pulpotec, a filling paste which contains polyoxymethylene, iodoform, dexamethasone acetate, formaldehyde, phenol, gaiacol and excipient, has been introduced to eliminate the risk of root canal infection. It can be used for simple, rapid, long term treatment of vital primary and permanent molars. The addition of pharmacological constituents ensures an aseptic treatment and induces cicatrization of the pulp stump at the chamber-canal interface whilst maintaining the structure of the underlying pulp. The composition of Pulpotec has been improved in a very large extent when compared to old Buckley's formula and its formaldehyde content has been dropped by more than 50% in order to take into account the result of the toxicological studies conducted [13]. The synergetic action of other ingredients in the Pulpotec cement like dexamethasone, a potent synthetic member of the glucocorticoid class of steroid drugs has an anti-inflammatory and immunosuppressant property [14]. Phenol has anti-inflammatory, antiviral, antibacterial, and anti-carcinogenic properties [15]. Iodoform; a pale yellow, crystalline, volatile substance used as a disinfectant makes it more patient friendly [16]. As long as very few studies have been published on this material, there is lack of evidence to conclude if it is an appropriate substitute to Fc for pulpotomy in primary teeth. Therefore, the purpose of this study was to evaluate the pulpal inflammatory response to Pulpotec versus Formocresol on pulpotomized primary teeth in puppies.

Materials and Methods

Sample

The study was conducted on 24 primary molars of three Mongrel puppies between the ages of six to ten weeks.

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Materials

- Formocresol: Buckley's Formula, 19% formaldehyde, 35% cresol, 17.5% glycerine. (Sultan Chemists, NJ, USA).
- Pulpotec: powder (polyoxymethylene, Iodoform, excipient) and Liquid (Dexamethasone Acetate, Formaldehyde, Phenol, Gaiacol, excipient) (Swiss quality dental product).
- Ketamine hydrochloride (Parke -Davis India).

Methods

Adequate measures were taken to minimize pain or discomfort to the experimental puppies. Each dog was anesthetized with ketamine hydrochloride, using a dose of 50 mg/kg body weight. Injection was done by intramuscular administration. The crowns of the canines were cut horizontally at the middle third with a fissure bur at low speed. The pulps of the molars were exposed through an occlusal preparation made with a round bur at low speed. Normal saline was used as both an irrigant and coolant during the preparations. The mouth of each dog was divided into two halves; the teeth on the left half were treated using the Pulpotec while those on the right half were treated with the Formocresol [17].

Pulpotomy using Pulpotec: After the coronal pulp was removed with a slow speed round bur and a sharp excavator, homeostasis was achieved using moist sterile cotton pellets for 15-30 sec, the Pulpotec powder and liquid were mixed to obtain the required thick, creamy consistency of the paste (One scoop of powder; one drop of liquid). The paste was inserted into the pulp chamber.

Formocresol pulpotomy (control group): After the coronal pulp was removed with a slow-speed round bur and a sharp excavator, homeostasis was achieved using moist sterile cotton pellets for 15-30 sec, and then a five minute application of Buckley's Formocresol with a moistened cotton pellet was done. The cotton pellet was removed of excess Formocresol by blotting it in a gauze square prior to placement over the pulp stumps. Reinforced zinc oxide eugenol was used to restore the teeth.

Postoperatively, the animals of the two groups were kept in the animal house, Faculty of Dentistry, Pharaos University. They were put on a balanced diet, which consisted of meat, milk, and bread with broth until the time of sacrifice. Puppies 1, 2 and 3 were sacrificed after performing the pulpotomy at 2, 4 and 6 weeks, respectively.

The portions of the jaws including pulpotomized teeth were carefully sectioned, washed in normal saline, and then fixed in 10% formalin for about three days. The specimens were then decalcified in 8% trichloroacetic acid, which was changed daily until complete decalcification took place. The specimens were then washed under running water for 24 hours, dehydrated in ascending grades of ethyl alcohol, and embedded in Paraffin blocks. Tissue sections were cut at four microns thickness, stained with hematoxylin and eosin (H & E) and examined histologically by light microscope (Olympus, Japan).

Histological evaluation parameter followed those previously published by Sahara et al. [18].

Results

Formocresol group; 2 weeks after pulpotomy

Examination of this group showed moderate increase in the inflammatory infiltrate occupying the pulp core, with absence

of the odontoblastic cell layer, cell free and cell rich zones. Areas of hydropic degeneration were also observed. Marked increase in the amount of fibrous tissue was noticed. The inner dentinal wall revealed resorption denoted by the presence of odontoclasts in Howship's lacunae (*Figure 1*).

Formocresol group; 4 weeks after pulpotomy

Microscopic examination revealed an increased amount of fibrosis and severe inflammation compared to the previous group. Congestion of blood vessels was evident in the pulp core, accompanied by internal root resorption (*Figure 2*). Areas of complete destruction and absence of pulp tissue were also noticed in the pulp core (data not shown). Some specimens showed severe internal root resorption, resulting in loss of dentinal wall.

Formocresol group; 6 weeks after pulpotomy

Complete resorption of the apical third of the root was revealed in this group causing severe inflammatory cell infiltrate to extend into the periodontal ligament. Odontoclasts were seen bordering the pulp-periodontal ligament interface (*Figure 3*).

Pulpotec group; 2 weeks after pulpotomy

Examination of this group showed absence of normal pulp architecture with complete disorganization of odontoblastic layer. Areas of hydropic degeneration and enlarged lymphatic vessels were observed in the pulp core (data not shown). Increased amount of fibrous tissue as well as mild increase in

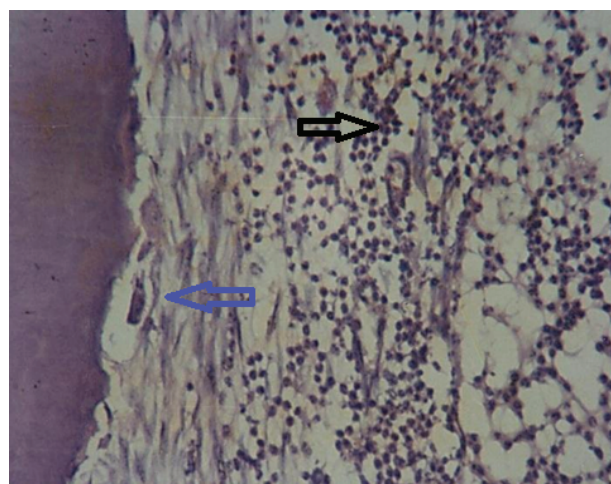


Figure 1: Two weeks after Formocresol pulpotomy: Inner dentinal wall resorption, increased fibrous tissue, and moderate inflammatory cells infiltration (black arrow). Note odontoclasts in Howship's lacunae (blue arrow) (H&E X400).

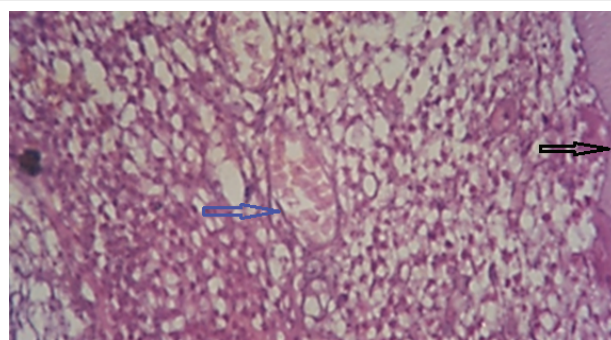


Figure 2: Four weeks after Formocresol pulpotomy: Areas of severe inflammatory cells infiltration, intermingling with increased fibrous tissue. Note the capillary dilatation (blue arrow) and internal root resorption (black arrow). (H&E X400).

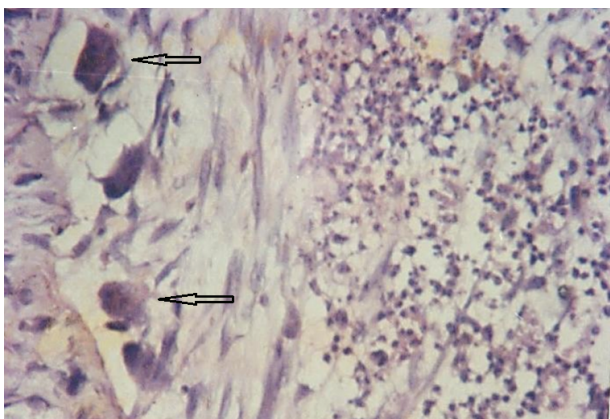


Figure 3: Six weeks after Formocresol pulpotomy: Multiple odontoclasts bordering the periodontal ligament (arrows), while the adjacent pulp tissue reveals severe inflammation and fibrosis. (H&E x400).

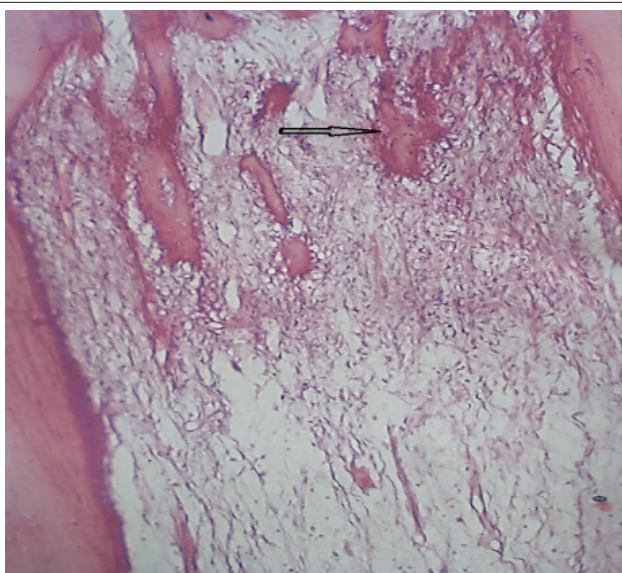


Figure 4: Two weeks after pulpotomy with Pulpotec: mild inflammatory cells infiltrate and marked fibrosis. Note the formation of calcified masses at the apical third of the root (arrow). (H&E. X 100).

inflammatory cellular infiltrate was also noticed in the mid-portion of the root, becoming more intense at the apical region. Some specimens showed formation of calcified masses at the apical portion of the root (Figure 4).

Pulpotec group; 4 weeks after pulpotomy

Loss of normal pulp architecture was revealed in this group. There was a complete loss of odontoblastic layer, cell-free and cell-rich zones. The pulp core became completely occupied by moderate inflammatory infiltrate and fibrosis (Figure 5). Root resorption was noticed along the inner dentinal wall

Pulpotec group; 6 weeks after pulpotomy

Microscopic examination revealed extensive amount of fibrosis occupying the pulp core, multinucleated giant cells were identified. Marked dilatation in lymphatic vessels was also detected in some specimens. Areas of calcified tissue deposition were noticed along the resorbed inner dentinal wall (Figure 6).

Discussion

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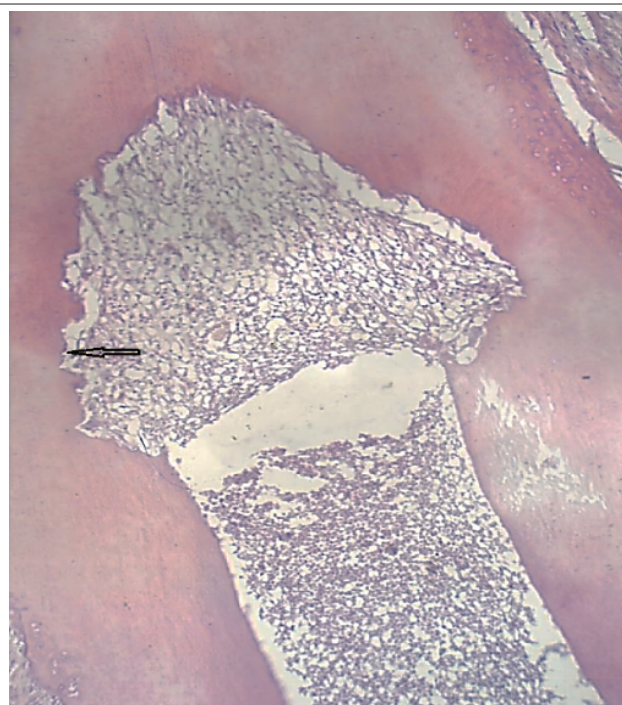


Figure 5: Four weeks after pulpotomy with Pulpotec: Complete absence of odontoblastic layer, moderate inflammation in pulp core. Note internal root resorption of dentin walls (arrow). (H&E. X 40).

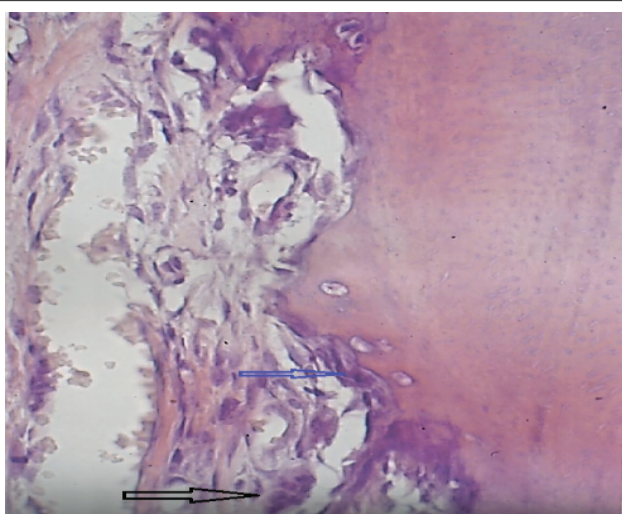


Figure 6: Six weeks after Pulpotec pulpotomy: Deposition of calcified masses at certain resorption sites (blue arrow). Note the presence of multinucleated giant cell adjacent to the resorbed area (black arrow) (H&E X 40).

on pulp therapy for primary teeth stated that a pulpotomy is a procedure in which the coronal pulp is amputated and the remaining radicular tissue is treated with a medicament to preserve the pulp health [19]. Due to the superior fixative properties of formocresol and its bacteriostatic properties, it is still considered the gold standard in primary tooth pulp therapy [20]. Recently, the National Institute for Occupational Safety and Health in the United States and the Organization for Economic Cooperation and Development have stated that “formaldehyde is not likely to be a potent carcinogen to humans when used under low exposure conditions” [21]. Consequently, Pulpotec has been selected for this study as it contains formaldehyde at a very low concentration. This was

an observational study to evaluate the pulpal inflammatory response of pulpotec versus Fc on pulpotomized primary teeth in puppies.

In this study, application of Fc at pulpotomy sites led to pulp inflammation that varied from moderate to severe, hydropic degeneration, increased fibrosis, capillary dilatation, disruption of odontoblastic layer and internal root resorption. These results were also noticed when using the Pulpotec at pulpotomy site with the detection of calcified tissues adjacent to the sites of resorption.

Increased fibrous tissue occupying the pulp core and dilatation of blood vessels were found in Fc group four weeks following pulpotomy. This may be due to the irritating effect of formaldehyde as it diffused down into the radicular portion of the pulp. This is in accordance with Sant'Anna et al. [22] who mentioned that Fc promoted coagulation necrosis, inflammatory process with mononuclear cells and dilatation of the vessels. Increased inflammatory response was detected especially in the mid and apical portion of the root. Indeed the presence of oedematous zones denoting hydropic degeneration occurred as a result of severe inflammation. These findings are comparable to those obtained by El-Meligy et al. [17] and Yorgancilar et al. [23] who stated that application of Fc resulted in infiltration of lymphomononuclear cells within connective tissues, thus it can modulate immune and inflammatory responses in dental pulp. Complete loss of normal arrangement of the pulp zones and disruption of odontoblastic layer was detected along most of the root length. Similar results were reported by Srinivasan and Javanthi [24] who mentioned that in Fc pulpotomized teeth, the odontoblastic layer was not intact throughout the dentin-pulp complex. This could be linked to the cytotoxicity and mutagenicity of formaldehyde as stated by Lewis [25]. Concerning Pulpotec, Areas of hydropic degeneration, disruption of odontoblastic layer and loss of pulp architecture in addition.

n of mild to moderate inflammation were observed in the second and fourth weeks following pulpotomy. This is in accordance with Khattab et al. [26] who mentioned that pulpotomy using Pulpotec resulted in destruction of odontoblastic cell layer, chronic inflammatory infiltrate and pulp necrosis. This also goes in line with Kakarla et al. [27]

who found diffuse chronic inflammatory cells after 1 week interval and obliteration of odontoblastic layer along dentinal surface.

Internal root resorption has been observed in Fc group two and four weeks after pulpotomy. This resorption appeared in an extensive form in the six weeks group resulting in pulp-periodontal ligament continuity in some specimens. These changes could be linked to chronic inflammation caused by bacterial invasion followed by application of Fc. Similarly, Toomarian et al. [28] stated that increased number of odontoclasts, sites of internal resorption, and other criteria observed with Formocresol group support the idea that Formocresol is a stimulating material with poor biocompatibility features. Furthermore, Maria et al. [29] mentioned that both bacterial infection and the contributing irritating effect of formaldehyde lead to death of odontoblasts and subsequent internal resorption. On the other hand, the internal root resorption was detected four and six weeks after application of Pulpotec. This may be attributed to pulp inflammation which can initiate internal inflammatory root resorption, as stated by Haapasalo and Endal [30]. Also, some specimens of the Pulpotec group revealed the deposition of calcified masses within the pulp two weeks following pulpotomy. This is considered as degenerative changes affecting inflamed pulp. Six weeks following the application of Pulpotec, deposition of a layer of calcified tissue over the site of inner root resorption was observed. This may be due to the activity of odontoblast-like cells arising from the undifferentiated mesenchymal cells of the pulp, depositing dentin-like material.

From the present study, we can conclude that there was no noticeable difference between the degenerative effects of both materials on the pulp tissue. At the same time, with the administration of Pulpotec, there was a deposition of calcified material on the site of internal root resorption, 6 weeks after pulpotomy. This could be considered as an initiation of a pulp healing process. The long term effect of Pulpotec on pulp healing process should be investigated.

Conflict of Interest

In the opinion of the authors, there was no conflict of interest. No external funds were used for this research.

References

1. Fuks AB. Current concepts in vital primary pulp therapy. *European Journal of Paediatric Dentistry*. 2002; **3**: 115-120.
2. Fuks AB. (2008) Vital pulp therapy with new materials for primary teeth: new directions and treatment perspectives. *Pediatric Dentistry*. 2008; **30**: 211-219.
3. Sweet CA. Treatment for deciduous teeth with exposed pulps. *Texas Dental Journal*. 1932; **54**: 6.
4. Primosch RE, Glom TA, Jerrell RG. Primary tooth pulp therapy as taught in predoctoral pediatric dental programs in the United States. *Pediatric Dentistry*. 1997; **19**: 118-122.
5. Vij R, Coll JA, Shelton P, Farooq NS. Caries control and other variables associated with success of primary molar vital pulp therapy. *Pediatric Dentistry*. 2004; **26**: 214-220.
6. Holgan G, Eidelman E, Fuks AB. Long- term evaluation of pulpotomy in primary molars using mineral trioxide aggregate and

Formocresol. *Pediatric Dentistry*. 2005; **27**: 129-136.

7. Yaman E, Gorken F, Erdem A Pinar, Sepet E, Aytepe Z. Effects of folk medicinal plant extract Ankaferd Blood Stopper[R] in vital primary molar pulpotomy. *European Archives of Paediatric Dentistry*. 2012; **13**: 197-202.

8. Sun HW, Feigal RJ, Messer HH. Cytotoxicity of glutaraldehyde and formaldehyde in relation to time of exposure and concentration. *Pediatric Dentistry*. 1990; **12**: 303-307.

9. Srinivasan V, Patchett CL, Waterhouse JP. Is there life after Buckley's Formocresol? Part I: a narrative review of alternative interventions and materials. *International Journal of Paediatric Dentistry*. 2006; 2004; **16**: 117-135.

10. International Agency for Research on cancer. Press release no. 153. Accessed (2013 June 15) at: <http://www.iarc.fr/ENG/PressReleases/pr153a.html>.

11. Milnes AR. Persuasive evidence that Formocresol use in

pediatric dentistry is safe. *Journal of Canadian Dental Association*. 2006; **72**: 247-248.

12. Rolling I, Thylstrup A. A three year follow- up study of pulpotomized primary molars treated with the Formocresol technique. *Scandinavian Journal of Dental Research*. 1975; **83**: 47-53.

13. Pulpitis on vital molars? A unique and efficient solution. Accessed at: http://www.Pulpotec.com/index_en.php.

14. McMaster A, Ray DW. Modelling the glucocorticoid receptor and producing therapeutic agents with anti-inflammatory effects but reduced side-effects. *Experimental Physiology*. 2007; **92**: 299-309.

15. Merkl R, Hradkova I, Filip V, Smidrkal J. Antimicrobial and antioxidant properties of phenolic acids alkyl esters. *Czech Journal of Food Sciences*. 2010; **28**: 275-279.

16. Estrela C, Estrela CR, Hollanda AC, Decurcio Dde A, Pecora JD. Influence of Iodoform on antimicrobial potential of calcium hydroxide. *Journal of Applied Oral Science*. 2006; **14**: 33-37.

17. El-Meligy O, Abdalla M, El-Barawy S, El-Tekya M, Dean JA. Histological evaluation of electrosurgery and Formocresol pulpotomy techniques in primary teeth in puppies. *Journal of Clinical Pediatric Dentistry*. 2001; **1**: 81-85.

18. Sahara N, Okafuji N, Toyoki A, Suzuki I, Deguchi T, et al. Odontoclastic Resorption at the Pulpal Surface of Coronal Dentin Prior to the Shedding of Human Deciduous Teeth. *Archives of Histology and Cytology*. 1992; **55**: 273-285.

19. American Academy of Pediatric Dentistry. Guidelines on pulp therapy for primary and permanent teeth: reference manual 2012-2013. *Pediatric Dentistry*. **30**: 2009; 170-174.

20. Patchett CL, Srinivasan V, Waterhouse PJ. Is there life after Buckley's formocresol? Part II-Development of a protocol for the management of extensive caries in the primary molar. *International Journal of Paediatric Dentistry*. 2006; **16**: 199-206.

21. Milnes AR. Is formocresol obsolete? A fresh look at the evidence concerning safety issues. *Journal of Endodontics*. 2008; **34**: S40-S46.

22. Sant' Anna AT, Spolidorio LC, Ramalho LTO. Histological Analysis of the Association between Formocresol and Endotoxin in the subcutaneous Tissue of Mice. *Brazilian Dental Journal*. 2008; **19**: 40-45.

23. Yorgancilar E, Deveci E, Deveci S. Effects of Formaldehyde on Respiratory Mucosa in Rats. *International Journal of Morphology*. 2012; **30**: 521-523.

24. Srinivasan D, Javanthi M. Comparative evaluation of Formocresol and mineral trioxide aggregate as Pulpotomy agents in deciduous teeth. *Indian Journal of Dental Research*. 2011; **22**: 385-390.

25. Lewis B. The obsolescence of Formocresol. *Journal of British Dental Association*. 2009; **11**: 525-528.

26. Khattab NM, El-Shehaby F AH, Madany NM (2010) A Histological and Bacteriological Evaluation of Pulpotec as a Pulp Medicament for Pulpotomized Primary Teeth. *Egyptian Dent j* **56**: 591.

27. Kakarla P, Avula JS, Mellela GM, Bandi S, Anche S (2013) Dental pulp response to collagen and pulpotec cement as pulpotomy agents in primary dentition: A histological study. *Journal of Conservative Dentistry*. 2013; **16**: 434-438.

28. Toomarian L, Fekrazad R, Sharifi D, Baghaei M, Rahimi H, Behnam E. Histopathological evaluation of pulpotomy with Er,Cr:YSGG laser vs Formocresol. *Lasers in Medical Science*. 2008; **23**: 443-450.

29. Maria R, Mantri V, Koolwal LS. Internal resorption: A review & case report. *Endodontics*. 2010; **22**: 100-108.

30. Haapasalo M., Endal U. Internal inflammatory root resorption: the unknown resorption of the tooth. *Endodontic Topics*. 2008; **14**: 60-79.