

COMPARATIVE EVALUATION OF JOJOBA OIL VERSUS FORMOCRESOL PULPOTOMY IN PRIMARY MOLARS — IN VITRO STUDY (Histopathological and Immunohistochemical)

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ABSTRACT

This study was conducted to evaluate and compare the histopathological and immunohistochemical responses of dog's dental pulp to formocresol pulpotomy versus jojoba. It was conducted on 32 primary molars of four dogs between the ages of one to two months. One of the dogs was used as control, while the rest of the molars were treated by formocresol and jojoba oil (pulpotomies). The animals were destroyed after one month. The pulp tissues of all the teeth were submitted to histological and immunohistochemical evaluation.

The pulp of the Jojoba oil treated group revealed slight hypervascularization, some blood vessels showed dilatation and areas of pulp calcification were detected. The cellularity of the pulp and its fibrous elements appeared normally with no inflammatory cell infiltration, while the pulp treated with formocresol was highly vascularized with high number of chronic inflammatory cells, areas of calcification with obvious increased collagen fiber density, in addition to numerous vacuoles and necrotic areas. In addition jojoba oil showed moderate immunoreactivity of von Willebrand factor in endothelial cells which may indicate increase in nitric oxide synthase. Histological and immunohistochemical reactions of dental pulp to Jojoba oil were more favorable than formocresol. The greater success rate of jojoba oil rather than FC pulpotomy in this study is attributed to anti inflammatory effect of jojoba oil compared to formocresol.

INTRODUCTION

Pulpotomy is a therapeutic procedure used in cases of reversible inflammation of the pulp of primary and immature permanent teeth, where the radicular portion of the pulp remain healthy and is capable to serve healthy for long time until normal exfoliation.¹⁻³

Ideal material used after amputation of coronal pulp should be bactericidal, harmless to pulp and surrounding structures, promote healing of remaining pulp, not interfere with the physiologic root resorption in primary dentition, has no systemic distribution and hazards possess, biologic and immunologic effect and has proper histological and radiographical and clinical outcomes.⁴⁻⁵

Historically, pulpotomy therapy for primary dentition has developed along three lines: devitalization, preservation and regeneration. Devitalization, where the intent to fix vital tissues by formocresol and

electrocautery. Preservation, the retention of maximum vital tissue with no induction of reparative dentin bridge is exemplified by glutaraldehyde and ferric sulfate treatment. Regeneration, the stimulation of dentin bridge, has long been associated with calcium hydroxide. Of the three categories, regeneration is expected to develop the most rapidly.⁶

Most of pediatric dentists use formocresol pulpotomy for vital primary pulp therapy⁷. Formocresol has been used as an acceptable, high successful and the most common capping material for fixation of the pulp for many years.⁸⁻⁹

Concerns about the safety of formocresol have been appearing in the dental and medical literature for more than 20 years.¹⁰⁻¹⁴ Cresol is locally destructive to vital tissue, but its potential for systemic distribution following pulpotomy treatment is negligible.¹⁵⁻¹⁶ The major concern has been with the formaldehyde component of formocresol. Although a 1: 5 dilution of formocresol is specified by many authors, most (78%)

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American pediatric dentists who use formocresol in primary tooth pulpotomy use it at full strength (19% or 48.5% formaldehyde).¹⁷⁻²⁰

In the United Kingdom, 54% of pediatric dentists reported concerns about possible sensitization, toxic, mutagenic or carcinogenic effect of formocresol.¹ These major concerns cause seeking for alternatives to formaldehyde derivatives for treating pulpotomized primary teeth. These alternatives must have efficacies equivalent to or better than formocresol technique and with a wider margin of safety.¹⁸⁻²⁰ These include electro-surgery, laser, glutaraldehyde, ferric sulfate, enriched collagen solution and mineral trioxide aggregate.²¹

Increased utilization of indigenous plant medicines in developing countries became a world health organization policy in 1970.²² Jojoba oil is extracted from ground crushed seeds of *Simmondsia chinensis*. It was introduced in Egypt in 1984 by Food and Agriculture Organization (FAO). However, no attention was given to this plant. In 1991, several Egyptian investors and companies succeeded in cultivation of *Simmondsia chinensis* and produced its seedlings and oil which is incorporated in the production of few pharmaceutical and cosmetic preparations.²³⁻²⁴

It possesses anti-inflammatory and anti-microbial actions. It is used as a cosmetic material and is considered non-irritant when applied to intact and abraded skin. It does not interfere with biological processes due to its indigestibility and purity.²⁵⁻²⁶ It had also powerful effects on reduction of the size of oral ulceration and healing of the edema or erythema in addition to relief of pain on the treatment of recurrent aphthous ulcers.²⁷⁻³⁰

Using it as pulp capping material, led to favorable healing pulp response similar to or sometimes better than the response manifested by the exposed pulps capped with calcium hydroxide.³¹

The aim of the present work was to evaluate and compare the histopathological and immunohistochemical responses of dog's dental pulp to formocresol pulpotomy versus jojoba.

MATERIALS AND METHODS

Four dogs aged 1-2 months were subjected to general anesthesia by intramuscular injection of 20mg/kg of ketamine HCl 50mg/ml and 10 mg/kg of xylazine 20mg for each dog. One of the dogs was used as control which will not receive any treatment. In the rest of the animals pulpotomy was done in their molars, and they were divided in two groups

according to the material used into jojoba oil group and formocresol group. The left side molars were treated with jojoba oil and the right side with formocresol.

Using a number 1 round carbide bur with low speed under water spray coolant, access cavities were prepared in occlusal surfaces and pulp was exposed, removal of the roof of the pulp was done followed by amputation of the coronal portion of the pulp. Bleeding was stopped with sterile moistened cotton pellets. Then, the pulp stumps were covered with cotton moistened with jojoba oil in the left molars and formocresol on the right molars, the cotton was left for 5 minutes.

Jojoba oil group (JB)

The paste was prepared by mixing the Jojoba oil with zinc oxide powder and eugenol on a clean dry glass slab. A thick creamy mix was applied to the coronal pulp after removing the cotton pellet and the cavity was sealed with zinc polycarboxylate.

Formocresol group (FC)

The paste was prepared by mixing the Formocresol with zinc oxide powder and eugenol on a clean dry glass slab. A thick creamy mix was applied to the coronal pulp after removing the cotton pellet and the cavity was sealed with zinc polycarboxylate.

At the end of the experiment after one month the animals were sacrificed by intravenous injection of 90 mg/kg body weight of thiopental sodium. The teeth with their alveolar bone were dissected out, cleaned from the surrounding soft tissues and fixed in 10% calciformal. The teeth with their alveolar bone were decalcified in solution containing equal parts of concentrated formic acid and 20% sodium citrate. The specimens were dehydrated in alcohol and embedded in paraffin; sections of 6µm were cut and stained with the following methods:

I-Hematoxylin and Eosin stain

To demonstrate the histological and histopathological changes of the tissues examined.

II-Histochemical staining

***Mallory's Trichrome stain:** used for collagen fibers identification.

III-Immunohistochemical staining

A-Factor VIII

The term factor VIII-related antigen has been replaced by the more precise designation von Will

brand Factor (vWF) *Marder et al.;1985*³². Factor VIII reacts with von Will brand factor present in endothelial cells and in the cytoplasm of megakaryocytes. The antibody may identify tumors derived from megakaryocytes or derived from endothelial cells.

B-Factor S-100

An anti-S-100 serum was obtained from a rabbit by injection S100 purified from bovine cerebra *Masuda et al; 1983*³³. The detailed characterization of anti-serum has been previously reported *Ushiki et al; 1984*³⁴. Factor S-100 used in the present study to demonstrate the neural elements in the pulp of the dog.

Immunohistochemistry procedure

Sets of 6 µm sections were deparafinized and rehydrated. Antigen retrieval was performed using citrate buffer solution (pH 6). After washing in PBS (pH 7.2), tissue endogenous peroxidases were blocked by hydrogen peroxide and unwanted background was reduced by incubation with 1.5% bovine serum albumin. Sections were incubated for 3 hours at 4° C with pre-diluted monoclonal antibodies an anti- factor VIII or an anti-S-100, purchased from Zymed (Ltd. San Francisco). Sections were incubated with Biotinylated-secondary antibody for 15 minutes. After washing, they were incubated with Streptavidin peroxidase enzyme for another 15 minutes. After washing, they were incubated with Streptavidin peroxidase enzyme for another 15 minutes. Sections were re-washed, the immunoreactivity was identified by using Diaminobenzidine Chromogen (DAB) until desired color was developed. Sections were washed, counter stained with hematoxylin, dehydrated, cleared and cover slipped by DPX.

RESULTS

I-Histological Results

*Hematoxylin and Eosin stain

In the control group

The structure of the pulp and dentin of the deciduous posterior teeth of the dogs were examined by light microscope.

In the control group stained with Hematoxylin and Eosin the pulp appeared with moderately vascularized and well organized odontoblast cells arranged side by side forming odontoblastic layer under a thin layer of predentin. The blood vessels were normally dispersed within fine oriented collagen fibers (Figs 1&2).

In jojoba oil group

The odontoblastic cells in the pulp showed normal arrangement with few degenerated cells. The predentin zone appeared relatively similar to the control group. Normal mesenchymal connective tissue was observed and no inflammatory reaction was noticed (Fig 3). The pulp began to show slight hypervascularization with new blood vessels formation. Some blood vessels appeared dilated with stagnation of blood and engorged with RBCs (Figs 3, 4 & 5). Moreover areas of pulp calcification surrounded by odontoblastic like cell away from the cavity were detected (Fig 5). The cellularity of the pulp and its fibrous elements more or less resemble normal pulp tissue (Fig 6).

In formocresol group

Exaggerated histological changes were detected in this group than jojoba oil group. The pulp showed more vascularization with increased vasoformative activity. High number of chronic inflammatory cells infiltration was observed. Many degenerated vacuoles in both odontoblastic cell layer and the pulpal connective tissue were obvious. Also collagen fiber density was markedly increased (Figs 7, 8, & 9). In many specimens the pulp tissue revealed necrosis in its cellular and extracellular elements (Fig 10). The presence of dilated blood vessels engorged with blood cells were detected. Moreover areas of hemorrhage and extravasated RBC's were numerous (Figs 7 & 9).

II-Histochemical staining

*Trichrome stain

In the control group, normal amount of collagen was detected throughout the pulp (Fig 2). However, slight increase in the collagen density was identified with jojoba oil treated group (Fig 6). While in formocresol treated group markedly increase in collagen density was detected (Figs 8 & 11). Also diffuse irregular calcific loci were obvious (Fig 11).

III-Immunohistochemical result

A-Factor S-100

The immunohistochemical detection of anti- S-100 was positive, with different intensity, in nerve fibers and bundles of both groups JB and FC. Increase in staining intensity of nerve fibers was observed in pulp treated by both jojoba oil and formocresol groups. The stain appeared as dots in pulp tissue treated by jojoba oil. Mesenchymal cells as fibroblasts and endothelial cells were devoid of anti- S-100 immunostaining in most specimens. Moreover some of the nerve fibers in the subodontoblastic plexus of the dental pulp

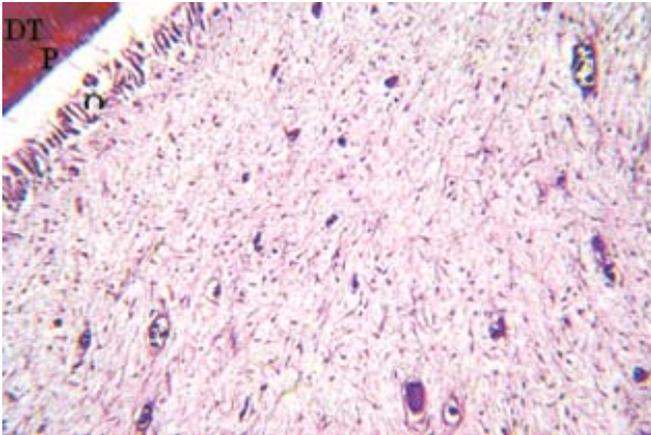


Fig 1: A photomicrograph of the pulp of control group showing normal pulp tissue with well distribution of cells & fibers, odontoblastic layer (O), predentin (P) and dentinal tubules (DT). (H&E X 200).

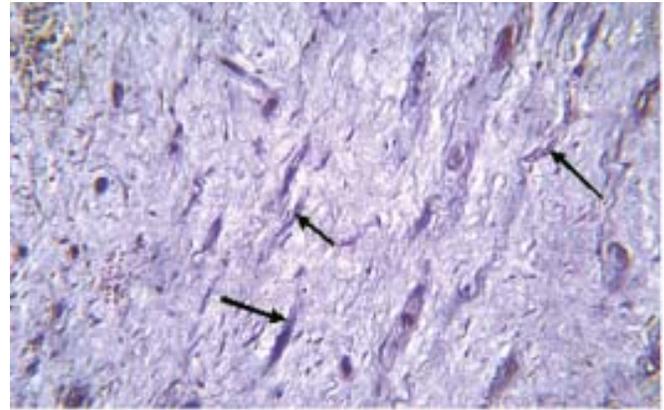


Fig 4: A photomicrograph of the pulp of JB group showing collagen bundles (arrows) (H&E X 200).

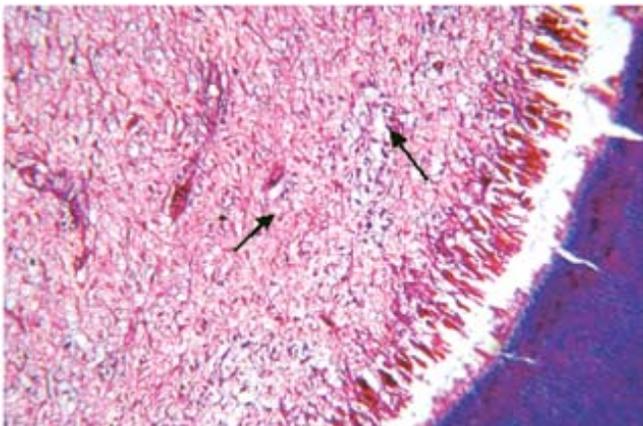


Fig 2: A photomicrograph of the pulp of control group showing normal amount of collagen fibers which denotes normal activity of fibroblasts (arrows). (Trichrome X 200).



Fig 5: A photomicrograph of the pulp of JB group showing area of dystrophic calcification(A) & dentin formation(D). (H&E X 200).

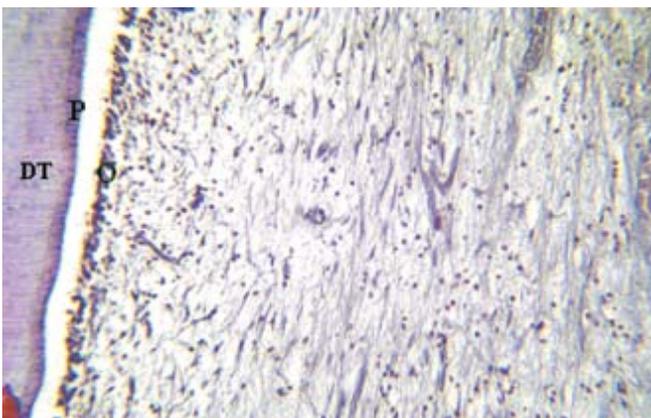


Fig 3: A photomicrograph of the pulp of JB group showing well distribution of cells and fibers with slight increase in collagen bundles, normal odontoblastic layer (O), predentin (P) and dentinal tubules (DT). (H&E X 200).

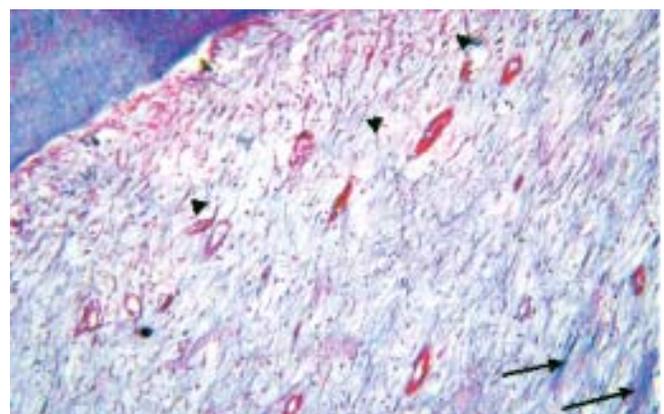


Fig 6: A photomicrograph of the pulp of JB group showing slight increase in amount of collagen fibers (arrow heads) & collagen bundles (arrows). (Trichrome X 200).

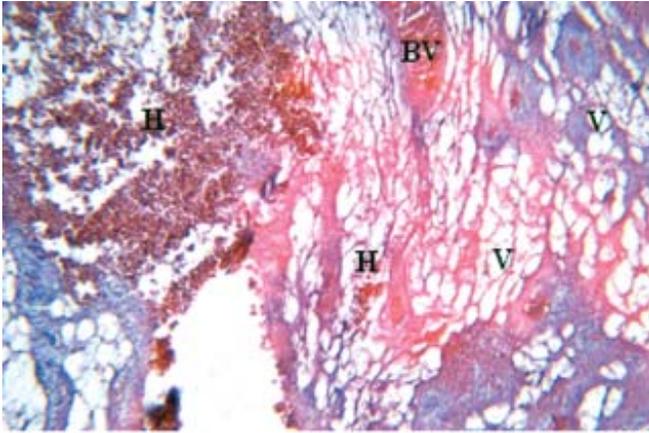


Fig 7: A photomicrograph of the pulp of FC group showing pulp with areas of vacuolation (V), fibrosis and chronic inflammatory cells & extreme dilatation of the blood vessels engorged with red blood cells (BV) & area of hemorrhage (H). (H&E X 200).

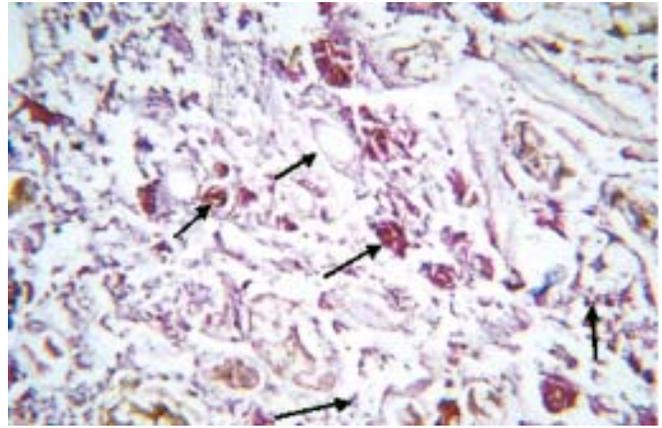


Fig 10: A photomicrograph of the pulp of FC group showing numerous degenerated and necrotic areas (arrows). (H&E X200)

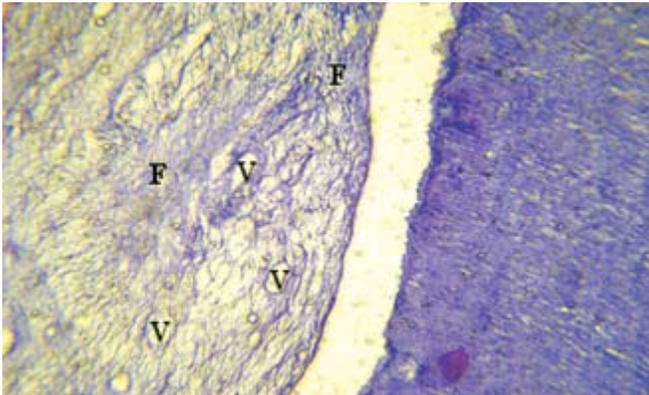


Fig 8: A photomicrograph of the pulp of FC group showing pulp with areas of vacuolation (V) & marked fibrosis (F). (H&E X200)

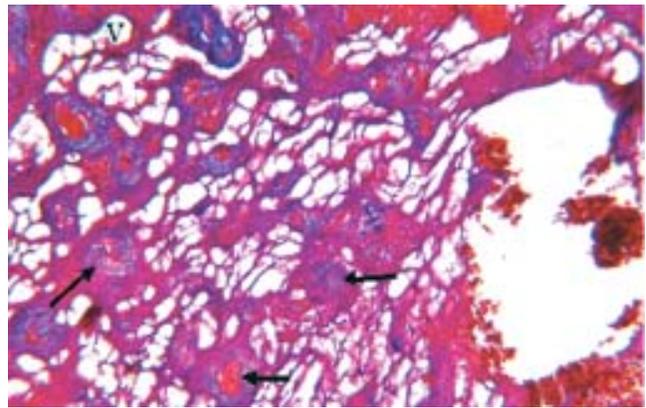


Fig 11: A photomicrograph of the pulp of FC group showing pulp with areas of calcification (arrows), vacuolation (V), fibrosis and collagen bundles. (Trichrome X 200).

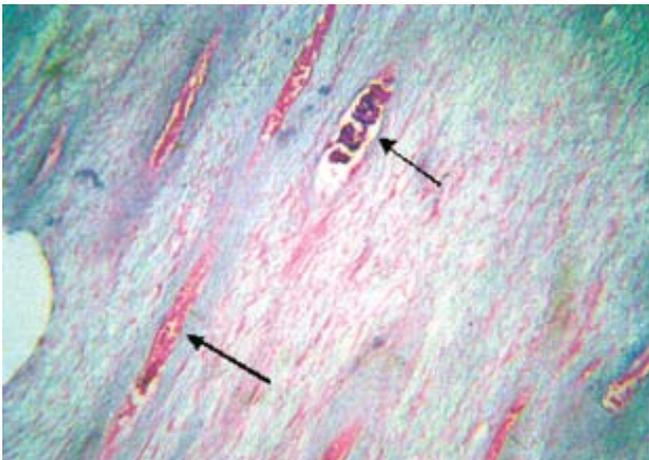


Fig 9: A photomicrograph of the pulp of FC group showing dilated blood vessels engorged with RBCs (arrows). (H&E X200)



Fig 12: A photomicrograph of S-100 immunostaining of the pulp of JB group showing strong expression of S-100 (arrows). (LAB-SA. X 200).

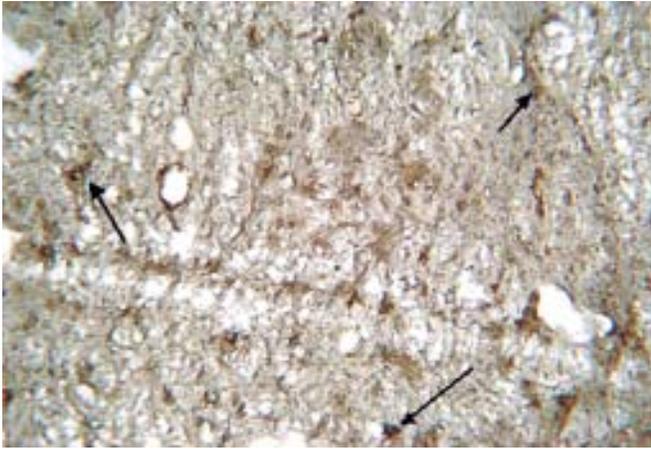


Fig 13: A photomicrograph of S-100 immunostaining of the pulp of FC group showing strong expression of S-100 in the pulp (arrows). (LAB-SA. X200).



Fig 14: A photomicrograph of factor VIII immunostaining of pulp of JB group showing moderate staining reaction of the wall of the blood vessels (V). (LAB-SA. X 200).

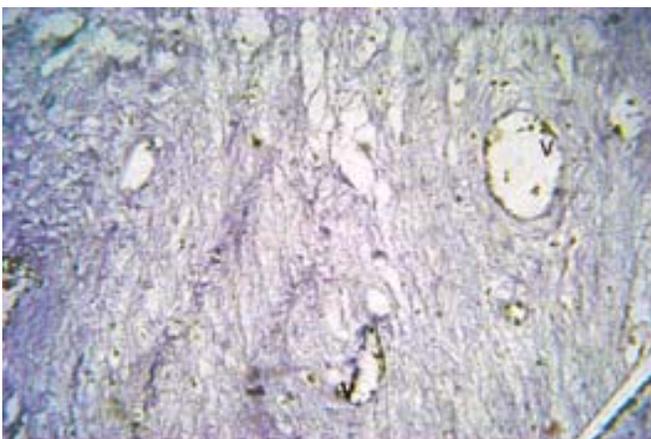


Fig 15: A photomicrograph of factor VIII immunostaining of FC group showing weak staining reaction of the wall of the blood vessels (V). (LAB-SA. X 200).

appeared positively stained by anti- S-100. The pulp revealed strong expression of anti- S-100 in JB group, while markedly increased expression of anti-S-100 was detected in pulp tissues of FC group (Figs 12 & 13).

B-Factor VIII

Examination of the immunostained sections of the pulp for factor VIII showed moderate to weak staining reaction in the endothelial cells of the wall of the blood vessels of JB group and FC group respectively (Figs 14 & 15). The blood vessels showed thickening of the walls and cytoplasmic vacuolization of the endothelial cells (Fig 14). Moreover the number of microvessels was significantly greater in the pulp of JB group than FC group.

DISCUSSION

Many traditional pulp treatment modalities for primary molars are still used. The current trend is reducing the toxicity of the standard materials or finding new biocompatible agents.⁶

Formaldehyde derivatives have been used as acceptable, high successful, and the most common capping material for the fixation of the pulp for many years.⁹

Success rate of pulpotomy with formocresol has been comprised with glutaraldehyde,³⁵ bone morphogenic proteins,³⁶ laser,³⁷ electrosurgery,³⁸ ferric sulfate³⁹ and mineral trioxide aggregate, bio active glass and hydroxyapatite.⁴⁰

Success rate of Formocresol pulpotomy has been between 70-97 percent in last decades.⁴¹

The major concerns that cause seeking for alternatives for formaldehyde derivatives are their potential mutagenicity, carcinogenicity, cytotoxicity, allergenicity and the other possible health hazards which have been attributed to them.¹

Jojoba has been used as a folk medicine for a variety of conditions including inflammatory diseases by the ancient natives of the American continents.⁴² Inflammation is the most common aspect of tissue pathology and has always taken a central role in medical practice.

The present work was performed to study histopathological, histochemical and immunohistochemical as well as to compare between the effect of formocresol and jojoba oil on the pulp.

In the present study, the pulp of the Jojoba oil treated group revealed slight hypervascularization,

some blood vessels showed dilatation and areas of pulp calcification were detected. The cellularity of the pulp and its fibrous elements appeared normal with no inflammatory cell infiltration. This finding could be explained according to *Sobhy and Mohamed (1997)*,²⁶ they reported that the mechanism by which Jojoba oil acts on pulp tissue is through the reduction in local inflammatory cell activity. Authors added that jojoba oil was considered to be non-irritant when applied to intact and abraded skin. Moreover, *Habashy et al; (2005)*⁴³ reported that jojoba liquid wax caused significant lowering of granulation tissue formation in the ear of the rat with normal epidermal and dermal tissues. So our results may be due to the anti-inflammatory effect of jojoba oil on the pulp tissue.

On the other hand the results of the present investigation proved that the destructive changes were more pronounced in formocresol treated group. The pulp was highly vascularized with high number of chronic inflammatory cells, areas of calcification with obvious increased collagen fiber density. In addition to numerous vacuoles and necrosis areas. From the previous findings it is apparent that formocresol resulted in inflammation and hyperemia of the pulp of the experimental groups.

Our findings agree with *Jabbarifar et al; (2007)*,⁴⁰ they revealed that moderate inflammatory changes and hyperemia were detected with formocresol. In addition tissue necrosis also was observed. The reasons of necrosis may be attributed to hazardous effect of these materials. Moreover, diffuse irregular calcified loci were detected which may be originated from biohistochemical properties of these materials and their reactions. Also *Shoji et al., (1985)*⁴⁴ explained that calcification and dentinal bridging could be a sign of either healing or irritation.

The nerve bundles entering the tooth pulp consists of sensory afferents of trigeminal nerve and sympathetic branches from the superior cervical ganglion. Each bundle contains both myelinated and unmyelinated axons with Schwann cells were present in dental pulp.⁴⁵

To form an image of the innervation of the dental pulp a general marker of interest is protein S-100, which shows the presence of myelinated tissue, or more specifically, of Schwann cells. Protein S-100 immunoreactive nerve fibers were numerous, both in jojoba oil and formocresol groups. Schwann cells were strongly immunostained by S-100 in both groups. Similar intense positively for S-100 of the nerve fibers was detected in the pulp of both groups which may indicate decrease in the teeth sensitivity during treatment.

In the present study the angiogenic changes, in the form of new blood vessels formation were observed by light microscopy and was confirmed by immunohistochemistry using factor VIII-related antigen marker for endothelial cells. In this study jojoba oil showed moderate immunoreaction of von Willebrand factor in endothelial cells which may indicate increase in nitric oxide synthase. This finding may be related to the anti-inflammatory effect of JB. Our finding goes in accordance with *Riba et al; (2006)*,⁴⁶ they explained that von Willebrand factor activates endothelial nitric oxide synthase through a specific Ca²⁺ dependent glycoprotein receptor-signaling cascade. Moreover, several studies have demonstrated that inflammation correlates with the level of nitric oxide *Miller & Grisham (1995)*.⁴⁷ Nitric oxide plays an important role in inflammation and nitric oxide synthase inhibitors can reverse several inflammatory symptoms *Amin (1995)*.⁴⁸

The greater success rate of jojoba oil rather than FC pulpotomy in this study is attributed to anti-inflammatory effect of jojoba oil compared to formocresol.

CONCLUSIONS

Histological and immunohistochemical reactions of dental pulp to Jojoba oil were more favorable than formocresol.

RECOMMENDATIONS

- 1- Jojoba oil could be a suitable alternative to formocresol in vital pulpotomy procedures in primary teeth due to low cost, biocompatibility and high success rate.
- 2- Further in-vivo studies are recommended to confirm efficacy of Jojoba oil.

REFERENCES

- 1 Camp JH and Barrett EJ: Endodontics treatment for primary and young dentition in Cohen, Burns RC. Pathway of the pulps. 8th Edition. St. Louis, Mosby 2000; 797-844.
- 2 Ingle J I : Endodontics. 4th edition, Philadelphia. Lea and Febrieger; 1984.
- 3 Khademi AA and Hasheminia M: Effects of amalgam and MTA on sealability involvement lateral perforation in cat canine. J of Res In Medical Science 1999; 34(3): 40-43.
- 4 Alcam A: Pulpal tissue changes following pulpotomies with formocresol, glutaraldehyde-calcium hydroxide, glutaraldehyde-zinc oxide eugenol pastes in primary teeth. J Pedodont 1989; 13(2): 123-133.
- 5 Stanely HR; Clark AE; Pameijer CH and Louw NP: Pulp capping with a modified bioglass formula (# Ab8-modified). Am J Dent 2001; 14(4): 227-232.
- 6 Ranly DM: Pulpotomy therapy in primary teeth : new modalities for old rationales. Pediatr Dent 1994; 16: 403-409.
- 7 Hunter ML and Hunter B: Vital pulpotomy in the primary dentition: attitudes and practices of specialists in pediatric

- dentistry practicing in the United Kingdom. *Int J Paediatr Dent* 2003; 13(4): 246-250.
- 8 Fucks AB: Pulptherapy for the primary dentition. In: Pinkham J; Casamassimo P; Fields HW; MC Tighe DJ and Nowak A: editors *Pediatric dentistry: Infancy through Adolescence*. 4th ed. Philadelphia: Mosby 2005; 341-353.
- 9 Smith NL; Seale NS and Nunn ME: Ferric sulfate pulpotomy in primary molars: a retrospective study. *Pediatr Dent* 2000; 22(3): 192-199.
- 10 Lewis BB and Chestner SB: Formaldehyde in dentistry: a review of mutagenic and carcinogenic potential. *JADA* 1981; 103 (3): 429-434.
- 11 Yodaiken RE: The uncertain consequences of formaldehyde toxicity. *JAMA* 1981; 264(5): 1677-1680.
- 12 Perara F and Petito C: Formaldehyde a question of cancer policy ? *Science* 1982, 216(4552): 1285-1291.
- 13 Judd PL and Kenny DJ: Formocresol concerns: a review. *J Can Dent Assoc* 1987; 53(5): 401-404.
- 14 Lewis BB: Formaldehyde in dentistry: a review for the millennium: *J Clin Ped Dent* 1998, 22(2); 167-177.
- 15 Ranly DM and Fulton R: Reaction of rat molar pulp tissue to formocresol, formaldehyde and cresol. *J Endodon* 1976; 2(6): 176-181.
- 16 Myers DR; Shoaf HR; Dirksen TR; Pashley DH; Whitford GM and Reynolds KE: Distribution of 14c- formaldehyde after pulpotomy with formocresol. *JADA* 1978; 96(5): 805-813.
- 17 King SRA; MC Whorter AG and Seale NS: Concentration of formocresol used by pediatric dentists in primary tooth pulpotomy. *Pediatr Dent* 2000; 24(2): 157-159.
- 18 Zarzar PA; Rosenblatt A; Takahashi CS; Takeuchi PL and Costa Junior LA: Formocresol mutagenicity following primary tooth pulp therapy: an in vivo study. *J Dent* 2003; 31(7): 479-485.
- 19 Ibricevic H and AL-Jame, Q: Ferric sulphate and formocresol in pulpotomy of primary molars: long term follow up study. *Eur J Paediatr Dent* 2003; 4(1): 28-32.
- 20 Loh A; O Hay P; Tran X; Charles R; Hughes A; Kubo K; and Messer LB: Evidence based assesment: evaluation of the formocresol versus ferric sulphate primary molar pulpotomy. *Paediatr Dent* 2004; 26(5): 401-409.
- 21 Fuks AB and Papagiannoulis L: Pulpotomy in primary teeth: review of the literature according to standardized criteria. *Eur Arch Paediat Dent* 2006; 7(2): 64-71.
- 22 Llewelyn DR: The pulp treatment of the primary dentition. *Int.J.Pead.Dent* 2000; 10: 248-252.
- 23 Schmitt D; Lee J and Bogen G: Multifacted use of TK/f Pro Root MTA root canal repair material. *Pead Dent* 2001; 23: 326-330.
- 24 Frans Worth NR: How can the well be dry when it is full of water? *Econ Botany* 1984; 38: 4-13.
- 25 Taguchi M and Kun MT: Toxicity studies on Jojoba oil for cosmetics and toiletries Koe perfumery Comp. In *Tokyocosmetics and toiletries* 1977; 92.
- 26 Sobhy H and Mohamed E: Some biological and pharmacological studies on Jojoba oil. *Bull Fac Pharm; Cairo Univ* 1997; 34:239.
- 27 Zania AA; Darah HY; Ibrahim CO and Naraini I: Jojoba leaf extracts: Potential as Antimicrobial and Antinetnatode agents. *First National Scientific Seminar on Jojoba (Simmondsia Chinensis) link schelder, A further Commercial corp in Malaysia, 1994*.
- 28 Amin, M; Salah El Din, M; Abbas, T and Ezz El Arab, A: Clinical evaluation of jojoba in management of recurrent aphthous ulceration. *Egypt.Dent.J* 1997; 43: 2569-2574.
- 29 Metwalli NE and Omar OM: Assessment of nutritional deficiencies and clinical evaluation of treatment of aphthous ulcer in children. *Egypt Dent J* 2002; 48(3): 1487-1494.
- 30 Abd El Sattar E and Saudi H: Comparative evaluation of low level laser therapy versus Jogel in the treatment of recurrent aphthous ulcers: A clinical and digital image analysis study. *Egypt Dent J* 2002; 48: 2227-2234.
- 31 Abi El-Hassan MH and El-Azab SM: Histological pulp response to an experimental capping material. *Egypt Dent J* 2000; 46(4): 2334-2342.
- 32 Marder VJ; Mannucci PM; Firkin BG; Hoyer LW; and Meyer D: Standard nomenclature for factor VIII and von Willebrand factor: a recommendation by the international committee on thrombosis and haemostasis. *Thromb haemostas* 1985; 54: 871-872.
- 33 Masuda T; Sakimura K; Yoshida Y; Kuwano R; Isobe T; Okuyama T and Takahashi Y: Development changes in the translatable m RNA for B subunit of S-100 protein in rat brain. *Biochem Biophys Acta* 1983; 740: 249-254.
- 34 Ushiki T; Iwanaga T; Masuda T; Takahashi Y and Fujita T: Distribution and ultrastructure of S-immunoreactive cells in human thymus. *Cell Tissue Res* 1984; 235: 509-514.
- 35 Jabbarifar E; Makarem A; and Khordimood M: Comparing Formocresol and electrosurgical pulpotomy in primary Molar teeth. *J Research in Medical Sciences* 1999; 3(4): 24-28.
- 36 Fei AL; Udin RD and Johnson R: A clinical study of ferric sulfate as a pulpotomy. *Pediatr Dent* 1991;13: 327-32.
- 37 Jabbarifar E: Clinical & radiographic evaluation ferricsulfate pulpotomy with Formocresol in primary molar teeth. *J Research in Medical Sciences* 2001; 3(6):
- 38 El-Meligy O; Abdalla M; El-Baraway S; El-Tekya, M and Dean JA: Histological evaluation of electrosurgery and formocresol pulpotomy techniques in primary teeth in dogs. *J Clinical Ped Dent* 2001; 26(1): 81-85.
- 39 Faraco IM and Holland R: The response of the pulp of dogs to capping with MTA or calcium hydroxide cement. *Dent Traumatol* 2001; 17(4): 163-166.
- 40 Jabbarifar, E; Mohammad S R and Ahmadi N: Histo-pathologic responses of dog's dental pulp to mineral trioxide aggregate, bio active glass, formocresol, hydroxyapatite. *Dent Res. J* 2007; 4(2): 83-7.
- 41 Pitt Ford T; Torabinejad M and Abedi H: Using MTA as a pulp capping material. *JADA* 1996; 127: 1991-9.
- 42 Yaron, A: Metabolism and physiological effects of jojoba oil. In: Wisniak J, editor. *The chemistry and technology of jojoba oil*. Champaign, IL. American Oil Chemists Society Press. 1987; 251-65.
- 43 Habashy, R; Ashraf, B; Amani, EK and Mohammed, M: Anti-inflammatory effects of jojoba liquid wax in experimental models. *Pharm.Res* 2005; 51:95-105.
- 44 Shoji, S; Nakamura, M and Horiuchi ,H: Histopathological changes in dental pulps irradiated by CO2 laser: A preliminary report on laser pulpotomy. *J. Endod* 1985; 11(9):379-384.
- 45 Ten Cate, AR: *Oral histology, development, structure and function*. Mosby. Year book, inc. 2004; 206-209.
- 46 Riba, R; Oberprieler, NG; Roberts,W and Naseem, KM: Von Willebrand factor activates endothelial nitric oxide synthase in blood platelets by a glycoprotein Ib-dependent mechanism. *J. Thromb. Haemost* 2006; 4(12):2636-44.
- 47 Miller, MJS and Grisham, MB: Nitric oxide as a mediator of inflammation ? You had better believe it. *Mediators inflamm* 1995; 4:109-42.
- 48 Amin, AR; Vyas, P; Attur, M and Leszcznsca-Piziak, J: The modes of action of aspirin-like drugs: effect on inducible nitric oxide synthase. *Proc.Natl.Acad. Sci: USA* 1995; 92: 7926-30.