Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

DENTAL PULP RESPONSE TO EPIGALLOCATECHIN GALLATE AS A VITAL PULPOTOMY AGENT

SHAIMAA MOHAMED MAHFOUZ OMER

Department of Paediatric Dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez University, Suez, Egypt. Email: shaimaa.mahfouz@den.suezuni.edu.eg

RANDA HAMED MOUSTAFA EL-SHERBINY *

Department of Oral Pathology, Faculty of Dentistry, Suez University, Suez, Egypt.
*Corresponding Author Email: randa.elsherbiny@den.suezuni.edu.eg

REHAM MOHAMED ALI ABDEL LATIF

Department of Paediatric Dentistry, Preventive Dentistry and Dental Public Health, Faculty of Dentistry, Suez Canal University, Ismailia, Egypt. Email: reham_mohamed@dent.suez.edu.eg.

Abstract

Background: A conservative procedure called a pulpotomy is used to keep decaying primary teeth in place until permanent teeth develop. The main polyphenol found in green tea, epigallocatechin gallate (EGCG), has several health benefits, including being an antioxidant, inflammatory reducer, antiseptic, and antibacterial. The objective of the present investigation was to histopathologically analyze and compare the dental pulp responses of dogs to EGCG and 1\5 concentration of Buckley's formula of FC as essential pulpotomy medicines. **Methods:** Two healthy Mongrel dogs' teeth were randomly selected for the experimental split-mouth study. EGCG and FC were used as vital pulpotomy agents. The treated teeth were assessed at four and nine weeks, and the pulp tissues were checked for fibrosis, inflammation, and other histological alterations. In addition to using an "independent samples t-test" to compare the means of two treated groups, a Paired t-test was performed to compare the means of the same group over two-time intervals. **Results:** When comparing the EGCG group at 4 weeks of treatment to the FC group, there was a noticeable reduction in pulp inflammation, vasodilation, and necrosis. After nine weeks of treatment, the FC group showed more destruction of the odontoblastic layer than the EGCG group, as well as substantial increase in fibrosis and necrosis. **Conclusion:** With respect to the study's limitations, the histological evaluation of the experimental animal model demonstrated that EGCG was more effective than FC as a pulp capping medication for vital pulpotomy.

Keywords: Green Tea, Pulpotomy, Epigallocatechin Gallate, Formocresol.

INTRODUCTION

One of the main goals of pediatric dentistry is to encourage primary teeth to stay in the mouth until their natural exfoliation phase.[1] Early primary tooth extraction has been found to cause space loss and malocclusion in both the mixed and permanent dentitions, which may later necessitate orthodontic treatment. As a result, it is more important to keep the child’s primary teeth than removing them to preserve the integrity of the arch by functioning as a natural space maintainer.[1] Identifying the causes and managing dental caries has received a lot of attention, as it is still the most frequent chronic disease globally, which leads to tooth loss in children. Dental caries leads to pulp exposure, necrosis as well as abscesses formation and later tooth loss. In these situations, pulp therapy is the first line of treatment since it maintains the radicular pulp's vitality and functionality.[2]

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

Pulpotomy is conducted in primary teeth with severe caries but no radicular disease. Pulp exposure may be performed during caries removal, after which the coronal pulp is removed, and the remaining healthy radicular pulp tissue is treated with a medicine such as ferric sulphate or Buckley's solution of formocresol (FC). [3] For many years, FC has been utilized successfully as a pulp dressing material. Since it has established bacteriostatic and fixative properties, it is the most frequently utilized medication in pulp treatment. Its composition is formaldehyde (19%), cresol (35%), glycerin (15%), and water. The International Agency for Research on Cancer (IARC) raised concerns about the substance's potential for carcinogenicity and toxicity in 2004; formaldehyde has been found to cause cancer in humans. As a result, experts are being advised to search for a less hazardous, safer pulp treatment that has a better success rate. [4]

Numerous pulp medicaments, including ferric sulphate, mineral trioxide aggregate (MTA), sodium hypochlorite, calcium hydroxide, laser, electrosurgery, freeze-dried bone, collagen, and bone morphogenic protein, have been clinically studied for the pulpotomy operation to replace FC.[5] However, no compromise regarding the proper pulpotomy material has arrived. To preserve radicular pulp tissues and encourage their healing, the perfect pulpotomy drug should include adequate sealing capability, bio-inductivity, biocompatibility, and antibacterial qualities.[6]

In contemporary dentistry, the use of herbal remedies has grown in popularity. The use of plants or plant extracts for medicinal purposes is known as phytotherapy, and it has become more popular in recent years.[7] Herbs' antibacterial and anti-inflammatory qualities have led to a rise in the usage of these remedies in the past few years for a variety of dental conditions.[8]

The antibacterial properties of green tea as herbal drug, and its unpolluted element and the effects of epigallocatechin gallate (EGCG) have been well studied. Approximately 59% of the total catechins found in green tea leaves are represented by the primary flavonoid component called EGCG. EGCG has been found to have several benefits on human physiological and pathological systems. It possesses broad-spectrum antibacterial, anti-inflammatory, antioxidant, and anticancer effects.[9,10] Furthermore, EGCG has been shown to have a reduction in the incidence of mutans streptococci, which is primarily accountable for the development of the caries lesion. [11]These results suggest that EGCG may inhibit the development of bacterial biofilms.[12]

Furthermore, in the coronal pulp, EGCG stimulates cell proliferation, reduces the inflammation that occurs in pulp stem cells, and withdraws their apoptosis in response to hypoxia destruction.[13] although previous studies have revealed that EGCG may prevent bacterial development, it is important to study the effects of EGCG as an important pulpotomy drug. The current investigation sought to examine and compare the histological responses of dental pulp to EGCG and a 1/5 concentration of Buckley's formula of FC as a vital pulpotomy capping agent.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

MATERIALS AND METHODS

Materials: At the Nawah Scientific Laboratory (Al-Mokattam St., Cairo, Egypt), the EGCG was extracted from green tea, next, EGCG was dissolved in a polyethylene glycol (PEG) hydrogel base (a blend of 80% PEG 400 and 20% PEG 4,000) at a concentration of 90 parts per million. Concentration of FC 1/5 (Indian supplier: PREVEST DenPro Limited). Riva Australia-SDI limited uses glass ionomer cement. Eugenol paste with zinc oxide produced by PREVEST DenPro Limited in India. The source of all other materials was Sigma-Aldrich, located in St. Louis, Missouri, USA.

Study Design: This work is comparative experimental animal study using split mouth technique. The same dog was evaluated for two critical pulpotomy treatments at two distinct time points (four and nine weeks).

Study Setting & Ethical Consideration: Experimental trial was implemented in the Faculty of Veterinary Medicine, Suez Canal University. The histological analysis was carried out by the Oral Pathology Department of the Suez University Faculty of Dentistry. The current study has been given ethical permission by the research ethics committee of the Suez Canal University Faculty of Dentistry (approved No. 397/2021), respecting the Helsinki Declaration of 1964 and any subsequent modifications. The ARRIVE (Animal Research: Reporting in Vivo Experiments) protocols for documenting animal research were followed in the conduct of this study.[14]

Sample Size Calculation: Using G*Power (version 3.1.9.6, University of Kiel, Germany), the sample size was estimated. With an alpha (α) level of 0.05 and a beta (β) level of 0.05, the effect size (d) was 0.70, meaning that the power was 95%, Twenty-four samples made up the estimated total sample size (n). Each group contained twelve samples, split equally; the first six samples were assessed after four weeks, and the remaining samples were assessed after nine weeks.

Eligibility Criteria: Two male mongrel dogs, weighing between 10 and 12 kg, and pathogen-free, provided twenty-four of their posterior teeth. There were no cavities or fractures in any of the posterior teeth. Dogs exhibiting signs of a systemic disease or wound infection were forbidden.

Randomization and Group Assignment (figure 1): For the four and nine-week intervals, two dogs were randomly selected using the coin flip method. For each capping material, a different mouth side was chosen at random for each dog. For each of the six posterior teeth (three maxillary and three mandibular) selected from each side, each capping material was applied utilizing two opposing quadrants (split mouth technique for each dog). The two medications were tried in the same animal on different sides of the mouth cavity.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

The selected teeth were split into two treated groups based on the kind of capping material that was employed as the following:

Group 1 (experimental group): twelve teeth received EGCG hydrogel pulpotomy treatment.

Group 2 (control group): twelve teeth received pulpotomy treatment utilizing FC.

Clinical Procedures: - General anesthesia was established in the Suez Canal University Faculty of Veterinary Medicine's Department of Anesthesia. A 6 mg/kg intramuscular injection of thiopental sodium (EIPICO business, Egypt) was administered to each dog.[15] all teeth in both jaws were sterilized with Betadine, then sealed off with a rubber barrier and quickly emptied to control saliva. After that, teeth were cleaned using PeridexTM, a 0.12% chlorhexidine gluconate solution from 3M, USA. A sterile large round bur was used to construct the coronal access cavity, which was subsequently cooled with water to perform pulpotomies. A large, sharp spoon excavator was used to extract the pulp's coronal portion. Following a thorough irrigation of the pulp chamber cavity with regular saline, the pulp stumps were treated with wet cotton pellets soaked in saline to stop the bleeding completely until it was under control. In the FC group, pulp stumps were covered for five minutes with a moistened & pressed tiny cotton pellet with 20% Buckley's FC (1/5 dilution). EGCG hydrogel was used to seal pulp stumps in the EGCG group. Following that, a 2 mm layer of zinc oxide-eugenol paste was applied to the pulp stumps in both groups, and glass ionomer filler was used to fill them.

Animal Housing

After the pulpotomy, the animals were given the care prescribed by the Faculty of Veterinary Medicine. While the dogs were being fed a typical diet of food and water in a 12-hour cycle of light and dark, a knowledgeable veterinary assistant took measurements of the microbiological control, temperature, ventilation, lightning, and noise.

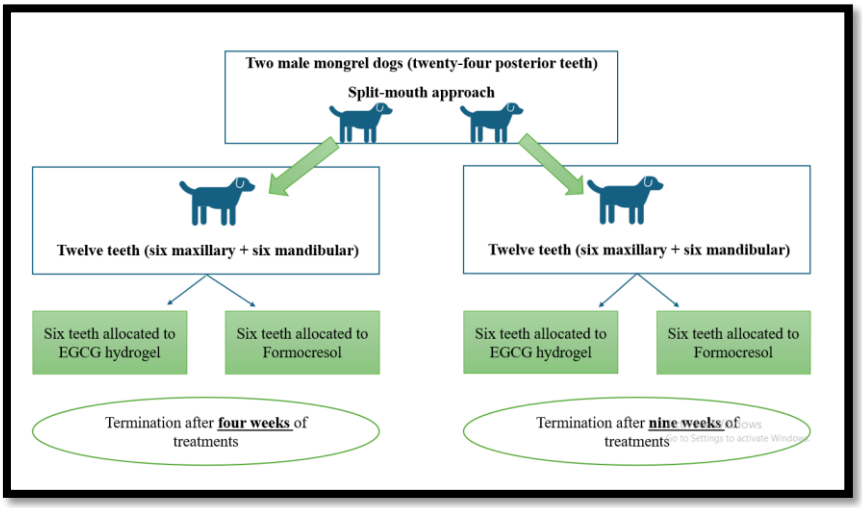

Figure 1: Randomization and Group Assignment

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

Histological Analysis:

One dog's treatment was stopped after four weeks, while the other dog after nine weeks. The dogs were given a 10% formalin injection into the common carotid artery. Every dog's two jaws were taken apart, and each region was cut into smaller pieces that each included a single tooth and its surrounding tissues. Then, to help the fixation solution spread rapidly, horizontal cuts beneath the apex at around 5 mm were done. Following that, teeth were placed for ten days in a fixative solution (10% neutral buffered formalin). Decalcification of teeth was done by Sodium citrate-buffered formic acid for a duration of 10 weeks. The teeth were then submerged in ethanol solutions at increasing concentrations (60, 70, 90, and 100%) to dehydrate them and washed with xylene clearing agent. After being placed into paraffin blocks at 60 °C, each specimen was given time to cool to 20 °C. Subsequently, a microtome was used to cut six consecutive segments of 5μm from each specimen in a bucco-lingual course, parallel to each tooth's vertical axis. Tissues were stained by the following:

A-Hematoxylin and Eosin (H&E): Fischer et al. staining methodology was done.[16] Following staining, each slide was examined under a light microscope (Japan's Olympus Co.; Olympus BX40) with Soft Imaging Analysis System (BX50F4) to analyze photomicrographs at 10x and 40x magnification. Evaluation of inflammatory cell response, vasodilation, pulp fibrosis and necrosis severity were evaluated in accordance with Heyeraas et al.[17] as follow: absent or normal tissue=0, mild=1, moderate=2, and severe=3. Besides, continuous odontoblastic layer=0 while interrupted=1

B-Masson Trichrome Stain: Slices were dyed in accordance with the methodology developed by Widbiller et al.[18] to identify the development of collagen fibers. After deparaffinizing, rehydration of the slices was done by descending concentrations of alcohol then stained by Weigert's iron haematoxylin working solution (10 minutes), Biebrich scarlet acid fuchsin solution (15 minutes), light green SF yellowish solution (10 minutes), followed by 1% acetic acid solution (5 minutes). Following that, they were dehydrated using 95% and 100% alcohol and cleaned with distilled water. They were eventually cleaned in xylene.

The degree of fibrosis was evaluated quantitatively using the "Area Percentage" method to precisely estimate the amount of fiber. Quantitative analysis along the tooth area as area % was carried out using the image analysis system (BX50F4) (six images/sample, 10 x Objective Lens). Mean ± Standard Error was used to express the area percentage results.

Statistical Analysis

The statistical package for social science (SPSS, 26.0; IBM Corp., Armonk, NY) was utilized to calculate, tabulate, and analyze all the data. Frequencies (n) and percentages (%) were used to present the qualitative data. Mean Standard Deviation (SD) and range (Max-Min) were the formula for calculating descriptive statistics. A paired sample T-test was used to compare the same groups at different time intervals. A P-value of less than 0.05 was accepted as statistically significant.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

RESULTS

Hematoxylin and Eosin Staining Results:

Histologic Changes Results in EGCG Group (Figure 2, Figure 3 [A&B] and Figure 4 [A&B]):

Following four weeks of treatment, in 83.3% of samples, the odontoblastic cells in the pulp were regular, and the pulp tissue demonstrated different degrees of inflammation from mild to moderate in an equal distribution. Vasodilation ranged from mild to moderate (66.7% and 33.3% respectively) and pulp necrosis was absent in most of the tissues (83.3%), and only 16.7% had a minor necrosis. Of the samples, mild to moderate fibrosis was present (66.7% and 33.3%, respectively). Following nine weeks of treatment, in all samples, the odontoblastic cells in the pulp exhibited a normal appearance and the pulp showed mild inflammatory cell infiltration in all samples. The cellularity and fibrous components of the pulp tissue were almost identical to the pulp architecture of the healthy pulp. The areas apical to the inflamed region included vital pulp tissues that appeared normal. Furthermore, vasodilation ranged from absence to mild present in tissues (16.7% and 83.3%, respectively). Only 16.7% of instances had minor pulp necrosis, whereas the majority (83.3%) showed no pulp necrosis at all. In 83.3% of the samples, mild pulp fibrosis was visible. In all samples, the odontoblastic cells in the pulp showed a continuous appearance. The results above show that there was no significant difference between the EGCG groups at 4 and 9 weeks (figure 6A).

Histologic Changes Results in FC Group (Figure 2, Figure 3 [C&D] and Figure 4 [C&D]):

Following four weeks of treatment, 66.7% of samples showed interrupted odontoblastic cell layer and 83.3% showed a dense infiltration of inflammatory cells as well as the production of micro-abscesses while 16.7% only showed moderate inflammation. Blood vessel vasodilation ranged from mild to severe, and there were different levels of pulp necrosis: mild (33.3%), moderate (16.7%), severe (33.3%), and nonexistent (16.7%). Mild, moderate, severe fibrosis were present (16.7% 66.7, 16.7%, respectively). Following nine weeks of treatment, 83.3% of the odontoblastic cells were arranged in a discontinuous manner and pulp tissues showed mild to moderate inflammation (66.7% and 33.3%, respectively). Vasodilation ranged from mild to moderate (66.7% and 33.3%, respectively). Most of samples (66.7%) showed severe pulp necrosis while in 33.3% of cases, it was moderate as well as the pulp showed vacuolated regions and degenerative changes. Of the samples, 16.6% had moderate pulp fibrosis and 83.3% had severe fibrosis. (Figures 2-D and 3-D). The results of the FC group demonstrated significant variations between four and nine weeks, with inflammation and vasodilation reducing dramatically at 9 weeks but fibrosis and necrosis increasing significantly, as seen in figure 6A. Based on all the previously mentioned data, after 4 weeks and compared to FC group, the EGCG group showed a significant decrease in inflammation, vasodilation, and necrosis (figure 6C) as well as after 9 weeks it showed highly significant reduction in

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

fibrosis and necrosis (figure 6B). Furthermore, odontoblastic layer degradation was significantly decreased compared to the FC group.

Masson's Trichrome Stain's Quantitative Assessment of the Degree of Fibrosis in the Pulp Tissue (Figure 5, Table 1):

After comparing the period intervals of each group independently, it was found that the mean values rose as the time intervals increased from 4 to 9 weeks. The only group to exhibit a significant difference between the 4- and 9-week time intervals was the FC group (30.95 ±1.588 and 68.207 ±0.588, respectively) (P<0.001). The EGCG and FC groups showed statistically significant differences at different time intervals. After 4 and 9 weeks, respectively, the mean values for the FC group were lower than those of the EGCG group as shown in table 2.

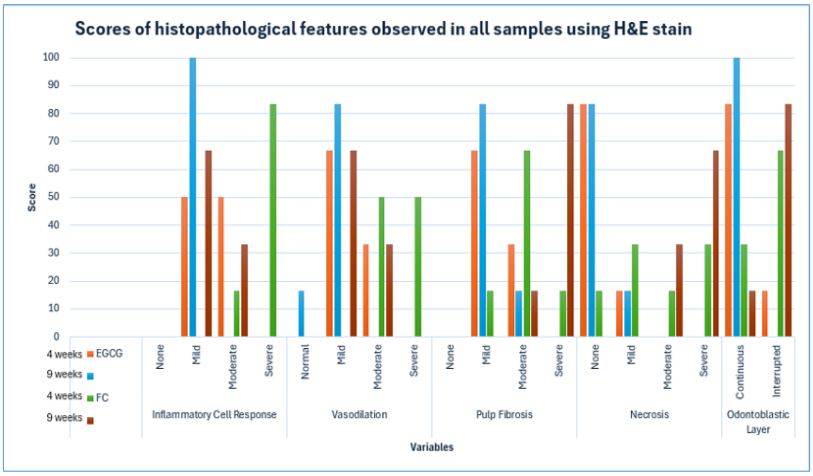

Figure 2: Scores of Histopathological Evaluations by using H&E Stain

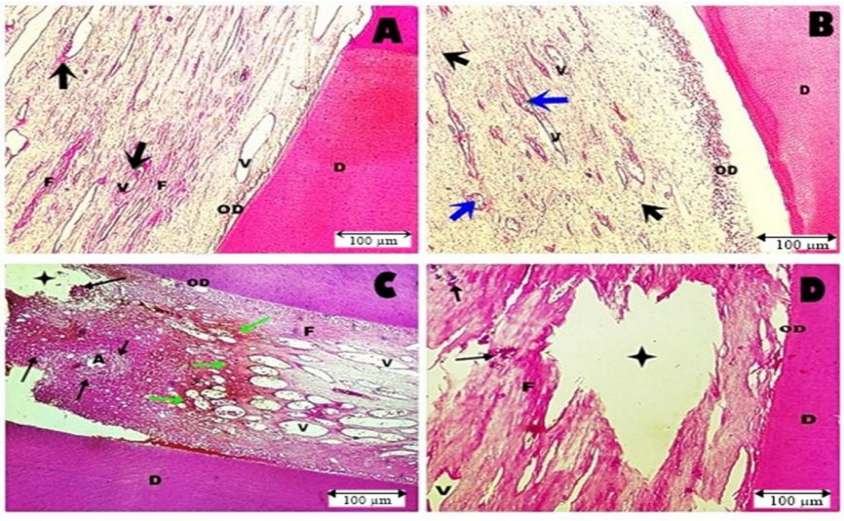

Figure 3: Photomicrographs show the Histopathology of Pulp Tissue in Several Groups

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

A: After four weeks, the pulp tissue in the EGCG group demonstrates mild to moderate pulp fibrosis (F) (black arrows), few extravasated red blood cells, mild to moderate vasodilation (V), and intact odontoblastic layer (OD). B: After nine weeks, the pulp tissue in the EGCG group has mild pulp fibrosis along with an intact odontoblastic layer (OD), mild vasodilated blood vessels (V), a few extravasated red blood cells (blue arrows), and a scattering of inflammatory cells (black arrows). C: Pulp tissue in the FC group (4 weeks) displays vacuolated regions (star), severe vasodilation (V), extravasated red blood cells (green arrows), dense acute chronic inflammatory cells (black arrows) with micro-abscess development (A), and significant pulp fibrosis. D: After nine weeks of treatment, the pulp tissue in the FC group exhibits significant pulp fibrosis, necrosis, and vacuolated regions (star), mild inflammation (black arrows), interrupted odontoblastic layer (OD), and moderate vasodilation (V). (H&E X 10)

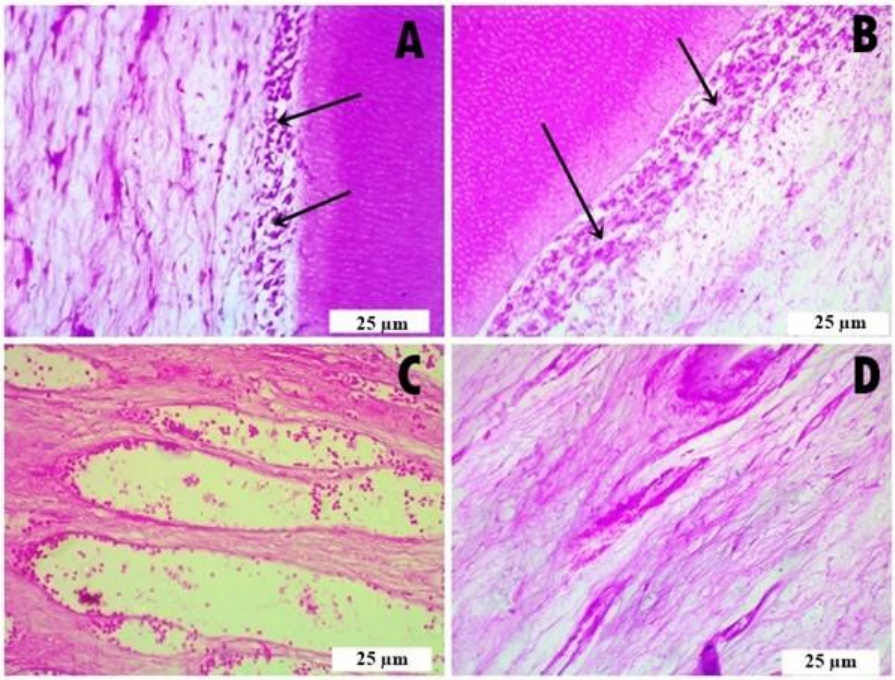

Figure 4: Higher Magnification of Histopathological Photographs of Pulp Tissue in Several Groups

A: The pulp tissue in the four-week EGCG group exhibits minor pulp fibrosis and an intact odontoblastic layer (arrows). B: The pulp tissue in the nine-week EGCG group has normal-appearing pulp tissue and an intact odontoblastic layer (arrows). C: After four weeks, the pulp tissue in the FC group exhibits significant vasodilation and extravasated red blood cells. D: After nine weeks of treatment, the pulp tissue in the FC group exhibits significant pulp fibrosis. (H&E X 40)

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

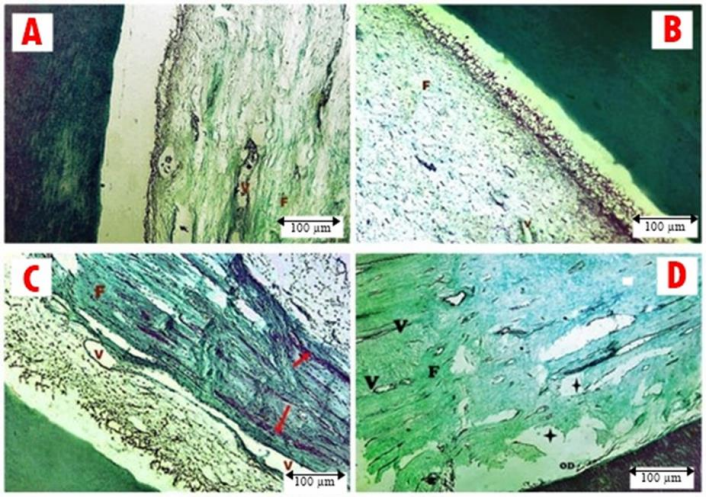

Figure 5: Photomicrographs of Pulp Tissue from Several Groups Stained with Masson's Trichrome

A: After four weeks of medication, the EGCG group exhibits mild to moderate fibrosis (F) and vasodilation (V). B: After nine weeks, the pulp tissue of the EGCG group has normal-appearing blood vessels (V) and a slight rise in fiber density (F). C: The FC group's (4-week) pulp tissue exhibits a significant increase in collagen fibers (F), moderately dilated blood vessels (V), and extravasated red blood cells (arrows). D: The pulp tissue from the FC group (9 weeks) exhibits significant increases in collagen fibers (F), mild vasodilated blood vessels (V), vacuolated and necrotic parts (stars), and remnants of the odontoblastic layer (OD). (Masson trichrome stain X10).

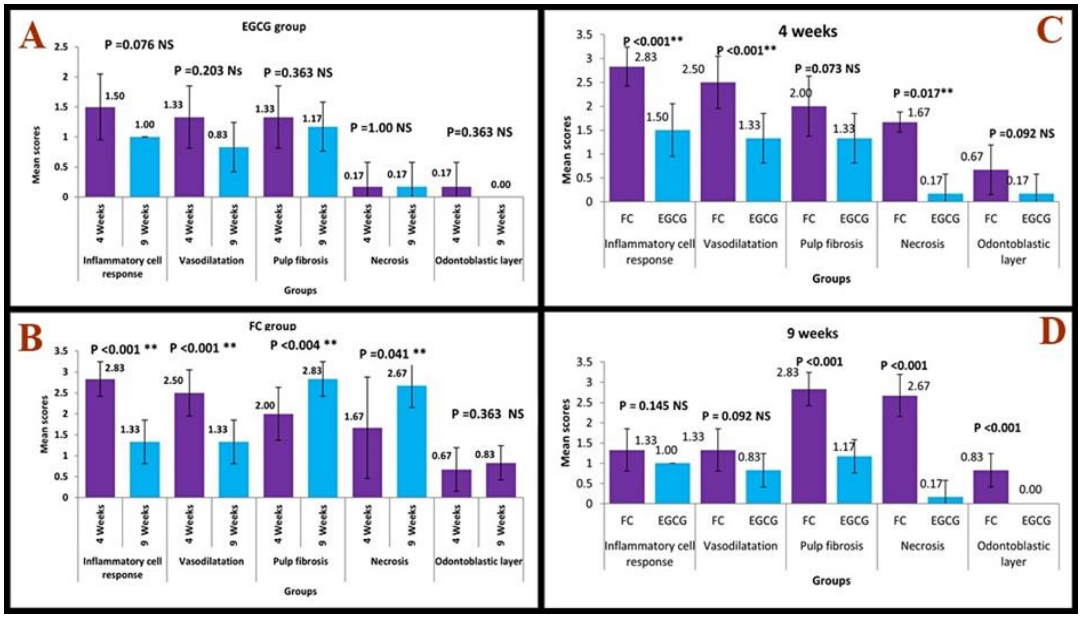

Figure 6: Statistical Analysis Results of H&E Stain to Coronal and Radicular Portions of the Pulp

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

A: EGCG group results in four and nine weeks. B: FC group results in four and nine weeks. C: EGCG and FC group results in four weeks. D: EGCG and FC group results in nine weeks.

Table 1: Degree of Fibrosis' Quantitative Analysis (Masson Trichrome Stain)

Groups \ Weeks	Four weeks		Nine weeks		Paired T-test	*P* value
	Mean	Standard Deviation	Mean	Standard Deviation		
Epigallocatechin gallate group	2.767	0.639	3.360	0.221	1.347	0.236
Formcresol group	30.952	1.588	68.207	0.588	32.52	<0.001**
Independent t-test	40.31		103.22			
P value	<0.001**		<0.001**			

DISCUSSION

In this preliminary experimental animal investigation, the histological effect of EGCG as a new pulp medication used in pulpotomy was measured in dogs' teeth. The main clinical features of successful pulpotomy are the absence of discomfort, preservation of pulp vitality, low levels of pulpal inflammation, the pulp's ability to self-preserve without degenerative consequences, and the lack of radicular and internal inflammatory resorptions.[19] A good pulpotomy medication should has bio-inductive properties and be biocompatible, antimicrobial as well as harmless for the pulp tissue and surrounding structures. It will additionally minimize bacterial microleakage, promote radicular pulp healing, while only minimally interfere with the normal process of resorption of roots.[20]

To examine and quantify the effect of EGCG as vital pulpotomy agent, the pulp's response to it was measured by histopathological assessment in dogs' teeth. Murray and Garcia-Godoy suggest that the best pre-clinical trial to assess a new material's biocompatibility on human tooth tissue is an experiment on experimental animals.[21] This study was conducted on dog teeth to evaluate and compare the pulpal tissue reaction to EGCG and FC as pulp capping drugs for essential pulpotomy. According to Omar et al., who studied the effect of *Nigella sativa* on the dogs' pulp tissue over a four-week period, in this experimental animal study, two assessment periods, each lasting four and nine weeks, are sufficient for evaluating the inflammatory tissue reaction and pulp response.[22] Additionally, a study using one-to nine-week follow-up intervals, El-Zekrid et al. evaluated autologous bone marrow's ability to repair dogs' teeth that had undergone partial pulpotomy.

The most widely used pulpotomy medication is formocresol, concerns with its cytotoxicity have prompted a number of investigations to find healthier alternatives. This study utilized EGCG, principal polyphenol in green tea,[23] as it has strong anti-inflammatory, antibacterial and antioxidant properties with low toxicity as well as high biocompatibility.[24] When pulp canals are being prepared for regenerative endodontic therapy, EGCG inhibits the growth of several bacterial species. According to Lee and Tan, *Enterococcus faecalis's planktonic* and biofilm systems are susceptible to the antibacterial effects of EGCG. These characteristics stop bacteria from growing and eliminate the presence of

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

genes linked to pathogenicity and the formation of biofilms.[25] The current work used injectable EGCG hydrogel because solid biomaterial insertion cement less effectively to dentine walls and is hence less suited for pulp-dentine complex regeneration, so EGCG hydrogel is preferred for dentin formation.[26] This corresponds to the application of EGCG hydrogel by Kwon et al. in regenerative endodontic treatment as a substitute for hard materials to promote the proliferation of human dental pulp cells as well as inhibit bacterial dissemination.[27] The 90 ppm concentration of EGCG hydrogels utilized in this investigation was consistent with the results of Ismiyatin et al., who discovered that a concentration of 90 ppm significantly increased the number of macrophage cells on day three of pulp inflammation caused by mechanical trauma. It prevented inflammation from progressing to pulp necrosis by shortening inflammation and promoting tissue regeneration through the proliferative process. [28]

Both H&E and Masson trichrome staining were utilized in this work to obtain an evaluation of dental pulp tissue. After four weeks, the histopathological examination of the FC-treated samples showed the development of micro-abscesses and dilatation of the blood vessels and abundant inflammatory cells in most of the samples with mild fibrosis of the pulp, necrosis spreading, and discontinuities in the odontoblast layer. Current results above, are in line with those of Omar et al. (2012), using H&E for histological assessment, noted interrupted odontoblastic cells of the pulp in group treated with FC. Furthermore, at nine weeks, the presence of vacuolated regions exacerbated pulpal necrosis and degenerative alterations. The odontoblastic cells in the pulp showed more irregularities than those at four weeks. The loss of integrity of the odontoblasts' layer suggests that the inability of the FC treated group to proliferate may have caused irreversible damage to the cells.[29] El-Tawil et al. reported that degenerative areas and fibrous tissue with inflammatory changes were present with FC medication.[30] The area of pulp exposure showed the presence of abscesses in the FC group. Additionally, FC caused the pulp to become chronically inflammatory, which increases the likelihood that an abscess may form.[31]

At four and nine weeks of treatment, the results for FC samples demonstrated a notable decrease in pulp vasodilation and inflammation. Furthermore, there was a notable rise in necrosis, which might be brought on by the detrimental effects of capping material, and a notable increase in pulpal fibrosis, which might point to a persistent inflammatory response of FC. This is accordance to the results of Jabbarifar et al., who observed that after three months, the FC group had much more necrosis than the groups that had hydroxyapatite and bioactive glass treatment.[32]

On the other side, after four and nine weeks of therapy, the mild to moderate inflammation and minor vasodilation were seen in the EGCG groups may have been caused by the compound's antibacterial and anti-inflammatory properties.[33] moreover, EGCG's antioxidant properties may reduce the duration of the inflammation by inhibiting the formation of inducible nitric oxide synthase as well as lowering nitric oxide levels which speeds up healing.[34] The majority of the samples showed no pulpal necrosis and a continuous arrangement of the pulp's odontoblastic cells. This could be because EGCG

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

has non-irritating properties. This contrasts with the study of Kwon et al. reported that EGCG by itself did not promote the growth of human dental pulp cells by odontogenic differentiation.

The FC group's persistent inflammation may be related to the significant rise in collagen fiber density that Masson's trichrome staining of the FC samples revealed. The histopathological findings are consistent with earlier research conducted by El-Tawil et al. (2009). Furthermore, at four weeks, Salako et al. noticed a significant amount of fibrosis in the FC group; this may suggest that FC stimulates pulpal tissue fibroblasts to create additional collagen fibers that indicate the development of chronic inflammation.[35] Although EGCG samples demonstrated that pulp tissue showed a small increase in collagen fiber density, this can be related to EGCG's ability to maintain pulp tissue vitality by limiting fibrous replacement of pulp. Overall, the findings demonstrated that EGCG is a significant pulpotomy agent that is more effective than FC. Nonetheless, there are certain disadvantages to this work. For example, applying EGCG hydrogel to pulp tissue was both expensive and complicated. Furthermore, split-mouth designs might be difficult to carry out and have a small sample size as well as the model employed in this study was not contagious and caries-free, resulting in an inaccurate simulation of pulp inflammation in carious teeth.

CONCLUSION

Topical application of EGCG hydrogel in tooth cavities of dogs showed very good results in preservation of the integrity of pulp tissue. Considering the EGCG biological activities including antioxidant, anti-inflammatory antimicrobial as well as bactericidal effects, EGCG hydrogel could be used as an alternative pulpotomy agent to FC.

RECOMMENDATION

It would be beneficial to conduct additional long-term clinical trials to evaluate the efficacy of EGCG as a pulp capping material in vial pulpotomy for both permanent and primary teeth. In the future, more infection model validation will be needed.

Acknowledgements

We especially thank Dr. Mohamed A.M. Hashem of the Suez Canal University Thank you to the Department of Anesthesiology and Radiology at the Faculty of Veterinary Medicine for your help.

Declarations

Funding

No particular grants from public, private, or governmental entities were used to finance this research.

Conflict of Interest

There was no conflict of interest, as far as the authors were concerned. This study was conducted without the assistance of outside funding.

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

Author Contributions

The methodology was conceived and conceptualized by Sh.M.M & R.H.M. For every dog, Sh.M.M. carried out the pulpotomy procedures. R.H.M., Sh.M.M, and R.M.A. conducted the histological examination, gathered data, ran statistical analyses, and wrote the first draft of the publication. The manuscript was edited, and the final version was prepared by Sh.M.M. R.H.M., and R.M.A. All authors have read and approved the final manuscript.

References

1) Kher MS, Rao A. Pulp therapy in primary teeth. In: contemporary treatment techniques in pediatric dentistry. Contemp Treat Tech Pediatr Dent 2019; 75–98. DOI: 10.1007/978-3-030-11860-0_3

2) Bhujel N, Duggal MS, Saini P, et al. The effect of premature extraction of primary teeth on the subsequent need for orthodontic treatment. Eur Arch Paediatr Dent 2016; 17(6):423–434. DOI: 10.1007/s40368-016- 0247-7

3) Smaïl-Faugeron V, Glenny AM, Courson F, et al. Pulp treatment for extensive decay in primary teeth. Cochrane Database Syst Rev 2018; 5(5):CD003220. DOI: 10.1002/14651858.CD003220.pub3

4) Seale, N.S. & Coll, J.A. Vital pulp therapy for the primary dentition. Gen Dent.58, 194-200 (2010).

5) Cogliano VJ, Grosse Y, Baan RA, et al. Meeting report: summary of IARC monographs on formaldehyde, 2-butoxyethanol, and 1-tertbutoxy- 2-propanol. Environ Health Perspect 2005; 113(9):1205–1208. DOI: 10.1289/EHP.7542

6) Bossù M, Iaculli F, Di Giorgio G, et al. Different pulp dressing materials for the pulpotomy of primary teeth: a systematic review of the literature. J Clin Med 2020; 9(3). DOI: 10.3390/jcm9030838

7) Samiei, M. et al. Zeolite-silver-zinc nanoparticles: Biocompatibility and their effect on the compressive strength of mineral trioxide aggregate. J Clin Exp Dent.9, e356-e360 (2017). https://doi: 10.4317/jced.53392.

8) Matole V, Thorat Y, Ghurghure S, et al. A brief review on herbal medicines. Res J Pharmacogn Phytochem 2021; 13(2):101–102. DOI: 10.52711/0975-4385.2021.00016

9) Nair, G.R. et al. Clinical effectiveness of aloe vera in the management of oral mucosal diseases- A systematic review. J Clin Diagn Res.10, ZE01-7(2016). https://doi: 10.7860/JCDR/2016/18142.8222.

10) Gan, R.Y. Li, H.B. Sui Z.Q., Corke H. Absorption, metabolism, anti-cancer effect and molecular targets of epigallocatechin gallate (EGCG): An updated review Critical Reviews in Food Science Nutrition, 58 (6) (2018), pp. 924-941,

11) Chu, C. Deng J., Man, Y. Qu Y. Green tea extracts epigallocatechin-3-gallate for different treatments Biomed Research International, 2017 (2017), Article 5615647,

12) Salama, M.T. & Alsughier, Z.A. Effect of green tea extract mouthwash on salivary streptococcus mutans counts in a group of preschool children: an in vivo study. Int J Clin Pediatr Dent.12,133-138 (2019). https://doi: 10.5005/jp-journals-10005-1610

13) Chakrawarti, L.; Agrawal, R.; Dang, S.; Gupta, S.; Gabrani, R. Therapeutic effects of EGCG: A patent review. Expert Opin. Ther. Pat. 2016, 26, 907–916.

14) Li, Y., Zhao, Y., Han, J., Wang, Y. & Lei, S. Effects of epigallocatechin gallate (EGCG) on the biological properties of human dental pulp stem cells and inflammatory pulp tissue. Arch Oral Biol.123:105034(2021). https://doi: 10.1016/j.archoralbio.2020.105034

15) Kilkenny, C., Browne, W., Cuthill, I.C., Emerson, M. & Altman, D.G. NC3Rs Reporting Guidelines Working Group. Animal research: reporting in vivo experiments: the ARRIVE guidelines. J Gene Med.12, 561-563(2010). https://doi: 10.1002/jgm.1473

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

16) El-Zekrid, M.H., Mahmoud, S.H., Ali, F.A., Helal, M.E.& Grawish, M.E. Healing capacity of autologous bone marrow-derived mesenchymal stem cells on partially pulpotomized dogs' teeth. J Endod. 45, 287-294 (2019). https://doi: 10.1016/j.joen.11.013

17) Fischer, A.H., Jacobson, K.A., Rose, J. & Zeller, R. Haematoxylin and eosin staining of tissue and cell sections. CSH Protoc., 1; 2008:pdb.prot4986. (2008) https://doi: 10.1101/pdb.prot4986

18) Heyeraas, K.J., Sveen, O.B. & Mjör, I.A. Pulp-dentin biology in restorative dentistry. Part 3: Pulpal inflammation and its sequelae. Quintessence Int.32, 611-625 (2001).

19) Widbiller, M. et al. Histology of human teeth: Standard and specific staining methods revisited. Arch Oral Biol.127, 105136. (2021). doi: 10.1016/j.archoralbio.2021.105136

20) Igna A. Vital Pulp Therapy in Primary Dentition: Pulpotomy-A 100-Year Challenge. Children (Basel). 2021 Sep 24; 8(10):841. doi: 10.3390/children8100841. PMID: 34682106; PMCID: PMC8534739.

21) Ratnakumari, N. & Thomas, B. A histopathological comparison of pulpal response to chitra-cpc and formocresol used as pulpotomy agents in primary teeth: A clinical trial. Int J Clin Pediatr Dent.5, 6-13 (2012). https://doi: 10.5005/jp-journals-10005-1126

22) Murray, P.E. & Garcia-Godoy, F. Comparison of the clinical and preclinical biocompatibility testing of dental materials: are the ISO usage tests meaningful? J Biomed Mater Res A.81, 51-58 (2007). https://doi: 10.1002/jbm.a.31015

23) Omar, O.M., Khattab, N.M. & Khater, D.S. Nigella sativa oil as a pulp medicament for pulpotomized teeth: a histopathological evaluation. J Clin Pediatr Dent. 36,335-341(2012). https://doi: 10.17796/jcpd.36.4.n6674435856q86w8

24) Vilela, M.M., Salvador, S.L., Teixeira, I.G.L., Del Arco, M.C.G. & De Rossi, A. Efficacy of green tea and its extract, epigallocatechin-3-gallate, in the reduction of cariogenic microbiota in children: a randomized clinical trial. Arch Oral Biol.114, 104727 (2020). https://doi: 10.1016/j.archoralbio.2020.104727

25) Jung, I.H. et al. Anti-inflammatory effect of (-)-epigallocatechin-3-gallate on Porphyromonas gingivalis lipopolysaccharide-stimulated fibroblasts and stem cells derived from human periodontal ligament. J Periodontal Implant Sci.42, 185-195 (2012). https://doi: 10.5051/jpis.2012.42.6.185

26) Lee, P. & Tan, K.S. Effects of Epigallocatechin gallate against Enterococcus faecalis biofilm and virulence. Arch Oral Biol.60, 393-399(2015). https://doi: 10.1016/j.archoralbio.2014.11.014

27) Gong, T., Heng, B.C., Lo, E.C. & Zhang, C. Current advance and future prospects of tissue engineering approach to dentin/pulp regenerative therapy. Stem cells int. 2016:9204574 (2016). https://doi: 10.1155/2016/9204574

28) Kwon, Y.S. et al. Effects of epigallocatechin gallate, an antibacterial cross-linking agent, on proliferation and differentiation of human dental pulp cells cultured in collagen scaffolds. J Endod.43, 289-296(2017). https://doi: 10.1016/j.joen.2016.10.017

29) Ismiyatin, K., Juniarti, D., Purwanto, D. & Primaza□ra, A. Effect of epigallocatechin-3-gallate (EGCG) on the number of macrophage cells in in□ammation of pulp with mechanical injury. Conserv DentJ.10, 31-35 (2020). https://doi: 10.20473/cdj.v10i1.2020.31-5.

30) Murray, P.E., Kitasako, Y., Tagami, J., Windsor, L.J. & Smith, A.J. Hierarchy of variables correlated to odontoblast-like cell numbers following pulp capping. J Dent.30, 297-304(2002). https://doi: 10.1016/s0300-5712(02)00024-6

31) El-Tawil, S.B., El-Dokki, N.A. & Aly, Z.H. Comparative evaluation of Jojoba oil versus formocresol pulpotomy in primary molars- in vitro study. Pak Oral Dental J. 29:85–92 (2009).

Tianjin Daxue Xuebao (Ziran Kexue yu Gongcheng Jishu Ban)/
Journal of Tianjin University Science and Technology
ISSN (Online):0493-2137
E-Publication: Online Open Access
Vol: 57 Issue: 07:2024
DOI: 10.5281/zenodo.12798170

32) Ranly, D.M. Pulpotomy therapy in primary teeth: new modalities for old rationales. Pediatr Dent.16, 403-409 (1994).

33) Jabbarifar, E., Razavi, S.M. & Ahmadi, N. Histopathologic responses of dog's dental pulp to mineral trioxide aggregate, bio active glass, formocresol, hydroxyapatite. DRJ.4, 83-87 (2007).

34) Hamilton-Miller, J.M.T. Anti-cariogenic properties of tea (Camellia sinensis). J Med Microbiol. 50, 299-302 (2001). https://doi: 10.1099/0022-1317-50-4-299

35) Silna, E., Krishnakumar, K., Nair, S.K., Narayanan, A. & Dineshkumar, B. Hydrogels in topical drug delivery – A review. IJIDD.6, 87-93 (2016).

36) Salako, N. et al. Comparison of bioactive glass, mineral trioxide aggregate, ferric sulfate, and formocresol as pulpotomy agents in rat molar. Dent Traumatol.19, 314-320 (2003). https://doi: 10.1046/j.1600-9657.2003.00204.x