EGYPTIAN DENTAL JOURNAL
www.eda-egypt.org
VOL. 69, 741:750, JANUARY, 2023
PRINT ISSN 0070-9484 • ONLINE ISSN 2090-2360
CONSERVATIVE DENTISTRY AND ENDODONTICS
Submit Date : 02-09-2022 • Accept Date : 14-11-2022 • Available online: 1-1-2023 • DOI : 10.21608/edj.2022.159147.2238

EFFECT OF PROPOLIS, NEEM AND CALCIUM HYDROXIDE AS INTRACANAL MEDICAMENTS ON THE SEALING ABILITY OF RESIN-BASED ENDODONTIC SEALER

Mai Sayed Hanafy* *and* Hebatallah Atef Fathallah**

ABSTRACT

Aim: This in-vitro study was conducted to evaluate the effect of 2 herbal extracts (Propolis, Neem) and calcium hydroxide, when used as intracanal medicaments, on the sealing ability of resin-based endodontic sealer.

Materials and Methods: A total of 60 extracted, human, permanent, single-rooted, maxillary, anterior teeth with fully formed apices and straight roots were collected for this study. All specimens were coronally resected to a standardized length of 15 ± 1 mm and root canals were mechanically prepared using crown down technique. The prepared teeth were divided into five groups: Group I, Positive control (teeth were instrumented but not root-canal filled); Group II, No intracanal medicament was used; Group III, Propolis gel was used; Group IV, Neem gel was used; Group V, Injectable calcium hydroxide was used. Intracanal medicaments were left for 7 days after which they were removed, and the specimens were obturated using gutta percha with AH Plus sealer by cold lateral compaction technique. Sealing ability was assessed using dye extraction method. Statistical analysis was carried out by one-way analysis of variance followed by Post-hoc Tukey test.

Results: The results showed that propolis group had better sealing ability than neem group but with no significant difference. Both propolis and neem medicated groups had statistically higher sealing ability for AH Plus sealer when compared to the calcium hydroxide medicated group.

Conclusion: The authors concluded that Propolis and Neem intracanal medicaments can be used as alternatives to calcium hydroxide without relatively affecting the obturated root canal final seal.

KEYWORDS: Sealing ability, Neem, Propolis, intracanal medicament, epoxy resin-based sealer.

* Lecturer of Endodontics, Faculty of Dentistry, Beni-Suef University, Egypt
** Lecturer of Endodontics, Faculty of Oral and Dental Medicine, Nahda University in Beni Suef, Egypt

INTRODUCTION

Fluid tight seal creation after eradication of microorganisms and their byproducts from infected root canal system is the essence of successful endodontic therapy [1,2]. Thorough biomechanical preparation of the infected root canal can help in reducing the number of microorganisms but not completely eliminating them [3]. This is owing to the inherent root canal anatomical complexity that hinders accessing the canal by instruments and irrigants for complete disinfection [4]. Hence, the adjunctive use of interappointment intracanal medicament (ICM) can assist in eradicating the remaining bacteria resulting in proper disinfection of the root canal [5].

Worldwide, calcium hydroxide (CH) is the most frequently used ICM due to its antimicrobial/anti-inflammatory properties with the ability to promote periradicular healing [6,7]. Yet, studies reported its limited action against Enterococcus faecalis and Candida albicans species which are main cause for failure of endodontic treatment [6,8,9].

Plant-derived natural biocompatible agents have strongly gained research interest in the endodontic field due to their markable antibacterial, anti-inflammatory and antioxidant properties [10]. These agents have been widely investigated as better, safer, and more potent alternative intracanal medicaments to overcome the drawbacks of CH [11].

Propolis, a natural wax-like antibiotic, is a flavonoid rich resinous material produced by bees [12]. When used as a root canal dressing, it has been proven to have potent antimicrobial action, anti-inflammatory effect, low toxicity and enhance healing as well [13-17]. Also, Neem (*Azadirachta indica*) is a well-known medicinal plant in India. Neem leaf extract was proven to have an antimicrobial and anticandidal action together with its biocompatibility and antioxidant effect [18-22].

Whatever the type of intracanal medicament used, complete removal before obturation is mandatory to obtain a final hermetic seal [23,24]. Any remnants of these medicaments will prevent endodontic sealer penetration into dentinal tubules thus jeopardizing the quality of apical seal and the final treatment outcome [25,26].

According to literature, limited studies evaluated the effect of propolis and neem intracanal medicaments on the sealing ability of endodontic sealers. Therefore, the purpose of this study was to investigate the effect of propolis, neem and CH when used as intracanal medicaments on sealing ability of resin-based endodontic sealer (AH Plus) using dye extraction method. The null hypothesis implied that there would be a no statistically significant difference between the three tested medicaments regarding their effect on AH Plus sealing ability.

MATERIALS AND METHODS

An approval from the Research Ethics Committee at Faculty of Dentistry -Beni-Suef University- was granted for the protocol of this study (*Approval number: #REC-FDBSU/04082022-01/SM*). Total of 60 human, permanent, single-rooted, maxillary anterior teeth extracted due to periodontal problems were collected to be used in this research. Teeth with immature root apices, root resorption, caries, fractures, cracks, previous restorations, multiple/curved/or calcified canals were not included in the study. Samples were cleaned, disinfected and stored in normal saline solution in air-tight containers until use to prevent desiccation.

Preparation of Specimens

For standardization purposes, all teeth were resected 15 ± 1 mm from the root apex using a diamond disc with water spray cooling. Teeth were accessed using size #4 round carbide bur followed by complete pulp chamber deroofing using an Endo-Z carbide bur.

Preparation of Root canals

Root canal patency was established using a size #15 K-type file. Root apices were sealed by wax to simulate the clinical situation. Mechanical preparation of the root canals was completed by crown-down technique using Revo-S rotary file system up to file AS40 taper 0.06. Irrigation with 2 ml of freshly prepared 2.6% NaOCl was performed between each successive file, followed by 5% of normal saline to neutralize the effect of NaOCl solution. Three ml of 17% EDTA were then used for 1 minute to remove smear layer and then 10 ml of distilled water were used as a final flush to prevent the erosion of dentinal tubules. The wax at the apex was then removed and canals dryness was carried out using absorbent paper point.

Preparation of Herbal medicaments

Propolis ICM preparation

Three hundred grams (300 g) of freeze-dried propolis (*Propolis, Imtenan Corporate Headquarters, Obour City, Egypt*) were macerated in 1 liter of 70% ethanol alcohol in the dark at room temperature for 2 weeks with frequent shaking. The aqueous ethanolic extract was then filtered twice through filter papers no.4 and no.1 (*Whatman, GE Health care, US*) then the filtrate was evaporated with a rotary evaporator *(Heidolph rotary evaporator, Heidolph, Germeny)* at 50°C. The resulted extract was manipulated using water and alcohol to control its viscosity then dried in an oven under vacuum (*Vaco Term, J.P Selecta, Spain*) at 50°C until the required creamy consistency was reached.

Neem ICM preparation

Mature fresh neem leaves were collected from the medicinal garden of Faculty of Pharmacy - Cairo University. Neem leaves were washed in sterile distilled water and dried using autoclave then ground to a powder form using a spice mill. Twenty grams (20 g) of the neem powder were macerated with 40 ml of monopropylene glycol solution to get a concentration of 50%. The extract was then filtrated through a white muslin cloth for coarse residue and through filter paper for finer residue. The infiltrate was then collected and stored at 4°C in a tightly closed container to preserve it from any contamination or decomposition until usage. At time of use, the extract was mixed with hydroxypropyl methylcellulose as a thickening agent in ratio (1:1) to get the gel form and then loaded in 30-gauge syringe.

Grouping of Specimens

A sample size of total 60 teeth - 12 in each group - with 0.5 effect size was enough to attain an 0.8 power and 0.05 significance level. An open-source software (*Gpower software 3.1, Universidad Düsseldorf, Düsseldorf, Germany*) was used to calculate the sample size. The specimens were randomly divided into five groups as follows:

- **Group I (Positive Control)**: Teeth were instrumented but not root-canal filled.
- **Group II**: Root canals were obturated without any prior placement of intracanal medication.
- **Group III**: Propolis gel was used as an intracanal medication.
- **Group IV**: Neem gel was used as an intracanal medication.
- **Group V**: Injectable calcium hydroxide was used as an intracanal medication.

In Propolis and Neem groups, the prepared medicaments were injected into the root canals after dryness using 30-gauge needle, inserted up to 2 mm from the root apex with continuous upward slow withdrawal of the needle till reaching the canal orifice, then the excess medication was removed by using sterile cotton rolls.

In CH group: A ready-made injectable CH paste (*Metapaste, Metabiomed.Co, LTD, Korea*) was introduced into the root canal until seen extruding through the root apex.

All samples were sealed with a cotton pellet and a 2 mm thick temporary filling material then were stored for 7 days at 37°C and 100% relative humidity.

Removal of intracanal medicaments:

After one week, the canals were re-opened and the medicaments in groups III, IV and V were removed by the help of hand K-file #40 used through the full working length in lateral strokes on the root canal walls in conjunction with copious irrigation. Five milliliters of 5.25% sodium hypochlorite followed by 5 ml of 17% EDTA were used, and each was agitated by a size #25 XP-endo Finisher with 0% taper (*FKG, La Chaux-de-Fonds, Switzerland*) for one minute using 7-8 mm vertical movements to the full working length. The XP-endo Finisher was operated at 800 rpm speed and 1 Ncm torque. A final flush with 5 ml sterile saline was applied to root canals followed by dryness using paper points to be ready for obturation.

Root canal obturation:

All root canals were obturated with gutta-percha/AH Plus sealer using cold lateral compaction technique. AH Plus was mixed to a creamy consistency according to manufacturer's instructions and applied to root canals using lentulo spiral. Radiographs were taken to ensure the quality of obturated root canals. Access was closed with 2 mm thick temporary filling material. Teeth were stored in an incubator for 72 hours at 37°C and 100% relative humidity to ensure proper setting of the sealer.

Apical microleakage assessment (Dye extraction method):

The specimens' external surfaces, except for the apical 2 mm, were polished with 2 layers of nail varnish. The teeth were totally immersed in aqueous solution of methylene blue (MB) with 2% w/v concentration. For MB proper diffusion in voids and unsealed gaps, teeth were kept in a vacuum chamber for 15 min under reduced pressure. Teeth were then properly rinsed with phosphate buffer saline to remove any dye leftovers. Afterwards, each tooth was placed in a screw-capped glass vial containing 65 wt% nitric acid to allow complete dissolution. After 3 days, the glass vial content was transferred into an eppendorf tube and centrifuged for 5 min at 14,000 rpm for 5 min to separate debris from the extracted dye.

One hundred microliters of the supernatant were taken from each eppendorf tube using a micropipette and added to a 96-well plate. Using a spectrophotometer, absorbance of the extracted dye in the supernatant was analyzed at 550 nm. Presence of unsealed dentinal tubules, pores or voids were interpreted by higher absorbance mean values confirming low sealing ability of the tested sealer and vice versa.

RESULTS

Data was statistically analyzed using SPSS version 17.0 statistics software. A significance level of $p\text{-value} < 0.05$ was applied to show any statistical difference between tested groups.

One-way analysis of variance (ANOVA) was used (Table [1]) for comparison of means followed by Post-hoc Tukey test (Table [2]) for multiple comparisons between groups.

The mean and standard deviation values of dye absorbance (microleakage) for all the five groups are presented in Table [3] and Figure [1]. Group I, the positive control group, displayed the highest statistically significant microleakage values (absorbance of 0.859±0.049); whereas, Group II, with no ICM, revealed the minimal microleakage values (0.253±0.069). Among the medicated groups, Group V with CH ICM exhibited statistically high microleakage values (0.510±0.124). Group III medicated with propolis and Group IV medicated with neem showed mean microleakage values of (0.370±0.049) and (0.385±0.044) respectively with no statistically significant difference between them ($p\text{-value} > 0.05$).

TABLE (1): One-way ANOVA

Groups	Sum of Squares	df	Mean Square	F	*p-value*
Between Groups	2.612	4	0.653		
Within Groups	0.297	55	0.005	120.960	<0.001
Total	2.909	59			

TABLE (2): Tukey Post-hoc test (Tukey HSD).

Groups	N	Subset for alpha = 0.05			
		1	2	3	4
Group II: No ICM	12	0.252667			
Group III: Propolis ICM	12		0.370083		
Group IV: Neem ICM	12		0.384500		
Group V: CH ICM	12			0.510417	
Group I: No Obt	12				0.859417
p-value		1.000	0.989	1.000	1.000

Means for groups in homogeneous subsets are displayed.

TABLE (3): Descriptive statistics for the dye absorbance values in different groups.

Groups	N	Mean	SD	95% CI for Mean		Min	Max
				Lower Bound	Upper Bound		
Group I: No Obt	12	0.859	0.049	0.828	0.890	0.793	0.921
Group II: No ICM	12	0.253	0.069	0.209	0.297	0.132	0.342
Group III: Propolis ICM	12	0.370	0.049	0.339	0.401	0.319	0.479
Group IV: Neem ICM	12	0.385	0.044	0.357	0.412	0.314	0.476
Group V: CH ICM	12	0.510	0.124	0.431	0.589	0.361	0.714
Total	60	0.475	0.222	0.418	0.533	0.132	0.921

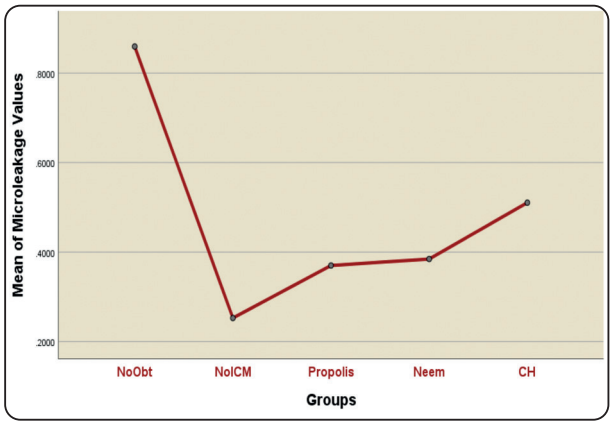

Fig. (1): A histogram showing the mean microleakage results in different groups.

DISCUSSION

Root canal system hermetic seal after cleaning and shaping is a prerequisite for a favorable endodontic treatment outcome [27]. Inadequate seal could result in microorganisms and tissue fluids leak inside the canal space leading to initiation of unwanted periapical inflammation [28]. That's why thorough removal of intracanal medicament before obturation is mandatory as any residuals might adversely affect the adaptation and the final seal of the root canal obturating material [24,29].

Various sealing ability assessment tests have been addressed in literature. Among which are dye-penetration, dye-extraction, and fluid-infiltration techniques that have been proven to be more representative and quantitative assessment methods[30]. The dye-extraction method was selected in the present study for sealing ability determination being a reliable, simple, and widely used technique[30,31]. When a tooth is submerged in MB solution, the small sized dye molecules can easily penetrate through all the gaps in the root canal walls/sealer interface [30,32].

The AH Plus epoxy resin-based sealer was used in the present study for obturation as it shows excellent physicochemical properties and is referenced as the gold standard for evaluating endodontic sealers [33,34]. Cold lateral compaction technique was used for obturation instead of warm gutta-percha obturation techniques as application of heat may alter the flow properties and the setting time of the sealer [35,36].

As stated before, every effort should be done to remove remnants of intracanal medicaments before final obturation of the root canals [23,24]. Despite of the various proposed techniques, no technique thoroughly removes intracanal dressings especially CH [37]. The XP-endo Finisher was used in this study for activation of irrigants for better removal of intracanal medicaments remnants. The adjunctive use of XP-endo Finisher was proved to be as effective as passive ultrasonic irrigation and superior to standard syringe irrigation, CanalBrush and EndoActivator techniques in in-vitro CH removal from root canal walls [38-40].

In the results of our study, the positive control group (Group I) with no obturation showed the highest mean microleakage values denoting the accuracy of the sealing ability assessment technique used [30,31]. The highest sealing ability was recorded in Group II that didn't receive any ICM. This is mainly due to the inherent properties of AH Plus sealer that wasn't altered by the presence of ICM as low solubility, little expansion, adhesion to dentin and the very good sealing ability [33]. It was clearly noted in our results that all the medicated groups showed variable amount of microleakage and the type of ICM had an impact on the sealing properties of the resin sealer used. This is in accordance with several studies that reported a difficulty in completely eliminating intracanal dressings from root canal walls during root canal therapy [40-42].

Calcium hydroxide medicated group showed the highest microleakage mean value among the medicated groups. This poor sealing ability may be attributed to the presence of medication residues that prevent proper bonding between the sealer and root canal dentin. It was highlighted by many authors that remnants of CH may inhibit the sealer penetration into the dentinal tubules and consequently affect the sealer's sealing ability [26,44]. Durate et al (2010) [44] also stated that an increase in the film thickness of AH Plus sealer occurs in the presence of CH leading to more voids incorporated in the final seal.

Two herbal extracts were used in this study as intracanal medicaments, propolis and neem. Propolis, a natural antibiotic, has been used for a while now as an endodontic intracanal dressing due to its wide-range antimicrobial action especially against resistant Enterococcus faecalis and Candida albicans [45,46]. It is a resin-like substance produced by bees and is highly rich in flavonoids (active ingredient) [12]. Neem *(Azadirachta Indica)* has been proven to have a potent antibacterial activity as well against the resistant Enterococcus faecalis strain [18,19]. This is owed to the presence of nimbidin and nimbolide as active ingredients in neem which possess antibacterial, antioxidant and antifungal properties [47].

Propolis medicated group reported lower mean microleakage values when compared to neem medicated group but with no significant difference. However, both showed improved sealing ability for

AH Plus sealer when compared to CH group. Some studies stated the difficulty in completely removing the experimental propolis pastes from root canal walls and in some cases, it was associated with the presence of precipitations which affected the root canal cleanliness [41]. The latter results were attributed to the hydrophilic components present in propolis paste that would favorably and firmly bind to the hydrophilic root canal dentinal surface. At the same time, the resinous sticky nature of propolis may result in some difficulty in its removal from the root canal walls. But what favors our results that the resin ingredients of propolis [48] may lead to effective binding with resin-based AH Plus, thus accounting for its relative high sealing ability findings when compared to the other medicaments.

For neem medicated group, there is limited literature addressing its effect on the seal of resin-based sealers. However, Christy and Nivedhitha (2022) [49] compared the effect of neem and CH as ICMs on AH Plus bond strength and found that neem didn't affect bonding of the sealer to dentin. Also, Goel et al (2019) [50] stated that neem when used as cavity disinfectant improved the resin-dentin bond durability of a resin-based restoration. These previously stated findings are in favor to our results that showed a statistically higher sealing ability of AH Plus in neem medicated groups in comparison to CH. Still, the possible chemical reaction that may occur between epoxy resin-based sealers and the ingredients of either neem or propolis should be in-depth investigated.

CONCLUSION

Based on the results of this study, the use of propolis and neem as intracanal medicaments improved the sealing ability of AH Plus endodontic sealer when compared to calcium hydroxide. So, they can be considered as alternatives to calcium hydroxide for root canal disinfection.

LIMITATIONS

- It was an in vitro study and accurate replication of clinical conditions were not achieved.
- It was not possible to directly compare studies due to difference in the methodology and technique of plant extraction used in the study. The present study reinforces the literature regarding the efficacy of neem and propolis as a better herbal ICM compared to CH. Further studies are required to corroborate the results of present study.

RECOMMENDATIONS

1. Further research should be directed to assess the effect of Propolis and Neem medicaments on the physicochemical properties of resin-based endodontic sealers and other types of sealers as well.
2. Herbal intracanal medicaments should undergo further assessment via clinical trials to investigate its clinical convenience.

REFERENCES

1. Aw V. Discuss the role of microorganisms in the aetiology and pathogenesis of periapical disease. Aust Endod J 2016; 42: 53-59.
2. Ørstavik D. Obturation of root canals. In: Chugal N, Lin LM editors. Endodontic Prognosis: Clinical Guide for Optimal Treatment Outcome. Springer International Publishing; 2017: 141-159.
3. Peters LB, van Winkelhoff AJ, Buijs JF, Wesselink PR. Effects of instrumentation, irrigation and dressing with calcium hydroxide on infection in pulpless teeth with periapical bone lesions. Int Endod J 2002; 35: 13-21.
4. Siqueira JF, Rocas IDN, Marceliano-Alves MF, Perez AR, Ricucci D. Unprepared root canal surface areas: causes, clinical implications, and therapeutic strategies. Braz Oral Res 2018; 32: e65.
5. Kumar A, Tamanna S, Iftekhar H. Intracanal medicaments-their use in modern endodontics: a narrative review. J Oral Res Rev 2019; 11: 94-99.

6. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011; 44: 697-730.

7. Tanomaru Filho M, Leonardo MR, da Silva LA. Effect of irrigating solution and calcium hydroxide root canal dressing on the repair of apical and periapical tissues of teeth with periapical lesion. J Endod 2002; 28: 295-299.

8. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part I. In vitro studies. Restor Dent Endod 2014; 39: 241-252.

9. Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis: the root canal survivor and star in post treatment disease. Endod Topics 2003; 6: 135-139.

10. Karobari MI, Adil AH, Assiry AA, Basheer SN, Noorani TY, Pawar AM, Marya A, Messina P, Scardina GA. Herbal medications in endodontics and its application-A review of literature. Materials 2022; 25; 15: 3111.

11. Pujar M, Mukandar S. Herbal usage in endodontics-a review. Int J Contemp Dent 2011; 2: 34-37.

12. Kosalec I, Pepeljnjak S, Bakmaz M, Vladimir-Knezević S. Flavonoid analysis and antimicrobial activity of commercially available propolis products. Acta Pharm 2005; 55: 423-430.

13. Kandaswamy D, Venkateshbabu N, Gogulnath D, Kindo AJ. Dentinal tubule disinfection with 2% chlorhexidine gel, propolis, morinda citrifolia juice, 2% povidone iodine, and calcium hydroxide. Int Endod J 2010; 43: 419-423.

14. Madhubala MM, Srinivasan N, Ahamed S. Comparative evaluation of propolis and triantibiotic mixture as an intracanal medicament against Enterococcus faecalis. J Endod 2011; 37: 1287-1289.

15. De Souza Ferreira SB, de Assis Dias BR, Obregón CS, Gomes CC, de Araújo Pereira RR, Ribeiro Godoy JS, Estivalet Svidzinski TI, Bruschi ML. Microparticles containing propolis and metronidazole: in vitro characterization, release study and antimicrobial activity against periodontal pathogens. Pharm Dev Technol 2014; 19: 173-180.

16. Neiva KG, Catalfamo DL, Holliday LS, Wallet SM, Pileggi R. Propolis decreases lipopolysaccharide-induced inflammatory mediators in pulp cells and osteoclasts. Dent Traumatol 2014; 30: 362-367.

17. Parolia A, Kundabala M, Rao NN, Acharya SR, Agrawal P, Mohan M, Thomas M. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. Aust Dent J 2010; 55: 59-64.

18. Nayak A, Nayak R, Soumya B, Bhat K, Kudalkar M. Evaluation of antibacterial and anticandidial efficacy of aqueous and alcoholic extract of Neem (Azadirachta indica) an in vitro study. Int J Res Ayurveda Pharm 2011; 2: 230-235.

19. Kusuma CS, Manjunath V, Gehlot PM. Comparative evaluation of Neem, Aloevera, Chlorhexidine and Calcium Hydroxide as an intracanal medicament against E. Faecalis-An in vitro study. J Clin Diagn Res 2018:12.

20. Raghavendra SS, Balsaraf KD. Antifungal efficacy of Azadirachta indica (neem) - An in vitro study. Braz J Oral Sci 2014; 13: 242-245.

21. Gupta A, Ansari S, Gupta S, Narwani M, Gupta M, Singh M. Therapeutics role of neem and its bioactive constituents in disease prevention and treatment. J Pharmacogn Phytochem 2019; 8: 680-691.

22. Alzohairy MA. Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. Evid Based Complement Alternat Med 2016: 1-11.

23. Tandan M, Hegde MN, Hegde P. Effect of four different intracanal medicaments on the apical seal of the root canal system: a dye extraction study. Indian J Dent Res 2014; 25: 607-612.

24. Pradhan PK, Sahoo KC, Dipallini S, Banerjee S, Lata S, Patri G. Effect of different intracanal medicaments on apical sealing ability of BioRoot RCS. J Contemp Dent Pract 2020; 21: 47-50.

25. Van der Sluis LW, Wu MK, Wesselink PR. The evaluation of removal of calcium hydroxide paste from an artificial standardized groove in the apical root canal using different irrigation methodologies. Int Endod J 2007; 40: 52-57.

26. Uzunoglu E, Erdoğan Ö, Aktemur Türker S. Effect of calcium hydroxide dressing on the dentinal tubule penetration of 2 different root canal sealers: A confocal laser scanning microscopic study. J Endod 2018; 44: 1018-1023.

27. García-Guerrero C, Delgado-Rodríguez CE, Molano-

González N, Pineda-Velandia GA, Marín-Zuluaga DJ, Leal-Fernandez MC, Gutmann JL. Predicting the outcome of initial non-surgical endodontic procedures by periapical status and quality of root canal filling: a cohort study. Odontology 2020; 108: 697-703.

28. Muliyar S, Shameem KA, Thankachan RP, Francis PG, Jayapalan CS, Hafiz KA. Microleakage in endodontics. J Int Oral Health 2014; 6: 99-104.

29. Chung HA, Titley K, Torneck CD, Lawrence HP, Friedman S. Adhesion of glass-ionomer cement sealers to bovine dentin conditioned with intracanal medications. J Endod 2001; 27: 85-88.

30. Kaya S, Yiğit Özer S, Adigüzel Ö, Oruçoğlu H, Değer Y, Tümen EC, Uysal İ. Comparison of apical microleakage of dual-curing resin cements with fluid-filtration and dye extraction techniques. Med Sci Monit 2015; 21: 937-944.

31. Camps J, Pashley D. Reliability of the dye penetration studies. J Endod 2003; 29: 592-594.

32. Sinhal TM, Shah RRP, Jais PS, Shah NC, Hadwani KD, Rothe T, Sinhal NN. An in vitro comparison and evaluation of sealing ability of newly introduced C-point system, cold lateral condensation, and thermoplasticized gutta-percha obturating technique: A dye extraction study. Contemp Clin Dent 2018; 9: 164-169.

33. Azar NG, Heidari M, Bahrami ZS, Shokri F. In vitro cytotoxicity of a new epoxy resin root canal sealer. J Endod 2000; 26: 462-465.

34. Marin-Bauza GA, Rached-Junior FJ, Souza-Gabriel AE, Sousa-Neto MD, Miranda CE, Silva-Sousa YT. Physicochemical properties of methacrylate resin-based root canal sealers. J Endod 2010; 36: 1531–1536.

35. Viapiana R, Moinzadeh AT, Camilleri L, Wesselink PR, Tanomaru Filho M, Camilleri J. Porosity and sealing ability of root fillings with gutta-percha and BioRoot RCS or AH Plus sealers. Evaluation by three ex vivo methods. Int Endod J 2016; 49: 774-782.

36. Camilleri J. Sealers and warm gutta-percha obturation techniques. J Endod 2015; 41: 72-78.

37. Capar ID, Ozcan E, Arslan H, Ertas H, Aydinbelge HA. Effect of different final irrigation methods on the removal of calcium hydroxide from an artificial standardized groove in the apical third of root canals. J Endod 2014; 40: 451-454.

38. Keskin C, Sariyilmaz E, Sariyilmaz Ö. Efficacy of XP-endo Finisher file in removing calcium hydroxide from simulated internal resorption cavity. J Endod 2017; 43: 126-130.

39. Uygun AD, Gündoğdu EC, Arslan H, Ersoy İ. Efficacy of XP-endo finisher and TRUShape 3D conforming file compared to conventional and ultrasonic irrigation in removing calcium hydroxide. Aust Endod J 2017; 43: 89-93.

40. Wigler R, Dvir R, Weisman A, Matalon S, Kfir A. Efficacy of XP-endo finisher files in the removal of calcium hydroxide paste from artificial standardized grooves in the apical third of oval root canals. Int Endod J 2017; 50: 700-705.

41. Victorino FR, Bramante CM, Zapata RO, Casaroto AR, Garcia RB, Moraes IG, Hidalgo MM. Removal efficiency of propolis paste dressing from the root canal. J Appl Oral Sci 2010; 18: 621-624.

42. Chawla A, Kumar V. Evaluating the efficacy of different techniques and irrigation solutions for removal of calcium hydroxide from the root canal system: A scanning electron microscope study. J Conserv Dent 2018; 21: 394-400.

43. Kanmaz, F, Altunbaş D. Evaluation of the effect of calcium hydroxide paste on sealer penetration into dentinal tubules by confocal laser scanning microscopy. Aust Endod J 2022; 48: 82-90.

44. Duarte MA, Ordinola-Zapata R, Bernardes RA, Bramante CM, Bernardineli N, Garcia RB, et al. Influence of calcium hydroxide association on the physical properties of AH Plus. J Endod 2010; 36: 1048–1051.

45. Arslan S, Ozbilge H, Kaya EG, Er O. In vitro antimicrobial activity of propolis, BioPure MTAD, sodium hypochlorite, and chlorhexidine on Enterococcus faecalis and Candida albicans. Saudi Med J 2011; 32: 479-483.

46. Saha S, Nair R, Asrani H. Comparative evaluation of propolis, metronidazole with chlorhexidine, calcium hydroxide and curcuma longa extract as intracanal medicament against E.faecalis- An invitro study. J Clin Diagn Res 2015; 9: ZC19-ZC21.

47. Khetarpal S, Bansal A, Kukreja N. Comparison of anti-bacterial and anti-inflammatory properties of neem, curcumin and aloe vera in conjunction with chlorhexidine as an intracanal medicament – An in-vivo study. Dent J Adv Stud 2014; 2, 130-137.

48. Hu F, Hepburn HR, Li Y, Chen M, Radloff SE, Daya S. Effects of ethanol and water extracts of propolis (bee glue) on acute inflammatory animal models. J Ethnopharmacol 2005; 100: 276-283.

49. Christy S, Nivedhitha MS.Effect of natural intracanal medicament on push out bond strength of resin-based sealer - An in vitro study. Int J Clin Dent 2022; 15: 233-240.

50. Goel S, Sinha DJ, Singh UP, Ahuja U, Haider N, Sharma N. Comparative evaluation of effect of chlorhexidine, Azadirachta indica (neem), and Aloe barbadensis miller (Aloe vera) on resin-dentin bond stabilization using shear bond testing: An in vitro study. J Conserv Dent 2019; 22: 300-304.