Original Article

Effect of Propolis Application in Root Canal Therapy for Decontamination; Reversible or Irreversible Coronal Discoloration?

Zohreh Ahangari[1], Mandana Naseri[2], Samin Banihashem[3], Sara Namjou[4], Leila Eftekhar[5]

[1]Professor, Department of Endodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2] Associate Professor, Department of Endodontics, Dental School, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3] General Practitioner.
[4]Endodontist, Private Practice
[5]Assistant Professor, Department of Pediatric Dentistry, Dental School, Alborz University of Medical Sciences, Karaj, Iran.

Received 6 February 2021 and Accepted 10 March 2021

Abstract

Introduction: Propolis is a resinous substance produced by honeybees. Despite antimicrobial properties, tooth discoloration has been reported during its application as intracanal medicament. The aim of this study was to assess the effect of intracanal propolis removal on crown discoloration. **Methods:** In this experimental study, after access cavity and canal preparation was performed in 40 intact anterior teeth, they were divided into three groups. In group 1 propolis was placed in the canal and pulp chamber while in group 2, it was applied into the canal only. The canals of third group were filled with distilled water as control. After six months, labial surfaces of all teeth were digitally photographed by a digital camera. Propolis was then completely removed and photography was repeated. Tooth color was evaluated in the labial surface using CIE Lab system and Photoshop software. **Results:** Overall color change (ΔE), change in lightness (ΔL), greenness-redness (Δa) and blueness-yellowness (Δb) were analyzed. ΔL and Δa values were significantly different in all three groups ($P<0.001$). The difference between groups 1 and 2 was not significant for ΔL or Δa, but groups 1 and 3 were significantly different in ΔL and Δa ($P<0.001$). Groups 2 and 3 were significantly different in ΔL and Δa ($P<0.001$). **Conclusion:** Coronal discoloration after six-month application of propolis as intracanal medicament was not reversed by its removal. Location of application of propolis (in the canal or both canal and pulp chamber) had no significant effect on degree of coronal discoloration.

Keywords: Intracanal medicaments, Propolis, Root canal, Tooth discoloration

---

Ahangari Z, Naseri M, Banihashem S, Namjou S, Eftekhar L. Effect of propolis application in root canal therapy for decontamination; Reversible or irreversible coronal discoloration? J Dent Mater Tech 2021; 10(2): 102-107.

Introduction

One of the major goals of endodontic treatment is to eliminate microorganisms from root canal system. During root canal therapy, number of microorganisms in the root canal is minimized by means of chemomechanical preparation; however, there is a chance that some part of microorganisms in the root canal system remain untouched(1, 2). Thus, various types of intracanal medicaments are used in-between treatment sessions to minimize microbial count in the root canal system (3-5). Calcium hydroxide has long been used as the conventional intracanal medicament (6-8). However, it has some limitations since it cannot eliminate all microorganisms from the root canal system (9, 10) and requires a long time to exert its antimicrobial effects (11). Moreover, due to high pH, it is potentially toxic and can cause soft tissue damage. It can also lead to chronic

inflammation and cell necrosis in its clinical application(12). Thus, researchers have been searching for more efficient medicaments for endodontic treatment with minimum toxicity and maximum antibacterial activity.

Propolis is a resinous substance produced by honeybees. It`s appearance and properties may vary depending on the origin(13). This substance is used by the honeybees to seal small gaps in the beehives or mummify the insects invading beehives. Also, due to having optimal disinfecting and antimicrobial properties, propolis can protect the honeybee colony from diseases(14). Several studies have evaluated the antibacterial and antifungal effects of propolis on resistant microorganisms and reported satisfactory results (15, 16). It has been reported that flavonoids present in propolis may induce the formation of reparative dentin. Moreover, propolis has less disruptive effect on fibroblast's activity (17, 18). On the other hand, flavonoids cause staining(19). The only study found on discoloration during application of propolis as intracanal medicament reported clinical discoloration of tooth crown at different time of applications(20). However, it was not evident whether this change of color was temporary or permanent. Thus, this study aimed to assess whether removal of propolis from the root canal system would reverse initial coronal discoloration or not.

Materials and Methods

This is an in-vitro experimental study that was approved in the ethics committee of Shahid Beheshti University of Medical Sciences. Forty sound extracted human anterior teeth with no caries, restorations, developmental defects, enamel cracks or external discoloration with straight roots were randomly divided into two experimental (n=15) and one control (n=10) groups. Soft tissue residues, calculus and debris were removed by a curette. For the purpose of disinfection, all teeth were immersed in 5.25% sodium hypochlorite (Vitex, Shamin Chemical Co.,Tehran, Iran) for 30 minutes and then were stored in sterile 0.9% saline (0.9% sodium chloride; Daroupakhsh, Tehran, Iran) at room temperature until used in the experiment.

After standard access cavity preparation by a fissure bur (Teeskavan, Hashtgerd, Iran) was performed, initial file was inserted into the canal until its tip was visible at the apex. Working length was measured by subtracting 0.5 mm of this length. Later, canals were instrumented with ProTaper Universal rotary system (Dentsply Maillefer, Ballaigues, Switzerland) according to the manufacturer's instructions to F3 rotary file. Smear layer was then removed using 2 ml of 17 % EDTA (META-biomed co, Republic of Korea) for one minute and canals were rinsed with 5.25% sodium hypochlorite (Vitex, Shamin Chemical Co., Tehran, Iran) after using each file. Final rinse of the canal was carried out with 5 ml of %2/5 saline (STERISOL-NS, producer SAMEN Co., Iran).

Pulp chambers and canals were dried with paper points (META-biomed co, Republic of Korea). Solid propolis obtained from Azarbaijan, Iran, was ground into powder. To prepare 30% propolis, 7 cc of 96% ethanol was homogenously mixed with 3 g of ground propolis powder using a magnetic stirrer. The 30% solution was filtered by a CA-20/25 filter to eliminate impurities. In the first experimental group, 30% propolis was injected into the canals up to the working length and pulp chambers using a 27-gage insulin needle(Supa co, Iran). In group 2, it was only injected into the canals to the level of the cementoenamel junction and canal orifices were cleaned by a cotton pellet dipped in alcohol. In group 3 (negative control), canals were filled with distilled water. Access cavities were restored and sealed with light-cured glass ionomer cement (CG –Gold Label, Japan). Teeth were separately placed in airtight plastic vials with 100% humidity at 37°C. This methodology was adopted from previous studies(21, 22). After six months, teeth were digitally photographed (Fujifilm, 5.0MP, 12X Optical, Tokyo, Japan). For the purpose of reproducibility of taking photographs in terms of the position of the teeth and the angle of radiation, a device was designed and fabricated and two light sources at 45° angle illuminated the tooth surface in a completely dark room. Also, a paste was used to fix the position of teeth when photographing their labial surface against a gray background. To adjust brightness and color of radiographs in the software, a small round disc was punched out of a cardboard and was placed on the most distant point of the root from the crown during photography at all steps. This middle gray cardboard disc had 18% reflectance and its color tone was perceptually about halfway between the pure black and pure white. It served as a neutral reference. Teeth canals were then instrumented with hand K files (Maillefer, Switzerland) and rinsed with sodium hypochlorite to ensure complete elimination of propolis from the root canals. Pulp chamber was cleaned by a cotton pellet dipped in alcohol. Teeth were then photographed again with similar protocol. Color parameters were measured using digital photography.

Images were imported to a computer for computer analysis and evaluated in Adobe Photoshop CS5 software. Color parameters were reported according to the CIE L*a*b* system. In this system, the L parameter indicates lightness, the a* parameter indicates green- red and the b* parameter indicates blue-yellow. Lightness, a and b parameters were assessed for each specimen in labial surface of tooth in images. The spectrum of Lab changes ranges from 0 to 255 in Adobe Photoshop CS5 software. In CIE L*a*b* system this spectrum range from 0 to 100 for lightness and from -120 to +120 for a

and b. The Lab values were converted to the CIE Lab system by following formula:

b*=b-128, a*=a-128,L*=Lx100/255

ΔE* was the total color change in the range of 0-100 and was calculated using the formula below (23):

$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{½}$

The Lab parameters for each tooth color along with color change (ΔE) were entered.

Statistical analysis

The Lab parameters for tooth color along with the color change (ΔE) were entered in version 16 of SPSS software. According to the shapiro-wilk test, distribution of ΔE data was normal in the three groups (P value; group A=0.350, group B=0.149, group C=0.758). One-way ANOVA was applied to compare the mean ΔE among the three groups.

Due to the abnormal distribution of the variances of ΔL, Δa and Δb in three groups using Levene test($P<0.05$), Kruskal-Wallis non-parametric test was used to compare the groups in terms of these components. Mann-Withney non-parametric test was also used to compare pairwise groups. The confidence level was considered 95% and P value<0.05 was considered statistically significant.

Results

Table I show mean ΔE in the three groups. The results showed that the three groups were not significantly different in terms of ΔE (P=0.149).

Table I. Mean and standard deviation of total color change (ΔE) in the three groups

Group*	Number	Minimum	Maximum	Mean± SD	P Value
Group A	15	1.17	13.66	6.23±0.93	
Group B	15	0.81	11.86	5.19±0.89	
Group C	10	0.88	7.17	3.64±0.61	0.149

* Group A: Propolis in the canals and pulp chamber, Group B: Propolis in canals, Group C: Control

Table II shows mean ΔL, Δa and Δb values in the three groups. Surveys revealed no significant difference among three groups in terms of Δb (P=0.057). However, the difference among groups was significant for ΔL and Δa (P<0.001). No significant difference was observed between groups 1 and 2 for ΔL (P=0.461) or Δa (P=0.683). However, groups 1 and 3 were significantly different in terms of ΔL and Δa and groups 2 and 3 were significantly different in terms of ΔL and Δa (P<0.001 for all four).

Table II**.** Mean ΔL, Δa and Δb values in the three groups

Parameter	Group*	Number	Minimum	Maximum	Mean ± SD	P Value
ΔL	Group A	15	0.41	8.97	4.11±0.71	P<0.001
	Group B	15	0.03	8.6	3.35±0.68	
	Group C	10	0.16	1.26	0.40±0.24	
Δa	Group A	15	0.3	9.44	3.61±0.72	P<0.001
	Group B	15	0.75	8.08	2.93±0.69	
	Group C	10	0	1.01	0.09±0.19	
Δb	Group A	15	0.41	4.75	0.78±0.64	P=0.057
	Group B	15	0.4	3.7	1.44±0.37	
	Group C	10	0.56	7.06	3.08±0.81	

* **Group A**: Propolis in the canals and pulp chamber, **Group B**: Propolis in canals, **Group C**: Control

Discussion

Discoloration of teeth, particularly in the anterior teeth, following application of intracanal medicaments during endodontic treatment is a common concern for patients and dentists (24, 25). Propolis as an intracanal medicament, similar to calcium hydroxide, with advantage of easy retrieval from tooth canal by rinsing the canal with sodium hypochlorite and using a endodontic file (25). But since propolis is produced by honeybees, its composition and physical, chemical and

biological properties may vary depending on the origin of plants and may have different colors (26). Therefore, considering the variability in color of propolis due to its origin, different results may be obtained in terms of coronal discoloration by using different types of propolis. However, considering similar overall chemical composition of propolis, the results of this study applies to various types of propolis. This study assessed coronal tooth discoloration six months after application of 30% propolis as an intracanal medicament and evaluated the effect of its removal on discoloration.

Visual assessment and electronic instruments are the two main methods for assessment of tooth discoloration. Several studies have compared these two methods. Visual assessment has the highest measurement error, and electronic instruments have been reported to be more accurate than human eye (27). In the current study, digital photography and assessment of the CIELab color parameters were performed to determine color change; this method has been used in previous studies (28, 29).

Krastl et al (30), in their review study showed that all endodontic materials cause tooth discoloration. Therefore, in order to decrease the risk of discoloration, they should be used with caution in the esthetic region. In the current study, all teeth showed discoloration compared to the control group.

In the current study, similar to that of Kim et al(31), coronal access cavity was prepared for placement of propolis as intracanal medicament, to simulate the clinical setting. In contrast, previous studies prepared canals through an apical access cavity, which is different from clinical setting (32, 33).

Kim et al. (31) evaluated the effect of Ledermix as intracanal medicament on tooth color and showed that less discoloration occurred when Ledermix was placed in the root canal to the level of the cementoenamel junction compared to when Ledermix was placed in the entire root canal and the pulp chamber. However, in the current study, location of placement of propolis (in the canal or in the canal and pulp chamber) caused no significant difference in lightness (L), greenness-redness (a) or blueness-yellowness (b) color parameters. This controversy in the results of the two studies may be due to the physical and chemical differences of propolis and Ledermix and greater penetration depth of propolis into the dentinal tubules. Also, using ethanol for cleaning of the pulp chamber can enhance penetration of propolis into the tubules. The constituents of propolis such as flavonoids and minerals such as iron can be responsible for the discoloration, and use of ethanol can enhance their penetration depth (20).

Kontogiannis et al.(34) evaluated the effect of calcium hydroxide and calcium hydroxide mixed with chlorhexidine on tooth discoloration and revealed that lightness (L) value in both groups significantly increased compared to the control group, which was in agreement with our findings. According to Kontogiannis et al. the time of remaining of intracanal medicament in the root canal is more important than the kind of medicament in causing discoloration(34). Nevertheless in the past study about propolis, the discoloration ability was shown(20).

One of the strengths of the current study was evaluation of discoloration immediately after removal of propolis from the canal. Previous study showed crown discoloration during application of propolis, but it was not clear if it was due to the shadow of that dark material in site which can be disappeared after its removal. Our results indicated that no change in color parameters occurred after removal of propolis from the canal. Stability of discoloration may be attributed to the penetration of propolis into the dentinal tubules and the inability to completely eliminate it from the tooth structure due to its resinous and sticky nature (35) .

Conclusion

Despite significant antimicrobial properties of propolis, application of propolis as an intracanal medicament can cause clinical discoloration in tooth crowns that does not diminish with medicament removal. Covering the inner walls of pulp chamber with resin or flowable composite in addition to complete removal of propolis from the pulp chamber can be beneficial. Further assessments are required to determine the pattern of discoloration and ways to prevent it.

Conflicts of interest:
None declared

Acknowledgment:
None

References

1. Ahangari Z, Eslami G, Ghannad S. In Vitro Antimicrobial Activity of Propolis in Comparison with Calcium Hydroxide against Enterococcus Faecalis. Journal Dental School, 2012(1): 9-17.

2. Byström A, Sundqvist G. Bacteriologic evaluation of the efficacy of mechanical root canal instrumentation in endodontic therapy. Eur J Oral Sci, 1981. 89(4): 321-328.

3. Awawdeh L, Al-Beitawi M, Hammad M. Effectiveness of propolis and calcium hydroxide as a short-term intracanal medicament against Enterococcus faecalis: a laboratory study. Aust Endod J, 2009. 35(2): 52-58.

4. de Souza CA, Teles RP, Souto R, Chaves MA, Colombo AP.Endodontic therapy associated with calcium hydroxide as an intracanal dressing: microbiologic evaluation by the checkerboard DNA-

DNA hybridization technique. J Endod, 2005. 31(2): 79-83.

5. Chugal NM, Clive JM, Spångberg LS. A prognostic model for assessment of the outcome of endodontic treatment: Effect of biologic and diagnostic variables. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 2001. 91(3): 342-352.

6. Fernandes FF, Dias AL, Ramos CL. The "in vitro" antifungal activity evaluation of propolis G12 ethanol extract on Cryptococcus neoformans. Rev Inst Med Trop Sao Paulo, 2007. 49(2): 93-5.

7. Ferreira CM, da Silva Rosa OP, Torres SA, Ferreira FB, Bernardinelli N. Activity of Endodontic Antibacterial Agents Against Selected Anaerobic Bacteria. Braz Dent J, 2002. 13(2): 118-122.

8. Zare Jahromi M, Navabi AA, Ekhtiari M. Comparing Coronal Discoloration Between AH26 and ZOE Sealers. Iran Endod J, 2011. 6(4): 146-149.

9. 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(4): 241-252.

10. Kim D, Kim E. Antimicrobial effect of calcium hydroxide as an intracanal medicament in root canal treatment: a literature review - Part II. in vivo studies. Restor Dent Endod, 2015. 40(2): 97-103.

11. Coldero LG, McHugh S, MacKenzie D, Saunders WP. Reduction in intracanal bacteria during root canal preparation with and without apical enlargement. Int Endod J, 2002. 35(5): 437-446.

12. Al-Shaher A, Wallace J, Agarwal S, Bretz W, Baugh D. Effect of propolis on human fibroblasts from the pulp and periodontal ligament. J Endod, 2004. 30(5): 359-361.

13. Kousedghi H, Ahangari Z, Eslami G, Ayatolahi A. Antibacterial activity of propolis and Ca(OH)2 against Lactobacillus, Entrococus faecalis, Peptostreptococus and Candida aalbicans. Afr J Microbiol Res, 2012. 6(14): 3510-3515.

14. Uzel A, Sorkun K, Onçağ O, Cogŭlu D, Gençay O, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian Propolis samples. Microbiol Res, 2005. 2160: 189-195.

15. Carbajal Mejía JB. Antimicrobial effects of calcium hydroxide, chlorhexidine, and propolis on Enterococcus faecalis and Candida albicans. J Investig Clin Dent, 2014. 5(3): 194-200.

16. Saxena D, Ganguly Saha S, Kanti Saha M. Dubey, S. Khatri, M. An in vitro evaluation of antimicrobial activity of five herbal extracts and comparison of their activity with 2.5% sodium hypochlorite against Enterococcus faecalis. Indian J Dent Sci, 2015. 26(5): 524-527.

17. Jahromi MZ, Ranjbarian P, Shiravi S. Cytotoxicity Evaluation of Iranian Propolis and Calcium Hydroxide on Dental Pulp Fibroblasts. J Dent Res Dent Clin Dent Prospects, 2014. 8(3): 130-133.

18. Sabir A, Tabbu CR, Agustiono P, Sosroseno W. Histological analysis of rat dental pulp tissue capped with propolis. J Oral Sci, 2005. 47(3): 135-138.

19. Masek A. Flavonoids as Natural Stabilizers and Color Indicators of Ageing for Polymeric Materials. Polymers, 2015. 7(6): 1127-1144.

20. Ahangari Z, Ghassemi A, Shamszadeh S, Naseri M. The Effects of Propolis on Discolouration of Teeth. Journal of Dental School, 2013. 31(1): 33-41.

21. Koursoumis AD, Kerezoudis NP, Kakaboura A. In vitro assesment of tooth color alternation by two different types of endodontic irrigant. J contemp dent pract., 2014. 15(5): 529-533.

22. Kontogiannis TG, Koursoumis A, Kerezoudis AP. in vitro evaluation of the staning effects of two intracanal medicaments over a 3-month period. J Stomatol, 2014. 4: 381-388.

23. Paravina RD, Ghinea R, Herrera LJ, Bona AD, Igiel C, Linninger M, et al. Herrera, Color difference thresholds in dentistry. J Esthet Restor Dent, 2015. 27(1): S1-S9.

24. 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(11): ZC19-21.

25. Shrivastava R, Rai VK. An in vitro Comparison of Endodontic Medicaments Propolis and Calcium Hydroxide alone and in Combination with Ciprofloxacin and Moxifloxacin against Enterococcus Faecalis. J Contemp Dent Pract, 2015. 16(5): 394-399.

26. Huang XY, Guo XL, Luo HL, Fang XW, Zhu TG, Zhang XL, et al. Fast Differential Analysis of Propolis Using Surface Desorption Atmospheric Pressure Chemical Ionization Mass Spectrometry. Int J Anal Chem, 2015. 2015.

27. Ma JF. Du RX, Wang SQ, Li YM. Effects of background, direction and intensity of ambient light, measuring position, and adjacent teeth, on anterior tooth colour measurement in vitro. Chin J Dent Res, 2010. 13(2): 147-152.

28. Lenherr P, Allgayer N, Weiger R, Filippi A, Attin T, Krastl G. Tooth discoloration induced by endodontic materials: a laboratory study. Int Endod J, 2012. 45(10): 942-994.

29. Gürel, MA. Kivanç, BH., Ekici, A. Alaçam, T. Evaluation of crown discoloration induced by endodontic sealers and colour change ratio determination after bleaching. Aust Endod J, 2016. 42(3): 119–123.

30. Krastl G, Allgayer N, Lenherr P, Filippi A, Taneja P, Weiger R.Tooth discoloration induced by endodontic materials: a literature review. Dent Traumatol, 2013. 29(1): 2-7.

31. Kim ST, Abbott PV, McGinley P. The effects of Ledermix paste on discolouration of mature teeth. Int Endod J, 2000. 33(3): 227-232.

32. Partovi M, Al-Havvaz AH, Soleimani B. In vitro computer analysis of crown discolouration from commonly used endodontic sealers. Aust Endod J, 2006. 32(3): 116-119.

33. van der Burgt TP, Eronat C, Plasschaert AJ. Staining patterns in teeth discolored by endodontic sealers. J Endod, 1986. 12(5): 187-191.

34. KontogiannisT G, Koursoumis A, Kerezoudis NP. In Vitro Evaluation of the Staining Effects of Two Intracanal Medicaments over a 3-Month Period. J Stomatol, 2014(4): 381-388.

35. Parolia A, Kundabala M, Rao N.N, Acharya S.R, Agrawal P, Mohan M, et al. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. Aust Dent J, 2010. 55(1): 59-64.

Corresponding Author
Leila Eftekhar
Assistant professor, Department of Pediatric Dentistry, Dental School, Alborz University of Medical Sciences, Karaj, Iran.
Tell: 09127363417
Email: Leila.eftekhar.a@gmail.com