

## The Effects of Propolis on Discoloration of Teeth

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### Abstract

**Objective:** Propolis is a resinous material produced by honeybees that has recently gained fame as an antimicrobial agent. This study sought to assess the effects of propolis as an intra-canal medicament on tooth discoloration. The effect of its application technique on the degree of discoloration was investigated as well.

**Methods:** This experimental study was conducted on 40 intact anterior human teeth. After access cavity and canal preparation, the teeth were randomly divided into three groups. In group A, propolis was placed inside the canals and pulp chamber. In group B, propolis was used as an intracanal medicament. Group C was considered as the control group and saline solution was injected into the canals. Labial surfaces of all teeth were digitally photographed using a digital camera (Fujifilm at one day, one week, 2 weeks, one month and 2 months time points. Color of teeth was assessed and measured at incisal, middle, and cervical segments using the CIELab system and Photoshop software. Collected data at different time points were statistically analyzed.

**Results:** The overall color change in the two groups of A and B was significantly different from the control group ( $P < 0.001$ ). Significant changes in color were also noticed in follow up sessions in groups A and B ( $P < 0.001$ ). At 2 months, the difference in overall color change between incisal, middle and cervical thirds of teeth in groups A and B was not statistically significant.

**Conclusion:** Based on the obtained results, application of propolis as an intracanal medicament can lead to clinical color changes in tooth crown. Its application technique has no effect on the degree of discoloration.

**Key words:** CIELab system, Intracanal medicament, Propolis, Tooth discoloration.

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### Introduction:

Bacteria are the main culprits responsible for development of pulpal and periapical inflammation (1). Thus, an important goal of endodontic therapy is to eliminate microorganisms from the infectious root canal systems. Endodontic treatment reduces the number of microorganisms in root canals to a great extent with the use of different instruments and medicaments through mechanical and chemical techniques (2, 3). However, there is a

possibility that some microorganisms remain in the root canal system. Therefore, different medicaments are often used in-between treatment sessions (4-6). Among microorganisms, *Enterococcus faecalis* has been introduced as a resistant pathogen against endodontic treatments (7). The efficacy of different intracanal medicaments against *E. faecalis* has been investigated in several studies and calcium hydroxide has been introduced as an effective agent against it (8-10). However, use of calcium hydroxide is associated with

some limitations as well because this medicament is not able to eliminate all the microorganisms from the root canal system (11) and requires a long time to exert its antimicrobial effects (5). Furthermore, it is potentially toxic due to its high pH and can damage the soft tissue as well. This issue can lead to chronic inflammation and cell necrosis in its clinical application (12). Thus, there is a clear need for more recent, suitable materials with minimal irritation and maximal antibacterial activity in endodontic treatments. Propolis is a newly introduced substance for this purpose. Propolis is a mixture collected by honeybees from the botanical sources around the beehive. It is used to reinforce the structural stability of the beehive and disinfect the beehive environment. The composition of propolis varies depending on the botanical sources found in the particular hive area and season of the year. It is a complex mixture of various chemical constituents with known biological effects including antibacterial, antifungal and restorative properties (13). In-vitro and animal studies conducted on this substance (14, 15) revealed that flavonoids present in propolis might stimulate the formation of secondary dentin (16). On the other hand, some studies demonstrated that calcium hydroxide in some cases has not been able to eliminate *E. faecalis* strains from the root canal system (17, 18) which, can lead to subsequent bacterial colonization in root apex and periapical tissue and impair the process of healing. This can have a negative impact on treatment prognosis (19). Following the recognition of some of the characteristics of propolis, its antimicrobial activity against some resistant strains became the subject of investigation for many researchers. The obtained results have mostly been favorable although achieving a sterile, germ-free root-canal system with the use of current techniques seems distant (20, 21). Considering the optimal characteristics of propolis, it may be a good alternative for an

intracanal medicament to increase the success rate of endodontic therapy. Aside from the chemical properties and antimicrobial efficacy of intracanal medicaments, an important issue to consider is the effect of their short- or long-term application on discoloration of tooth crown. To the best of our knowledge, no study has investigated the color change due to the application of newly introduced propolis intracanal medicament (22). Therefore, the present study sought to assess tooth crown discoloration due to the application of propolis intracanal medicament at different follow up time points.

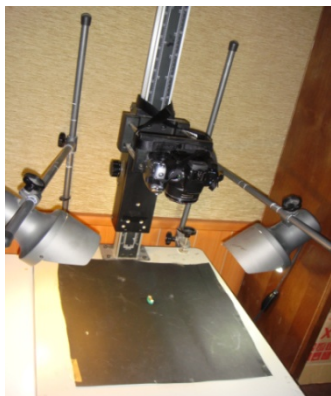
### **Methods:**

In this in-vitro experimental study, a total of 40 sound anterior human teeth with no caries, restoration, developmental defect, enamel crack or external discoloration were selected and evaluated. The teeth had been recently extracted due to periodontal problems or orthodontic treatment plan. For surface disinfection, samples were placed in 5.25% sodium hypochlorite for 30 minutes (Vitex, Shamin Chemical Co., Tehran, Iran) and then stored in 0.9% sterile saline solution at room temperature until the experiment. Standard access cavities were prepared. Cleaning and shaping of root canals were carried out to size #45 hand file (Maillefer, Switzerland) to the working length using the Step-back technique. Recapitulation and irrigation with 10cc of 5.25% sodium hypochlorite solution were performed in between every two files. Canals were then dried with paper points. In order to obtain 30% propolis, 7 cc of 96% ethanol was mixed with 3 g propolis. A filter (CA-20/25) was used to separate the impurities of the 30% solution. The teeth were digitally photographed at baseline using a digital camera (Fujifilm, 5.0 MP, 12X Optical, Tokyo, Japan). To avoid possible errors, the teeth were randomly divided into three

groups of A and B (15 samples each) and C (control group, 10 specimens). Using a sterile insulin syringe, 30% propolis was injected into the canals and pulp chamber in group A and into the canals alone in group B. Canal orifice was cleaned with a cotton pellet. In group C, root canals were filled with distilled water. Access cavity in all teeth was sealed with temporary dressing. Digital photographs were obtained at one day, one week, 2 weeks, one month and 2 months following the placement of medicaments. Assessment of color parameters was done by digital photography at follow up time points. Tooth color parameters were reported according to the CIE L\*a\*b\* color scale. L (lightness) is the mean lightness, positive a is red, negative a is green, positive b is yellow, and negative b is blue.  $\Delta E$  is the overall color change and ranges from 0-100.  $\Delta E$  was calculated according to the following formula (23):

$$\Delta E = [(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2]^{1/2}$$

When taking the photographs, the lighting of the room, position of the light source and angle of lighting were constant. A device was specifically made for this purpose that had two light sources at both sides lighting the specimen surface at 45° angles in a completely dark room (Figure 1).



**Figure 1- The specifically designed device (position of the light source and specimen)**

Putty was used to fix the teeth in the same

position to photograph their labial surface against a grey background.

A small round disc was punched out of a cardboard and placed at the most distant point of the root from the tooth crown and was constant at all phases to adjust the brightness and color of all photographs in the software.

For computer analysis, obtained images were transferred to a computer and assessed in Adobe Photoshop CS5 software. Labial surface of teeth in images was divided into three equal segments of incisal, middle and cervical thirds and lightness, a and b parameters were separately calculated for each segment. The spectrum of Lab changes in Adobe Photoshop software varies from 0 to 255. This range in CIELab system varies from 0 to 100 for lightness and from -120 to +120 for a and b. The following formula was used to convert the Lab values to the CIE system:

$$b^* = b - 128/a^* = a - 128/L^* = L \times 100/255$$

The Lab parameters for tooth color along with the color change ( $\Delta E$ ) were entered SPSS version 16 software. Changes in Lab color parameters and overall color change ( $\Delta E$ ) were compared with Repeated Measures ANOVA. Change in parameters between each two time points was also assessed using tests of within-subjects factors. In these comparisons, type 1 error was considered as 0.05 and type 2 error as equal or less than 0.05.  $p < 0.05$  was considered statistically significant.



**Figure 2- Tooth specimen at baseline**



**Figure 3- Tooth specimen at 2 months follow up**

### Results:

This study evaluated the change in tooth color parameters following the use of 30% propolis as the intracanal medicament at different follow up time points. For data analysis, facial surfaces of teeth were divided into three equal segments of incisal, middle and cervical thirds and each zone was analyzed separately. Significant mutual associations existed between the assessment time points and understudy groups ( $p < 0.001$ )

mainly attributed to the no color change in the control group over time. In the control group, the values for L, a and b parameters were 101.155, 0.03 and 0.09, respectively and no change in these values and consequently in  $\Delta E$  occurred at 2 months; whereas,  $\Delta E$  significantly increased in the remaining two groups. Thus, to further analyze the obtained data, the control group was eliminated and the calculations were continued.

#### *Incisal third:*

Color change value increased in this group over time ( $p < 0.001$ ) and the effect of interaction between the color change in the two remaining groups and time was not statistically significant ( $p = 0.21$ ). Pair-wise comparison of time points in terms of  $\Delta E$  by Bonferroni method revealed that the color change value had an increasing trend. In other words, statistically significant differences were found between time points in this respect. The effect of study group on color change was not significant ( $p = 0.816$ ).  $\Delta E$  values in the two groups of A and B are demonstrated in Table 1.

**Table 1-  $\Delta E$  values for incisal, middle and cervical thirds in each group at different time points**

	Incisal				Middle				Cervical			
	Group A (Propolis in the canals and pulp chamber)		Group B (Propolis in canals)		Group A (Propolis in the canals and pulp chamber)		Group B (Propolis in canals)		Group A (Propolis in the canals and pulp chamber)		Group B (Propolis in canals)	
Time	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
At one day	9.543	1.756	5.850	1.696	7.261	1.348	5.067	1.348	7.211	1.586	3.566	1.586
At one week	15.786	2.916	16.811	2.817	10.677	1.814	7.975	1.814	10.595	2.971	10.425	2.971
At two weeks	28.657	3.505	30.820	3.386	22.264	2.604	16.489	2.504	21.707	3.989	20.345	3.989
At one month	35.002	3.787	39.793	3.639	31.086	3.908	28.326	3.906	30.935	4.516	36.834	4.516
At two months	42.839	2.921	42.671	2.822	45.195	3.734	37.638	2.734	41.933	3.948	42.418	3.948

#### *Middle third:*

Color change value showed an increasing trend

over time ( $p < 0.001$ ) and the effect of interaction between the two intervention groups and time on

color change was not statistically significant ( $p=0.472$ ). Pair-wise comparison of time points in terms of  $\Delta E$  by using Bonferroni test revealed the increasing trend of color change and the difference between time points was statistically significant. The intervention group had no effect on color change ( $p=0.211$ ).  $\Delta E$  values in groups A and B are demonstrated in Table 1.

*Cervical third:*

Color change value increased over time ( $p<0.001$ ). The mutual effect of intervention and time on color change was not significant ( $p=0.302$ ). Pair-wise comparison of time points according to Bonferoni method in terms of  $\Delta E$  revealed the increasing trend of  $\Delta E$  and the difference between time points in this regard was statistically significant. The intervention group had no significant effect on color change ( $p=0.954$ ).  $\Delta E$  values in groups A and B are shown in Table 1. The overall color change in incisal, middle and cervical thirds of teeth in groups A and B was similar at follow up time points.

**Discussion:**

Bacteria play a pivotal role in development of pulp and periapical diseases. Elimination of bacteria, their products and the residual pulp tissue from the root canal system are among the important goals of endodontic therapy. Intracanal medicaments play an important role in this regard. In vital pulp therapy, there is no need for the use of intracanal medicaments. But, in case of time shortage, they may be used in between treatment sessions. However, their role is more prominent in necrotic teeth and cases of apical periodontitis for disinfection of root canal system and reducing the pain in between treatment sessions (24).

Preventing discoloration especially in the anterior teeth following short- or long-term application of intracanal medicaments is a major concern for both patient and dentist. Role of root

canal filling materials and sealers has been investigated in this regard as well. Propolis is a new substance that has recently gained fame in medicine. Due to its antimicrobial activity, it has been suggested for use as an intracanal medicament (25, 26). Similar to calcium hydroxide, propolis has the advantage of being washed off by irrigation with sodium hypochlorite and filing (27). Due to its nature and different types resulted from the activity of honeybees, propolis does not have constant physical, chemical or biological characteristics and has various compositions and colors. Therefore, considering the lack of a specific formula and difference in color due to its source of production, different results may be obtained by using another type of propolis. However, considering the overall similarities of all types, the results of this study can, to a great extent, be generalized to other types. It seems that the original color of propolis also affects its coloring potential as yellow Ledermix or gray MTA can cause tooth discoloration (28, 29). The original color of propolis is amber; which per se can be responsible for color change. In this study, color change of teeth due to the application of 30% propolis intracanal medicament was evaluated at different follow up time points. Several techniques have been suggested for color change assessment in teeth. In the present study, digital photography and measurement of color parameters (CIElab) were used for determination of color change. This is a popular technique and has been used in numerous studies such as those of Zare Jahromi in 2011 (30), Partovi in 2006 (31) and Kim in 2000 (28). Use of CIElab scale as an important index for the assessment of quantitative color characteristics is another advantage of the current study. In previous studies on color change due to endodontic sealers, Partovi (2006) and Van derBurgt (1986) used similar techniques for root canal preparation and obturation (31, 32). But, in their study, canal preparation was carried out through

an apical access cavity which is not clinically feasible; whereas, in the present study similar to that of Kim *et al.* (2000), coronal access cavity was prepared for placement of intracanal medicament (28) providing conditions similar to clinical setting. No specific time point has been set for color change due to intracanal medicaments. A previous study assessed discoloration due to the application of Ledermix intracanal medicament for 2 months (28); which is in accord with the present study (time-wise). Time required for clinical tooth discoloration due to intracanal medicament depends on several factors including the thickness of remaining dentin, presence or absence of smear layer and quality and quantity of the intracanal medicament (32). In Van derBurgt study (1986) color change due to sealers occurred sooner which may be attributed to smear layer removal (32).

In the present study, following the application of 30% propolis as intracanal medicament, facial surface of teeth in the test groups was divided into three equal thirds. Studies have demonstrated that propolis can cause significant discoloration in coronal tooth surfaces over time ( $P < 0.001$ ). Also, the degree of this color change was equal in incisal, middle and cervical thirds. Color change followed the same path in groups A and B.

When Ledermix was placed as a medicament in root canals and pulp chamber, the degree of color change was greater than the situation where the medicament was only placed inside the root canals (28). This finding is in contrast to the results of the present study and may be attributed to physical and chemical differences between Ledermix and propolis as well as the deeper penetration of propolis. Furthermore, cleaning the orifices with ethanol can expedite

the diffusion of medicament resulting in similar degree of color change in groups A and B.

On the other hand, propolis constituents can be responsible for this color change. Flavonoids and minerals such as iron can leave stains and ethanol facilitates their diffusion. Considering the fact that color change due to propolis still remained after 2 months, there was no need for longer evaluation periods. Also, technique of application of medicament (in root canals alone or root canals plus pulp chamber) caused no difference in discoloration. Considering the potential of propolis in causing discoloration and despite its antimicrobial properties, this material is not suggested for use as an intracanal medicament in between treatment sessions especially in the anterior teeth. Further evaluations are recommended to illuminate the pattern of color change due to propolis.

### **Conclusion:**

Based on the obtained results, use of propolis as an intracanal medicament can cause clinical discoloration in tooth crowns. Different techniques of medicament application have no effect on degree of discoloration.

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### **Conflict of Interest: “None Declared”**

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