Original Study

EVALUATING THE EFFECTIVENESS OF PROPOLIS EXTRACT ON OCCLUSION OF DENTINE TUBULES: AN SEM STUDY

Arabnejad R,[1] Eskandarizadeh A,[2] Hoseinifar R,[3] Hamzeh F [3]

1. *Post Graduate Student, Deaprtment of Operative Dentistry, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran*
2. *Associate Professor, Department of Operative Dentistry, Oral and Dental Diseases Research Center , Kerman Social Determinants on Oral Health Research Center, Kerman, Iran.*
3. *Assistant Professor, Department of Operative Dentistry, School of Dentistry, Kerman University of Medical Sciences, Kerman, Iran.*

ABSTRACT

Aim: Due to the cost-effective and clinical safety of propolis, beside the lack of adequate data, the aim of this study includes evaluating the ability of Iranian propolis for dentinal tubule occlusion incorporating Scanning Electron Microscopy (SEM).

Materials & Method: Twenty one mid-coronal human dentine samples were conditioned by 17% EDTA prior to randomly divided into seven groups treated as follows (n=3): Group 1: Normal Saline (NS) – Group 2: 30% Ethanolic Extract of Propolis (EEP) – Group 3: EEP 30%+Citric Acid (CA)- Group 4: Flouride Varnish (FV) -Group 5: FV + CA -Group 6: Artificial Saliva (AS) – Group 7: AS + CA. Subsequently, four 53.7×53.7 µm micrograph images were obtained for each specimen using SEM. Two blinded restorative dentists evaluated the images and the number of open dentinal tubules was recorded. Ultimately, the data were analyzed using One Way ANOVA followed by Tukey Post Hoc test (α= 0.05).

Results: The NS had significant difference with all groups except the AS (p= 0.000 and 0.99 respectively). However, the groups 2,3,4 and 5 did not showed any statistical significance difference with each other (p= 1.00 in all mentioned pair wise comparisons).

Conclusion: The propolis could occlude the dentinal tubule as effective as fluoride varnish while the formed precipitation in both of these agents were resistance to acid challenge.

Key words: Dentine hypersensitivity, Dentine tubule, Dentine permeability, Propolis, Flouride varnish, SEM.

Introduction

Dentin hypersensitivity (DH),which is defined as short sharp pain resulting from a thermal, chemical, evaporative, tactile,or osmotic stimulus that contact with exposed dentin,[1] is still remained as a major concern in patients visiting dental offices.[2] Among various hypotheses corresponded for DH, the most widely accepted theory has been proposed as hydrodynamic theory in which the moving fluid in dental tubules is responsible for the resulting pain.[3,4] Therefore, in DH situations, one of the best solutions includes as tubule obstruction for isolating them from the oral environment and declining dentinal fluid movement.[5] This strategy has been the topic of numerous investigations in dentistry field seeking for an efficient remedy in DH cases.[2,5-10]

Recently, in occlusive treatments, several agents such as bioactive glass,[8] fluoride,[10,11] hydroxyapatite,[10] amorphous calcium phosphate- caseine phophopeptide (ACP-CCP),[9] and potassium oxalate[11] are provided in either form of toothpaste, gels, varnishes or etc.[12] However, these materials have some black points. For instance, the available dentifrices produce some plugs on dentine those are not stable and cannot withstand neigther the mechanical abrasions nor the acidic chemical compounds in the daily diet.[5,6] Accordingly, growing number of researches are conducted scoping on DH management while innovative desensitizing agent is till required.[2,9,11,13,14]

Nowadays, the ascending interest in alternative and complementary medicine has encouraged many dental researchers to evaluate different properties of natural products in dentistry especially because most of these agents are not only cost-effective but also safe clinically.[15-18] Among these natural gifts, the propolis is introduced as one of the best choices due to its wide possible application in medicine including antimicrobial, antitumor, anesthetic, anti-inflammatory, antiviral, healing properties, dental caries prevention, and reducing pulpal inflammation with no major side effect and without any contraindication in human.[9]

Regarding to the chemical composition of the propolis (which is consisted of approximately 50% of resin and vegetable balm)[19] and due to its natural role (which is employed by bees to seal the hive as a defensive mechanism),[19,20] this agent was considered as a possible curing for DH. On this object, few recent literatures have been published comparing the occlusive property of propolis with different chemical agents.[2,9-11,13,14] However, the available data is quite sparse and more studies are suggested. Moreover, the chemical composition of propolis is directly related to its botanical origin that may results to diverse properties based on the chemistry.[2]

Accordingly, the aim of this study includes evaluating the ability of Iranian propolis for dentinal tubule occlusion incorporating Scanning Electron Microscopy (SEM).

Materials & Method

Propolis extraction

The propolis was harvested by hand in spring season from beehives situated in Kerman province that is roughly situated in the south-east of Iran. The samples were desiccated and stored at 4°C prior to the beginning of the study.

The ethanolic extraction of propolis (EEP) was prepared adjusted to the Bosio *et al* method.[21] Accordingly, the propolis was added to ethanol 95% (v/v) and shacked for 7

days at room temperature. Then, the whole mixture was centrifuged and filtered using a #4 Whatman paper. Afterward, the solution was desiccated and a powder was obtained. Finally, the powder was diluted by ethanol to produce a 30% solution.

Sample preparation

This research was approved by the Research Ethics Committee of Kerman University of Medical Science(IR.KMU.REC.1396.2454) (2017). Regarding to this approval, eleven healthy human third molar were used in this study, those were extracted via surgery procedure due to the their impaction. The teeth were stored in tap water that was replaced once a week. At the beginning, the teeth were disinfected by immersing in 5.25% sodium hypochlorite solution for 1-hour. After cleaning with a low-speed handpiece using brushes and slurry of pumice, they were examined by a restorative dentist to discard the cracked or decayed samples.

The selected teeth were mounted in clear polyester resin prior to sectioning horizontally (perpendicular to the long axis) from mid coronal area with a diamond saw (Iso Met® 1000 Percision Saw; Buehler, Lake Bluff, IL, USA). Consequently, the exposed mid coronal dentine surfaces were polished using silicon carbide paper serially (Soflex, 3M, USA), sonicated for 10 minutes to remove the polishing abrasive while they were immersed in distilled water, and rinsed with saline. Finally, in order to open the dentinal tubules thoroughly, the mid coronal dentine samples were treated by 17% Ethylenediaminetetraacetic acid (EDTA) (MORVABON Co.) for 2 minutes, rinsed with distilled water for 1 minute, and sonicated again for 5 min.

Experimental design

The twenty-one prepared mid coronal dentine specimens were randomly divided into seven groups receiving treatment solutions as follows:

- Group1: Normal Saline (NS)
- Group2: EEP 30% (30 min)
- Group3: EEP 30% (30 min) followed by Citric Acid (CA) for one minute
- Group4: Flouride Varnish (FV) (30 min)
- Group5: FV (30 min) followed by CA for one minute
- Group6: Artificial Saliva (AS) (30 min)
- Group7: AS (30 Min) followed by CA for one minute

Finally, all the samples were washed by normal saline for 2 minutes. It should be noted that immersing in citric acid were accomplished to evaluate the stability of the possibly formed precipitation in the tubules.[22]

Scanning Electron microscopy analysis (SEM)

Ultimately, for SEM analysis, the treated dentine surfaces were mounted on the aluminum stub using carbon-coated double-sided adhesive tape and then coated with gold using sputter coater.

Photomicrographs representative of dentin surface areas were obtained using SEM (Camscan MV2300, Czechoslovakia) at a standard magnification of ×3000 in four 53.7×53.7 µm areas of each specimen. Two blinded restorative dentists evaluated the images and the number of open dentinal tubules in each micrograph was recorded.[10]

Statistical methods

The data were analyzed statistically using One Way ANOVA followed by Tukey Post Hoc test (α= 0.05).

Results

Some sample micrographs obtained from this study are displayed in Figure 1. As can be seen, incorporating the EEP and FV has led to noticeable occlusion of dentine tubules comparing to the NS and AS. Moreover, acid challenge did not visibly affect the EEP or FV treated surface.

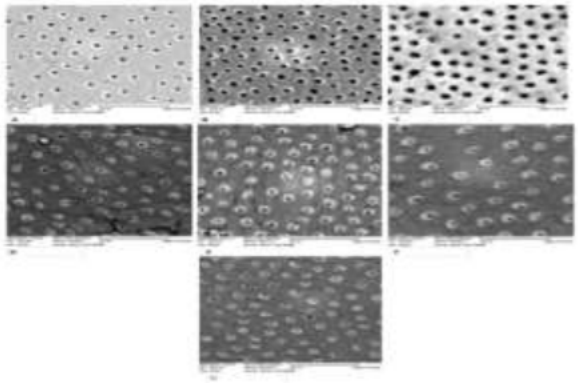

Figure 1: Sample SEM micrograph representing the open or obstructed tubule in each dentine specimen (A, B, C, D, E, F and G are respectively demonstrate Normal saline, Artificial saliva, Artificial saliva+Citric acid, Flouride varnish, Flouride varnish+Citric acid, Ethanolic Extract of propolis, and Ethanolic extract of propolis+Citric Acid).

On the other hand, the mean ± S.D of all subgroups are represents in Figure 2.

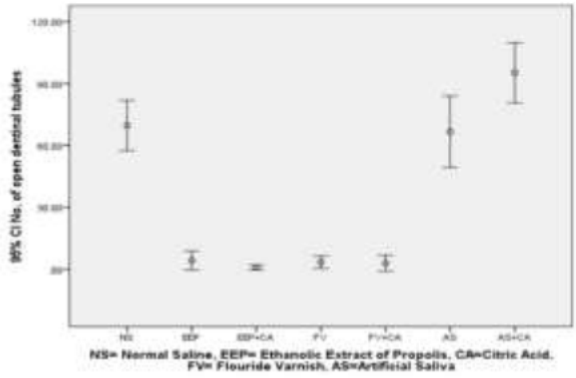

Figure 2: The mean number of open dentinal tubules ± S.D of all subgroups.

Accordingly, the statistical analysis revealed that there was significance difference between the studied groups (p= 0.00). More precisely, the p values related to pair wise comparison of different groups are demonstrated in Table.1.

	EEP	EEP+CA	FV	FV+CA	AS	AS+CA
NS	.000	.000	.000	.000	.999	.006
EEP		1.00	1.00	1.00	.000	.000
EEP+CA			1.00	1.00	.000	.000
FV				1.00	.000	.000
FV+CA					.000	.000
AS						.001
AS+CA						

NS: Normal Saline, EEP: Ethanolic Extract of Propolis, CA: Citric Acid, FV: Flouride Varnish, AS: Artificial Saliva.

Table 1: The P values related to pairwise comparison of different groups.

As it is shown, the NS has significant difference with all groups except the AS. It means that all the groups had protective effect on dentine except the NS and the AS groups. However, the AS+CA showed the highest number of open dentinal tubule [Figure 2] while it is obvious from the Table.1 that there is statistically significant difference between the AS and AS+CA groups. Therefore, the un-protective effect of AS and the destroying capacity of CA was confirmed on dentine.

Nevertheless, the EEP, EEP+CA, FV and FV+CA groups did not have any significant difference with each other. [Table.1] Hence, both the EEP and FV treatment had led to strong precipitation on dentine surface, which was not noticeably altered by acid attack

Discussion

The results of this study revealed that the experimental EEP and FV have strong ability to occlude the dentine tubules and this layer is resistance to acid challenge. Therefore, both the propolis extract and the fluoride varnish could desensitize the dentine.

Our result is in agreement with Chen *et al* who reported effective tubule occlusion for propolis extract.[2] However, they compared the red propolis extract (RPE) with calcium sodium phosphosilicate (Novamin) and arginine-calcium carbonate (ACC).[2] Interestingly, they found superior effect of RPE comparing to Novamin. In addition, they used RPE with concentration of 10% while we studied the 30% extract, which could be more effective. However, Sales-Peres *et al.* showed that there was no significant difference among 10% and 30% propolis extract in reducing dentine permeability.[11] They compared the effectiveness of two experimental gels containing these 10% and 30% alcoholic extract of propolis with oxa gel and acidulated flourophosphate gel reporting desensitizing effect for all groups while there was no significant difference among them.[11] Accordingly, their observations confirmed our results comparing the EEP and FV for tubule occlusion.

The acid challenge was performed in the present study to mimic the clinical situations in which the treated surface would be exposed to different dietary substances.[2,11] Fortunately, our results regarding to the acid challenge showed that both the EEP and the FV was completely resistance while this finding is in accordance with previous documentations.[2,11]

Other publications also confirmed the protective effect of propolis against dentine in their in-vitro studies.[9,23] Moreover, some clinical investigations, reported beneficial application of propolis in cases of dentine hypersentivity.[13,14] Although several other researches are needed on this subject, propolis could be suggested as a valuable agent for treating dentine hypersensitivity, because it has other advantageous in dentistry field including cariostatic effect and inhibition of plaque formation.[24-26] Therefore, in hyperseneitive patients it could be applied not only for rendering their pain but also as a preventive agent for possible future decay.[13,14,24-26] Especially that the protective layer would be resistance to acidic conditions.[2,11] Actually, the propolis is a unique natural gift in dentistry field because of its wide application.[20]

The occluding potential of propolis extract on dentinal tubules could be explained by different mechanisms. First of all, the propolis has a resinous architecture, which could penetrate into microprosities and deep into dentinal tubules; this mechanism is quite similar to dental adhesives resins and since this penetration is somehow deep, the durable effect would be resulted.[27,28] Secondly, the high concentration of flavonoids in both propolis and dentine would led to interaction with each other besides that they results to crystal formation occluding the dentinal tubules.[11] Moreover, as the third mechanism, the propolis could stimulate transforming growth factors (TGF)-β1 in dentine, which is a dominant factor for differentiation of odontoblast-like cells.[29] This phenomenon was confirmed by researches those investigate the effect of propolis in direct pulp capping aspects.[30] Therefore, reparative dentinogenesis stimulated by the propolis could contribute to dentine tubule occlusion.[2]

On the other hand, our finding about the FV is in accordance with many previous publications that discovered the noticeable effect of fluoride in dentine occlusion and treatment of dentine hypersensitivity.[12]

In this research, we studied the dentine occlusion via SEM micrographs. This is a quite valid and precise method for analyzing the topographical aspects of a surface and providing non-destructive high-resolution three-dimentional images.[2] This technique was frequently incorporated in literatures investigating the effect of various agents on dentine permeability, dentinal tubule occlusion and compensating dentine hypersensitivity.[2]

Overwhelmingly, although the In-vitro tests do not reproduce the real clinical situations, our study demonstrated that the propolis extracted could be incorporated as an effective agent in preventive dentistry. However more researches are strongly suggested to resemble the clinical effectiveness. Furthermore, complementary investigations are recommended to detect the detailed chemical composition of different propolis and comparing their effect with each other.

Conclusion

Significant dentine tubule occlusion was observed in both the EEP and FV groups comparing to NS and AS while there was no significant difference between EEP and FV. Moreover, the protective layer formed in both the EEP and FV groups were resistance to acid challenge.

References

1. Chiang YC, Wang YL, Lin PY, Chen YY, Chien CY, Lin HP *et al*. A mesoporous biomaterial for biomimetic crystallization in dentinal tubules without impairing the bonding of a self-etch resin to dentin. J Formos Med Assoc 2016;115(6):455-462.
2. Sabir A, Tabbu CR, Agustiono P, Sosroseno W.Histological analysis of rat dental pulp tissue capped with propolis. J Oral Sci 2005;47(3):135-8.
3. Gillam DG. Current diagnosis of dentin hypersensitivity in the dental office: an overview. Clin Oral Investig 2013;17(Suppl 1):S21-29.
4. Leonard RH Jr, Smith LR, Garland GE, Caplan DJ. Desensitizing agent efficacy during whitening in an at-risk population. J Esthet Restor Dent 2004;16(1):49-55.
5. Liu X, Barnes V, DeVizio W, Yang H, Malmstrom H, Ren Y. Effects of dentin tubule occlusion by dentifrice containing a PVM/MA bioadhesive copolymer in a silica base. J Dent 2011;39(4):293-301.
6. Absi EG, Addy M, Adams D. Dentin hypersensitivity: uptake of toothpastes onto dentine and effects of brushing, washing and dietray acid-SEM in vitro study. J Oral Rehabil 1995;22(3):175-82.
7. Addy M, West N. Etiology, mechanisms, and management of dentine hypersensitivity. Curr Opin Periodontol 1994;71-7.
8. Chen WC, Kung JC, Chen CH, Hsiao YC, Shih CJ, Chien CS. Effects of bioactive glass with and without mesoporous structures on desensitization in dentinal tubule occlusion. Appl Surf Sci 2013;283:833-842.
9. Morris MF, Davis RD, Richardson BW. Clinical efficacy of two dentin desensitizing agents. Am J Dent 1999;12(2):72-76.
10. Farooq I, Moheet IA, AlShwaimi E. In vitro dentin tubule occlusion and remineralization competence of various toothpastes. Arch Oral Biol 2015;60(9):1246-1253
11. Rimondini L, Baroni C, Carrassi A. Ultrastructure of hypersensitive and non-sensitive dentine. J Clin Periodontol 1995;22(12):899-902.
12. Miglani S, Aggarwal V, Ahuja B, Dentin hypersensitivity: Recent trends in management. J Conserv Dent 2010;13(4):218-224.
13. Madhavan S, Nayak M, Shenoy A, Shetty R, Prasad K. Dentinal hypersensitivity: A comparative clinical evaluation of CPP-ACP F, sodium fluoride, propolis, and placebo. J Conserv Dent 2012;15(4):315.
14. Torwane NA, Hongal S, Goel P, B R C, Jain M, Saxena E *et al*. Effect of two desensitizing agents in reducing dentin hypersensitivity: An in-vivo comparative clinical trial. J Clin Diagn Res 2013;7(9):2042-6.
15. Fani M, Kohanteb J. Inhibitory activity of aloe vera gel on some clinically isolated cariogenic and periodontopathic bacteria. J Oral Sci 2012;54(1):15-21.
16. Fereidooni M, Samani MK, Amiri A, Ahmadi MH. Comparison of the effect of propolis and traditional toothpaste on bacterial plaque. J Babol University of Med Sci 2014;16(2):17-22.
17. Palombo EA. Traditional medicinal plant extracts and natural products with activity against oral bacteria: potential application in the prevention and treatment of oral diseases. Evid Based Complement Alternat Med 2011; 2011:680354.
18. Rezvani MB, Hemmati MA, Norouzi M, Bakhtiari R, Azinpour F, Hamzeh F. Evaluating the effect of Kandovan propolis against Streptococcus Mutans. J Oral Health Oral Epidemiol 2018;7(2):94-98.
19. Dziedzic A, Kubina R, Wojtyczka RD, Kabala-Dzik A, Tanasiewicz M, Morawiec T. The antibacterial effect of ethanol extract of polish propolis on mutans streptococci and lactobacilli isolated from saliva. Evid Based Complement Alternat Med 2013;2013:681891.
20. Toreti, V.C., *et al*., Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med 2013;2013:697390.
21. Bosio K, Avanzini C, D'Avolio A, Ozino O, Savoia D. In vitro activity of propolis against Streptococcus pyogenes. Lett Appl Microbiol 2000;31(2):174-177.
22. Wang Z, Sa Y, Sauro S, Chen H, Xing W, Ma X *et al*. Effect of desensitising toothpastes on dentinal tubule occlusion: a dentine permeability measurement and SEM in vitro study. J Dent 2010;38(5):400-410.
23. Almas K, Mahmoud A, Dahlan A. A comparative study of propolis and saline application on human dentin. A SEM study. Indian J Dent Res 2001;12(1):21-27.
24. Hayacibara MF, Koo H, Rosalen PL, Duarte S, Franco EM, Bowen WH *et al*. In vitro and in vivo effects of isolated fractions of Brazilian propolis on

caries development. J Ethnopharmacol 2005;101(1-3):110-5.
25. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. Caries Res 1991;25(5):347-351.
26. Koo H, Pearson SK, Scott-Anne K, Abranches J, Cury JA, Rosalen PL *et al*. Effects of apigenin and tt-farnesol on glucosyltransferase activity, biofilm viability and caries development in rats. Oral Microbiol Immunol 2002;17(6):337-343.
27. Montero JC, Mori GG. Assessment of ion diffusion from a calcium hydroxide-propolis paste through dentin. Braz Oral Res 2012;26(4):318-322.
28. van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P *et al*. Adhesion to enamel and dentin: current status and future challenges. Oper Dent 2003;28(3):215-235.
29. Ansorge S, Reinhold D, Lendeckel U. Propolis and some of its constituents down-regulate DNA synthesis and inflammatory cytokine production but induce TGF-β1 production of human immune cells. Z Naturforsch C 2003;58(7-8):580-589.
30. Parolia A, Kundabala M, Rao NN, Acharya SR, Agrawal P, Mohan M *et al*. A comparative histological analysis of human pulp following direct pulp capping with Propolis, mineral trioxide aggregate and Dycal. Aus Dent J 2010;55(1):59-64.

Corresponding Author

Dr.Faezeh Hamzeh
Assistant Professor,
Department of Operative Dentistry,
School of Dentistry,
Kerman University of Medical Sciences,
Kerman, IRAN
E-mail: faeze.hamze@gmail.com