Journal of Taibah University Medical Sciences (2021) 16(2), 283–287

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Experimental Article

Efficacy of propolis in remineralising artificially induced demineralisation of human enamel - An in-vitro study

Saqib Ali, MSc [a], Imran Farooq, MSc [a,*], Amr Bugshan, PhD [a], Intisar Ahmad Siddiqui, MSc [b], Khalifa S. Al-Khalifa, DrPH [c] and Mohammed Al-Hariri, PhD [d]

[a] *Department of Biomedical Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, KSA*
[b] *Department of Quality Assurance, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, KSA*
[c] *Department of Preventive Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, KSA*
[d] *Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, KSA*

Received 21 August 2020; revised 25 September 2020; accepted 3 October 2020; Available online 14 December 2020

الملخص

أهداف البحث: في هذه الدراسة في المختبر، قمنا بتحليل إمكانية قدرة مادة البروبوليسعلى إعادة بناء طبقة المينا.

طرق البحث: تم تقسيم ٢٠ عينة من المينا بشكل عشوائي إلى مجموعتين (كل مجموعة ١٠) المجموعة ١ (التحكم): حيث تم دهن عينات المينا باللعاب الصناعي، والمجموعة ٢: تم دهن عينات المينا بزيت البروبوليس. تعرضت جميع عينات المينا لنزع المعادن عن طريق تعريضها إلى حمض الستريك بنسبة تركيز ٦٪ (الرقم الهيدروجيني = ٢.٢) لمدة ٥ دقائق. وتم إجراء تفريش للأسنان داخل آلة محاكاة تفريش الأسنان باستخدام فرش الأسنان اليدوية وتلقت كل عينة ٥٠٠٠ ضربة طولية. تم إجراء تحليل الصلابة الدقيقة للسطح على كل عينة على ثلاث فترات مختلفة قبل نزع المعادن (الأساس)، وبعد نزع المعادن، وبعد إعادة المعادن للحصول على عدد صلابة فيكرز.

النتائج: أظهرت النتائج تحسنا في الصلابة الدقيقة لعينات المينا بعد تفريش الأسنان بزيت البروبوليس مقارنة بالعينات التي تم تفريشها فقط. وبالنسبة لمجموعة التحكم، كان متوسط خط الأساس لعدد صلابة فيكرز هو ٥٨٣.٦٦، الذي انخفض إلى ١١٦.٢٣ بعد نزع المعادن، في حين تمت زيادة عدد صلابة فيكرز إلى ١٨٤.٠٢ بعد فترة إعادة المعادن. وكان متوسط خط الأساس لعدد صلابة فيكرز للمجموعة ٢ هو ٥٠٦.٩١، الذي انخفض إلى ٣١٧.٦ بعد نزع المعادن، ثم ارتفع إلى ٤٣٥.١٩ بعد إعادة المعادن. كشفت قيم عدد صلابة فيكرز لكلا المجموعتين في مقارنات المجموعة المشتركة وداخل المجموعة فروق ذات دلالة إحصائية.

الاستنتاجات: أظهرت دراستنا أن دهن عينات المينا بالبروبوليس ساعد في تحسين مستويات الصلابة الدقيقة، أكثر من مجموعة التحكم. الدراسات المستقبلية ضرورية للتحقق من الآلية الدقيقة للآثار المفيدة للبروبوليس على طبقة المينا.

الكلمات المفتاحية: اعادة المعادن؛ العكبر؛ الصلابة المجهرية؛ المينا؛ نزع المعادن

Abstract

Objective: In this *in vitro* study, we aimed to analyse the enamel-remineralisation potential of propolis.

Materials and methods: Twenty enamel blocks (N = 20) were randomly divided into two groups (n = 10). In group 1 (control), enamel blocks were brushed with artificial saliva (AS). In group 2, they were brushed with propolis oil. All the blocks were demineralised by exposing them to 6 wt% citric acid (pH: 2.2) for 5 min. Brushing was performed inside a tooth brushing simulation machine with manual toothbrushes. Each sample received 5,000 linear strokes. Surface microhardness analysis was performed for each sample at three time intervals (pre-demineralisation or baseline, post-demineralisation, and post-remineralisation) to obtain the Vickers hardness numbers (VHNs).

Results: An enhancement in the microhardness of the enamel samples was observed after brushing with propolis oil when compared with brushing using AS alone. In

* Corresponding address: Department of Biomedical Dental Sciences, College of Dentistry, Imam Abdulrahman Bin Faisal University, Dammam, 31441, KSA.
E-mail: drimranfarooq@gmail.com (I. Farooq)
Peer review under responsibility of Taibah University.

ELSEVIER Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2020.10.022

group 1 (control group), the mean baseline VHN was 583.66. It decreased to 116.23 after demineralisation and increased to 184.02 after remineralisation. The mean baseline VHN of group 2 was 506.91. It decreased to 317.60 after demineralisation and increased to 435.19 after remineralisation. The VHN values of both the groups revealed statistically significant differences ($p < 0.05$) in inter-group and intra-group comparisons.

Conclusion: Brushing of enamel blocks with propolis led to a greater enhancement in their microhardness levels when compared with the control group. Future studies are essential to validate the exact mechanism of the beneficial effects of propolis on enamel.

Keywords: Demineralisation; Enamel; Microhardness; Propolis; Remineralisation

Introduction

Dental enamel constitutes the outer protective coating of teeth.[1] Odontoblasts (cells responsible for producing dentine) are available throughout the life of a person. However, the ultimate fate of ameloblasts (cells that synthesise enamel) is apoptosis and regression.[2] Thus, enamel cannot be biologically repaired or replaced after its formation.[3] Human teeth are continuously subjected to dynamic cycles of demineralisation and remineralisation.[4] A balance between these two coupled processes is crucial for maintaining the tooth structure.[5] When this equilibrium shifts in favour of demineralisation, the risk of tooth surface loss is increased.[4] Interaction among multiple factors such as cumulative salivary flow, dietary habits of a person, and the oral microflora can significantly shift the balance towards demineralisation.[6] Moreover, constant consumption of acidic foods or drinks and low salivary flow rate over time generate an acidic environment with low pH, resulting in demineralisation that can eventually lead to dental caries or erosion.[7] Certain systemic diseases such as gastro-oesophageal reflux disease are also associated with demineralisation of teeth, ultimately resulting in dental erosion.[8]

Interestingly, demineralisation can be reserved in its early stages if the intensity and the frequency of acidic attack is reduced together with the creation of an oral environment that favours remineralisation.[4] The potential of tooth surface remineralisation could be increased naturally through salivary ions or it could be induced by therapeutic materials.[9] The currently available remineralisation therapies act either by increasing the minerals around the demineralised lesions or by affecting the volume and the composition of the dental biofilm around the lesions.[9] Among the currently available therapies, fluoride application is the standard management for remineralisation of demineralised lesions, as it has the ability to replace hydroxyl ions in the apatite structure, changing it from hydroxyapatite (HAP) to fluorapatite (FAP). Chemically, FAP is more stable and resistant to acidic exposures.[10]

Recently, products have been developed to enhance the remineralisation ability by utilising potential elements for remineralisation such as calcium, phosphate, sodium trimetaphosphate, triclosan, and xylitol.[9] Propolis is an important natural product that has already shown its efficacy as an anti-inflammatory agent, anti-infective agent, and antioxidant.[11] It is a natural resinous substance that has shown potential for bio-dental applications due to its beneficial properties.[12] It has also been reported as an anticaries agent,[13] root canal irrigant,[14] pulpotomy agent,[15] intracanal medicament,[16] and pulp-capping agent.[17]

It is evident from the previous studies that propolis is a promising agent that can be used in the dental field. However, there is a scarcity of studies in the available literature regarding its ability as a potential remineralising agent for the enamel. Therefore, the aim of the present study was to evaluate the remineralisation potential of propolis on artificially demineralised human enamel.

Materials and Methods

The present study was planned in agreement with the protocols of the Declaration of Helsinki. Propolis oil was used in the brushing experiments.

Artificial saliva (AS) formulation

AS was formulated by mixing 0.400 g NaCl and KCl, 0.69 g NaH_2PO_4. H_2O, 0.79 g $CaCl_2$. H_2O, and 0.005 g Na_2S. $9H_2O$ in 1000 mL of deionised water, as proposed by Fusayama et al.[18] The pH of the newly synthesised AS was 5.4, which was adjusted to a neutral pH of 7.0 by adding 1 M of NaOH, as suggested by Farooq et al.[19]

Preparation and grouping of enamel blocks

Twenty extracted human third molars (N = 20) were acquired from the dental hospital of the institute. Teeth that were devoid of white spot lesions, cavitation, and restorations were carefully selected. The teeth were cut mesiodistally just above the cementoenamel junction using a high-speed handpiece (EXPERTmatic® LUX E15L, KaVo Dental GmbH & Co. KG, Germany) fitted with diamond bur having a head length of 1.2 mm, a diameter of 0.3 mm, and an overall length of 19 mm (WR-13, Prima®, Prima Dental Group, Gloucester, United Kingdom). After the teeth were cut into two sections, the roots were discarded and the anatomic crown of each tooth was entrenched in an acrylic block ensuring that only the buccal surface of the enamel was exposed. With the help of a nail varnish, a window of approximately 4 mm × 4 mm was created on this surface to ensure that all experiments and analyses were performed only in this area. As the natural contour of the enamel surface is not flat, the marked surface was ground and polished with the help of a grinding and polishing machine (MetaServ 250 Grinder-Polisher with Vector Power Head, BUEHLER®, Lake Bluff, IL, USA). Twenty enamel blocks were randomly and equally divided into two groups. Group 1 (control) included ten enamel blocks brushed with AS and group 2 included ten enamel blocks brushed with propolis oil.

Artificial demineralisation

All enamel blocks were subjected to demineralisation by exposing them to 6 wt% citric acid (pH: 2.2) for 5 min. To mimic dynamic *in vivo* conditions, glass beakers containing 500 mL of citric acid with enamel blocks completely immersed in them were placed over a rotating orbital shaker (CO-Z® Orbital Shaker, USA). After the acidic challenge, enamel blocks were washed with distilled water for 1 min and were allowed to air-dry overnight.

Simulated brushing protocol

Manual toothbrushes from a single brand (Trisa®, Triengen, Switzerland) were used in this study. Tooth brushing was performed inside a brushing simulator (ZM-3.8, SD Mechatronik, Feldkirchen-Westerham, Germany). Each sample was brushed with 5,000 linear strokes, which is comparable to *in vivo* brushing for 6 months.[20] The load applied to the enamel samples was 250 g and the linear distance covered by the toothbrushes was set to 30 mm. After every 1,000 strokes, AS or propolis oil was added on the surface of the samples from the respective groups. After brushing, samples were washed with distilled water for 1 min and air-dried before the microhardness measurements.

Surface microhardness analysis

Surface microhardness investigation was conducted on each sample at three time intervals: pre-demineralisation (baseline values), post-demineralisation, and post-remineralisation. Vickers surface hardness was calculated using a digital microhardness tester (FM-ARS 9000; Future-Tech Corp, Kawasaki, Japan). Each enamel block received three indentations using a Vickers diamond indenter under a load of 250 g with a dwell time of 10 s. The average value of the three indentations was used for the analysis.

Statistical analysis

Data analysis was performed using IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA). Numerical data were presented as mean and standard deviation. Normality of data distribution was checked using the Kolmogorov−Smirnov test. Since the data showed non-Gaussian distribution, non-parametric Wilcoxon Mann−Whitney U test was used for the comparison of microhardness between the groups. Wilcoxon signed-rank test was applied to assess the significance of the differences within each group (comparison of baseline values with post-demineralisation and post-remineralisation values). P-values ≤ 0.05 were considered statistically significant.

Results

The microhardness of the enamel blocks was assessed with the help of Vickers indentation. Each sample received three indentations (Figure 1) on the marked unexposed buccal surface. An enhancement in the microhardness levels was observed in both group 1 and group 2 after brushing. Although brushing with AS in group 1 resulted in remineralisation of the enamel surface after artificially induced demineralisation, the improvement in the microhardness levels after remineralisation was lower than that in group 2 (Table 1).

The mean Vickers hardness number (VHN) in group 1 (AS group) was 583.66 at baseline, 116.23 after demineralisation, and 184.02 after brushing. The mean VHN in group 2 (propolis group) was 506.91 at baseline, 317.60 after demineralisation, and 435.19 after brushing. Significant differences were observed in the inter-group and the intra-group comparisons (Table 1).

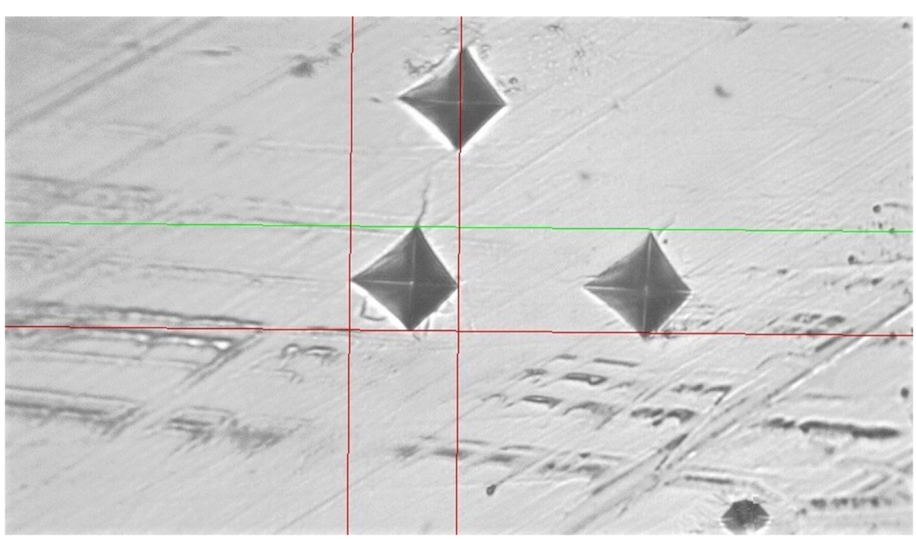

Figure 1: Indentations on the enamel surface to obtain the Vickers hardness numbers.

Table 1: Mean VHN values for both the groups measured at baseline, after demineralisation, and after brushing.

Microhardness	Vickers hardness number mean (standard deviation)		*P-value*
	Group-1	Group-2	
Baseline	583.66 (15.80)[a]	506.91 (87.41)[a]	0.012
Post-demineralisation	116.23 (6.84)[a,b]	317.60 (82.06)[a,b]	0.001
Post-brushing	184.02 (32.94)[a,b]	435.19 (105.44)[a]	0.001
^*P-value*	0.012	0.012	–
^^ *P-value*	0.012	0.208	–

^P-value: Within-group significance (baseline versus post-demineralisation).
^^P-value: Within-group significance (baseline versus post-brushing).
[a] Denotes the significance of means in the horizontal direction (inter-group) by employing the Wilcoxon Mann Whitney U test at 5% level of significance.
[b] Denotes the significance of means in the vertical direction (intra-group) by employing the Wilcoxon signed-rank test at 5% level of significance.

Discussion

In the present study, propolis displayed promising results in terms of remineralisation after artificially induced demineralisation of human enamel according to the surface microhardness analysis. In the current era of novel dental materials, researchers are looking to explore and utilise biocompatible natural products that possess beneficial properties for desired functions.[16] Propolis is a natural material synthesised by bees for the assembly and defence of their hives.[12] It is used by bees to disinfect the hive and to preserve its optimal internal temperature.[11] The composition of propolis includes plant deposits, wax, and pollen. Other important constituents of propolis include minerals, vitamins, and amino acids.[21] It has been researched extensively in the field of medicine and dentistry and has many clinical applications.[22]

Dental caries initiate with the formation of a white spot lesion that can be reversed if the equilibrium shifts in favour of remineralisation.[3] The metabolic products of bacterial species residing in the oral cavity play an important role in the demineralisation process.[4] The acquired enamel pellicle is the first line of protection against caries. If it is modified with natural products containing polyphenolic compounds, it can prevent bacterial colonisation.[23] Propolis is rich in phenolic compounds and flavonoids.[24] Its antimicrobial role has been studied extensively in the past. Reportedly, it could interfere with cell division and enzyme activity of microbes and could also retard the adhesion of bacteria.[25] Therefore, it has been suggested that propolis has the potential to modify the pellicle positively and could have a defensive influence against cariogenic bacteria.

In the present study, enamel samples brushed with propolis exhibited enhanced surface microhardness when compared with samples brushed with AS alone. This finding could be attributed to the fact that propolis enhances the availability and application of calcium and phosphate minerals.[26] Dental enamel is composed of HAP crystals, which are primarily made up of calcium and phosphate minerals.[20] In the present study, the application of propolis might have enhanced the absorption of these minerals back into the enamel surface after brushing, resulting in enhanced remineralisation. In a previous study, Wassel and Khattab reported that varnishes containing propolis slowed down the demineralisation of enamel.[27] Our results are consistent with the results of their study. Another study reported that surface microhardness of enamel samples increased upon immersion in propolis solution.[28] Our findings demonstrated strengthening and improved integrity of enamel upon exposure to propolis and are consistent with the results of the aforementioned study, although we used another form of exposure (simulated tooth brushing with propolis), which is closer to the actual *in vivo* conditions.

One of the limitations of our study was its *in vitro* nature. Propolis needs to be tested with actual human saliva, as the interaction of the ingredients and the proteins of saliva with propolis could show different results. Another limitation was the difficulty in obtaining the microhardness readings from exactly the same point at different intervals. To minimise variation and to ensure standardisation, enamel surfaces were marked before the start of the experiment. Thus, brushing was performed on the same area and measurements were obtained only from the marked enamel surface.

To the best of our knowledge, this is the first study utilising propolis for simulated tooth brushing of enamel samples to study their microhardness levels. Propolis can be acquired easily and is cost effective. Moreover, it can also be extracted by simple real-life methods. With the rising costs of dental products, the clinical potential and cost-effectiveness of propolis should be considered while synthesising novel dental products with antimicrobial and remineralising properties.

Conclusion

The results of the present study suggested that propolis has good remineralisation potential to improve the microhardness of enamel surface after artificial demineralisation. Future studies and clinical trials are anticipated to test the potential of propolis under more dynamic *in vivo* conditions.

Recommendations

Based on the results of this study, the potential use of propolis in toothpastes should be investigated in future studies. Laboratory investigations and clinical trials are warranted in the future to understand its beneficial properties related to dentistry.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or non-profit sectors.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

This project was approved to be conducted at The College of Dentistry, Imam Abdulrahman Bin Faisal University and ethical approval was obtained and all protocols were firmly shadowed. Ethical approval [EA: 2018001−22-11-2017].

Authors' contributions

SA and IF conceived and designed the study, conducted the research, provided research materials, performed the experiments, and collected and organised the data. AB and IAS analysed and interpreted the data and helped in writing the manuscript. KAK and MA wrote the initial and the final drafts of the article and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the contents and the similarity index of the manuscript.

References

1. Ali S, Farooq I. A review of the role of amelogenin protein in enamel formation and novel techniques to study its function. **Protein Pept Lett 2019**; 26: 880−886.
2. Lacruz RS, Habelitz S, Wright JT, Paine ML. Dental enamel formation and implications for oral health and disease. **Physiol Rev 2017**; 97: 939−993.
3. Alhussain AM, Alhaddad AA, Ghazwi MM, Farooq I. Remineralization of artificial carious lesions using a novel fluoride incorporated bioactive glass dentifrice. **Dent Med Probl 2018**; 55: 379−382.
4. Abou Neel EA, Aljabo A, Strange A. Demineralization-remineralization dynamics in teeth and bone. **Int J Nanomed 2016**; 11: 4743−4763.
5. Farooq I, Ali S, Siddiqui IA. Influence of thymoquinone exposure on the micro-hardness of dental enamel: an in vitro study. **Eur J Dent 2019**; 13(3): 318−322.
6. Tahmassebi JF, Duggal MS, Malik-Kotru G, Curzon ME. Soft drinks and dental health: a review of the current literature. **J Dent 2006**; 34: 2−11.
7. Barbour ME, Finke M, Parker DM. The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures. **J Dent 2006**; 34: 207−213.
8. Cengiz S, Cengiz MI, Saraç YS. Dental erosion caused by gastroesophageal reflux disease: a case report. **Cases J 2009**; 2: 8018.
9. González-Cabezas C, Fernández CE. Recent advances in remineralization therapies for caries lesions. **Adv Dent Res 2018**; 29: 55−59.
10. Amaechi BT, van Loveren C. Fluorides and non-fluoride remineralization systems. **Monogr Oral Sci 2013**; 23: 15−26.
11. Abbasi AJ, Mohammadi F, Bayat M. Applications of propolis in dentistry: a review. **Ethiop J Health Sci 2018**; 28: 505−512.
12. Khurshid Z, Naseem M, Zafar MS, Najeeb S, Zohaib S. Propolis: a natural biomaterial for dental and oral healthcare. **J Dent Res Dent Clin Dent Prospects 2017**; 11: 265−274.
13. Ikeno K, Ikeno T, Miyazawa C. Effects of propolis on dental caries in rats. **Caries Res 1991**; 25: 347−351.
14. Jolly M, Singh N, Rathore M, Tandon S, Banerjee M. Propolis and commonly used intracanal irrigants: comparative evaluation of antimicrobial potential. **J Clin Pediatr Dent 2013**; 37: 243−249.
15. Ozório JE, Carvalho LF, de Oliveira DA, de Sousa-Neto MD, Perez DE. Standardized propolis extract and calcium hydroxide as pulpotomy agents in primary pig teeth. **J Dent Child 2012**; 79: 53−58 (Chic).
16. Shabbir J, Qazi F, Farooqui W. Effect of Chinese propolis as an intracanal medicament on post-operative endodontic pain: a double-blind randomized controlled trial. **Int J Environ Res Publ Health 2020**; 17: 445.
17. Parolia A, Kundabala M, Rao NN. 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. Fusayama T, Katayori T, Nomoto S. Corrosion of gold and amalgam placed in contact with each other. **J Dent Res 1963**; 42: 1183−1197.
19. Farooq I, Moheet IA, AlShwaimi E. In vitro dentin tubule occlusion and remineralization competence of various toothpastes. **Arch Oral Biol 2015**; 60: 1246−1253.
20. AlShehab AH, AlHazoom AA, Alowa MH, AlAli HA, Abdulmohsen AA, Farooq I. Effect of bristle stiffness of manual toothbrushes on normal and demineralized human enamel-an in vitro profilometric study. **Int J Dent Hyg 2018**; 16: e128−e132.
21. Sardana D, InduShekar K, Manchanda S, Saraf BG, Sheoran N. Role of propolis in dentistry: review of the literature. **Focus Alternative Compl Ther 2013**; 18: 118−125.
22. Wassel MO, Sherief DI. Ion release and enamel remineralizing potential of miswak, propolis and chitosan nano-particles based dental varnishes. **Pediatr Dent J 2019**; 29: 1−10.
23. Siqueira WL, Bakkal M, Xiao Y, Sutton JN, Mendes FM. Quantitative proteomic analysis of the effect of fluoride on the acquired enamel pellicle. **PloS One 2012**; 7:e42204.
24. Galeotti F, Maccari F, Fachini A, Volpi N. Chemical composition and antioxidant activity of propolis prepared in different forms and in different solvents useful for finished products. **Foods 2018**; 7: 41.
25. Habluetzel A, Schmid C, Carvalho TS. Impact of honey on dental erosion and adhesion of early bacterial colonizers. **Sci Rep 2018**; 8: 10936.
26. Haro A, López-Aliaga I, Lisbona F. Beneficial effect of pollen and/or propolis on the metabolism of iron, calcium, phosphorus, and magnesium in rats with nutritional ferropenic anemia. **J Agric Food Chem 2000**; 48: 5715−5722.
27. Wassel MO, Khattab MA. Antibacterial activity against Streptococcus mutans and inhibition of bacterial induced enamel demineralization of propolis, miswak, and chitosan nanoparticles based dental varnishes. **J Adv Res 2017**; 8: 387−392.
28. Giamalia I, Steinberg D, Grobler S, Gedalia I. The effect of propolis exposure on microhardness of human enamel in vitro. **J Oral Rehabil 1999**; 26: 941−943.

How to cite this article: Ali S, Farooq I, Bugshan A, Siddiqui IA, Al-Khalifa KS, Al-Hariri M. Efficacy of propolis in remineralising artificially induced demineralisation of human enamel - An in-vitro study. J Taibah Univ Med Sc 2021;16(2):283−287.