# *In Vitro* Evaluation of Microleakage and Microhardness of Ethanolic Extracts of Propolis in Different Proportions Added to Glass Ionomer Cement

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***Objective:*** *To evaluate the effect of ethanolic extracts of propolis (EEP) addition in different proportions to glass ionomer cement (GIC) on microleakage and microhardness of GIC.* ***Study design:*** *The cement was divided into four groups: one using the original composition and three with 10%, 25%, and 50% EEP added to the liquid and then manipulated. For microleakage assessment, sixty primary molars were randomly divided into four groups (n=15). Standard Class II cavities were prepared and then filled with EEP in different proportions added to GICs. Microleakage test was performed using a dye penetration method. The data were analyzed using one-way ANOVA and Mann - Whitney U tests (α = 0.05). Disc shaped specimens were prepared from the tested* GIC *to determine Vickers hardness (VHN). The data were analyzed using one-way ANOVA and post hoc Tukey test (α = 0.05).* ***Results:*** *There were no statistically significant differences between the groups in terms of microleakage (p > 0.05). There were statistically significant differences between the VHN values of groups (p < 0.05). Increasing addition of EEP to GIC statistically significantly increased VHN value of GIC (p < 0.05).* ***Conclusions:*** *The addition of EEP to GIC increased the microhardness of the GIC and did not adversely affect the microleakage. Thus, it might be used during routine dental practice due to its antibacterial properties*

*Key words: Ethanolic extracts of propolis, glass ionomer cement, microhardness microleakage*

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## INTRODUCTION

Dental caries is one of the most prevalent disease affecting humans. Its incidence is especially high during childhood.[1] In the last decade, new approaches for caries management have gained great importance in dentistry. These approaches consist of decreased sugar intake, usage of fluoride containing toothpastes, topical fluoride application, usage of anti-plaque and antibacterial solutions[2] and increasing the antibacterial properties of dental materials. However, dental caries is still a serious health problem in developing and the least developed countries. Thus, new techniques were developed in caries treatment. One of them is Atraumatic Restorative Treatment (ART) which depends on maximum prevention and minimall invasive procedures. This technique consists of removal of infected carious dentin with hand instruments and restoration with the glass ionomer cements (GIC).[3,4] The favorable characteristics of GICs include continuous fluoride release, inhibition of bacterial acid metabolism and activity, biocompatibility, chemical bonding to both enamel and dentin, and effective bonding in a moist environment without the need for an additional bonding agent layer.[5-7] Despite its advantages, GICs have antibacterial effects against a small spectrum of microorganisms and low bactericidal potential.[8]

Antimicrobial substances such as propolis, chlorhexidine and antibiotics are added to GICs to increase their antibacterial effects and they were investigated in previous studies.[8-13] The substance added to a dental material as an antibacterial agent is important in

terms of its biocompatibility. Propolis which is a natural resinous substance made by honey bees[14], has many pharmacologic properties such as antioxidant, antifungal, antiviral, anti-inflammatory, and antibacterial effects.[15] There are different forms of propolis in commercial market, such as lyophilized and ethanolic form. Ethanolic extract of propolis (EEP) is the most commonly used, where ethanol works as a solvent or vehicle.[10] EEP has been used commercially on the market as an antibacterial component of mouth rinses, toothpastes, lozenges, and so forth. It is reported that EEP demonstrates antibacterial effect on *Streptococcus mutans* which is a main contributor to tooth decay caused by biofilm formation.[2, 8] EEP can be used in clinical dentistry due to its antimicrobial activity against cariogenic bacteria and inhibition of glucosyltransferase activity[16]. Although several studies were published about antibacterial activity of propolis on oral microorganisms[2, 8, 9, 11, 14, 16], there are limited studies in terms of evaluating effects of propolis on mechanical properties of glass ionomer cement. To our knowledge, to date, shear-peel band strength (SPBS)[8], diametral tensile strength, water sorption and solubility[10] of extracts of propolis added GIC were investigated. However, microleakage and microhardness of propolis added to GIC were not evaluated. Thus, the aim of this study was to evaluate the effect of ethanolic extracts of propolis (EEP) addition in different proportions to GIC on microleakage and microhardness of GIC.

## MATERIALS AND METHOD

This study was approved by the ethical committee of Sifa University.

### Preparation of propolis extract

Solid-state propolis was produced by honeybees (*Apis mellifera* L.) in the region of western Anatolia, Kayseri in Turkey. It was left cooling after having dissolved inside boiling ethanol with the help of an extractor and separated from its wax after filtration. This filtrated product was then mixed at room temperature using an evaporator until it took a thick paste form. EEP was obtained after dissolving this extract in ethanol.

### Preparation of propolis containing GIC

EEP was added to liquid of conventional GIC (Imicryl SC, Imicryl Diş Malz San. Tic. AŞ, Konya, Turkey) with the proportions of 10%, 25%, and 50%. In this way three new solutions were prepared. Original liquid of a GIC was used as a control.

Sixty primary molar teeth were used for microleakage assessment. The teeth were divided randomly into four groups of 15 teeth each. The teeth were cleaned with pumice slurry and were washed with running water to eliminate pumice residues prior to use. Standardized class II cavities were prepared on the mesial or distal surfaces of each tooth with a carbide bur under water cooling. The dimensions of prepared cavities were 2.5 mm occlusal-gingival extension and 2.0 mm buccal-lingual extension. The powder and each liquid prepared before were mixed according to the manufacturer's instructions and pastes were put into the cavities by pressing down with a glass side. After setting, all specimens were stored in a humid environment at 37 °C for 24 h. Then, the specimens were covered with nail varnish up to 1 mm from the cavity margins to prevent dye infiltration. All specimens were immersed in a 5% basic fuchsin dye solution for 24 h. Following immersion in the dye solution, the teeth were washed under running tap water to remove excess solution. Next, the specimens were sectioned buccolingually and parallel to the long axis with a low speed handpiece into 3 fragments for microleakage evaluation and the depth of dye penetration in each section was examined under a stereomicroscope with 20× magnification. Microleakage was scored for the degree of dye penetration at the occlusal and cervical walls.[17]

Scoring for dye penetration for marginal microleakage on the occlusal wall:

0 – No dye penetration.1 – Dye penetration into enamel. 2 – Dye penetration beyond the dentinoenamel junction. 3 – Dye penetration into the pulpal wall.

Scoring for dye penetration for marginal microleakage on the cervical wall:

0 – No dye penetration. 1 – Dye penetration into half extension of cervical wall. 2 – Dye penetration into more than half or complete extension of the cervical wall. 3 – Dye penetration into cervical and axial walls toward the pulp.

The results were recorded and analyzed using the statistical package SPSS 17.0 program (SPSS, Chicago, IL). The statistical evaluation was carried out using one-way ANOVA and Mann - Whitney U tests at a significance level of $p < 0.05$.

### Microhardness assessment

The powder and each liquid were mixed according to the manufacturer's instructions and pastes were put into disc shaped teflon mold. The upper surfaces of the samples were pressed with thin plates to provide flat surfaces until setting. In this wise, five disc shaped specimens (6 mm in diameter and 2 mm in thickness) were obtained from each group. Surface of the cements were covered with varnish after the completion of the setting reaction. All specimens were stored in a humid environment at 37 °C for 24 h for Vickers hardness test. The hardness of the specimens was measured using a microhardness testing machine (Q10, QNESS GMBH, Tokyo, Japan) on the top of the surface of each specimen and recorded. Vickers diamond indentations were performed under a load of 300 g and 15 s. Each sample was subjected to at least three indentations located 200 μm far from each other, and the mean hardness values were recorded. The diagonal length of the impressions were measured and the hardness (HV) was calculated according to the standard formula H=1.854P/d2. The data were analyzed using one-way ANOVA and post hoc Tukey test ($\alpha = 0.05$).

## RESULTS

The distribution of microleakage scores of groups is presented in Table 1. The mean microleakage score of control group was higher than those of other groups. However, there were no statistically significant differences among groups ($p > 0.05$). Cervical and occlusal walls of each group showed similar mean microleakage values ($p > 0.05$) (Table 1).

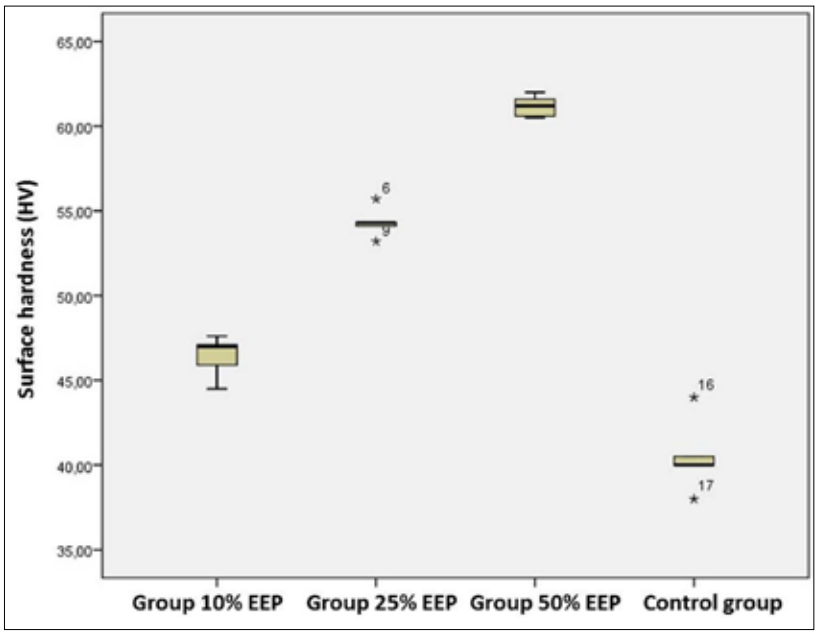
The mean and standard deviations of VHN values of groups are presented in Fig. 1. There were statistically significant differences among groups in terms of microhardness ($p < 0.05$). Groups 50%, 25%, and 10% showed higher VHN values than control group ($p < 0.05$). Group 50% showed the highest VHN value whereas group 10% showed the lowest ($p < 0.05$) (Fig. 1).

**Table 1. Distribution of microleakage scores of occlusal and cervical walls according to groups**

| | | | Scores | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Groups** | **N** | **0** | **1** | **2** | **3** | **Mean(SD)** | **Significance*** |
| Control group | Cervical | 15 | 0 | 1 | 6 | 8 | 2.3(0.5) | A |
| | Occlusal | 15 | 0 | 0 | 9 | 6 | 2.5(0.6) | A |
| Group 10% EEP | Cervical | 15 | 0 | 2 | 9 | 4 | 2.3(0.8) | A |
| | Occlusal | 15 | 0 | 3 | 5 | 7 | 2.1(0.6) | A |
| Group 25% EEP | Cervical | 15 | 0 | 3 | 7 | 5 | 2.2(0.7) | A |
| | Occlusal | 15 | 0 | 2 | 8 | 5 | 2.1(0.7) | A |
| Group 50% EEP | Cervical | 15 | 0 | 4 | 7 | 4 | 2.0(0.5) | A |
| | Occlusal | 15 | 0 | 2 | 11 | 2 | 2.0(0.8) | A |

Same uppercase letters indicate statistically similar means ($p<0.05$)

**Fig 1. Vickers hardness number (VHN) values of groups (median, maximum, minimum, 25th percentile, 75th percentile and outlier)**




## DISCUSSION

GICs are widely used in dentistry especially in pedodontics. They can absorb or release fluoride, and they can bond to moist environments, eliminating the need to keep the teeth dry during bonding. [18] These properties are advantageous for dental fillings in children which is a technique-sensitive procedure and that requires isolation. Due to their capability of releasing fluoride, GIC contributes to some reduction in the number of residual bacteria in cavities as well as remineralization of affected dentin. [8, 9, 19]

The requirements of an ideal restorative material should include good antibacterial effects on oral cariogenic bacteria and an ability to withstand the occlusal forces.[20] GICs do not completely fulfil these requirements and its mechanical properties should be improved. With this regard, it must be kept in mind to improve the physical properties of dental materials as well as their biocompatibility.

Following insertion into the cavity, GICs release 10 ppm of fluoride in the first 48 h. [21] However, this amount is inefficient for achieving the desired antibacterial effects. [19] In order to improve the antibacterial characteristics of GIC, several antibacterial materials were added to its content. [8-13, 22] One of these materials is called EEP which is an easily available, cheap natural substance which can be a great option in dental treatment.[10] Moreover, it showed considerable antimicrobial activities against *Streptococcus mutans*.[2, 8] In previous studies, addition of EEP to GIC with concentrations of 10%, 25%, and 50% were found to increase antibacterial activity of GIC.[8, 9] Considering these factors, EEP' s concentrations tested in the present study were previosly found sufficient in terms of reducing bacteriaes.

In the present study, EEP added GICs were evaluated in terms of microleakage and microhardness. Surface hardness which provides information about wear resistance and long term durability of materials is one of the most important properties of restorative dental materials.[23]. The surface of the dental material is considered to be directly affected by oral conditions[24]. In vitro

microleakage test is one of the valuable tools for evaluating physical properties of dental materials Microleakage assessment with a dye penetration is the most widely used method for marginal leakage. Because this technique is easily available, nontoxic and cheap.[25] In the current study, three slices of each tooth were obtained and mean degree of microleakage of the slices were recorded to increase the reliability of the evaluation.

No data are available in the literature about the microleakage and microhardness evaluation of EEP added to GIC. However, there are only two studies on other mechanical properties of it. Troca et al.[10] evaluated diametral tensile bond strength and solubility of EEP added to three GICs and reported that EEP added to GIC increased the water sorption of all GICs and decreased the diametral bond strength of two of them. The concentration of EEP in that study was 1%. Hatunoğlu et al.[8] evaluated the SPBS of EEP with concentrations of 10%, 25%, and 50% added to GIC. They found that adding EEP to GIC insignificantly increased the SPBS. In the present study, adding EEP with different concentrations to GIC did not statistically affect the microleakage.

Various antibacterial agents such as Epigallocatechin-3-gallate (EGCG), cetrimide, chlorhexidine, benzalkonium chloride and cetylpyridinum were added to GIC to evaluate the surface hardness.[22, 26] Hu *et al* [22] reported that the concentration of 0.1% EGCG increased the microhardness of GIC however, concentration of 0.1% CHX had no effect on microhardness. Tuzuner et al.[26] reported that adding cetrimide, chlorhexidine, benzalkonium chloride and cetylpyridinum to GIC decreased the microhardness of GIC. In this study, adding EEP to GIC increased the microhardness value. Moreover, with the increased ratio of EEP added, VHNs linearly increased.

The curing of GIC depends on neutralisation reaction, which requires the mixing of liquid and powder. The crosslinking of GIC occurs with the interaction of $Al^{3+}$ and $Ca^{2+}$ ions with the COOH groups on the acidic polymers. Generally, COOH groups cannot participate in these complexes because of the vitrification of GIC [27]. The advancement of the microhardness of EEP added GIC specimens is more obvious than GIC specimens. This may be resulted from the combination of GIC and EEP molecules. Many aromatic fatty acids and phenolic compounds are present in EEP molecule. It was indicated by many researchers that polyphenols have various favourable properties due to their high activity.[28, 29] A chelation reaction occurs between this phenolic hydroxyl and carboxyl group of GIC [30]. EEP can act like a spacer for dissociative carboxyl, providing high activity poly-salt bridging and cross-linking. The increase in surface microhardness of EEP added GIC could be due to the high activity of EEP. As a result, the complexity of GIC increases due to the increase in cross-links. The interstitial packing is reduced when greater amount of acid reacts with powder. The intensity of the molecules on the surface increases as fewer gaps exist between the crosslink networks due to the greater amount of poly-salt bridges following the incorporation of EEP.[22, 31, 32]

The color change of GIC to yellow can be attributed to EEP. It could not be problem in use as a base or liner, however when used in the anterior region it might negatively affect aesthetics. The pulp response of GIC is very important when the caries is very deep.[22] Thus, long term mechanical properties and pulpal response of GIC incorporated with EEP should be studied in further investigations.

## CONCLUSION

Within the limitations of this *in vitro* study, addition of EEP to GIC increased the microhardness of the GIC and did not adversely affect the microleakage. Thus, it might be used during routine dental practice due to its antibacterial properties.

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