Effect of ethanolic extract of propolis on antibacterial and microshear bond strength of glass-ionomer restorations to dentin

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A R T I C L E   I N F O

A B S T R A C T

Objectives: This study was conducted to evaluate the effect of ethanolic extract of propolis on antibacterial and microshear bond strength of glass ionomer restorations to dentin.

Materials and methods: Conventional glass ionomer cement (Equia forte, GC Tokyo, Japan), resin-modified glass ionomer (Fuji II LC, GC Tokyo, Japan) and propolis powder (dried extract from honey bees) materials were used in this study. Both conventional glass ionomer and resin-modified glass ionomer were modified by two different concentrations of ethanolic extract of propolis (10 % and 25 % EEP). For antibacterial test, Streptococcus mutans strain was spread on agar petri dishes using a sterile swab. Discs of both glass ionomer restorative materials (without adding EEP, with 10 % EEP and with 25 % EEP) were fabricated within the agar plates. Antibacterial activity was evaluated by measuring the inhibition zones around each disc. For microshear bond strength test, 60 healthy human permanent molars were prepared by cutting occlusal surface and expose the dentin at the height of contour of all teeth then conditioned using poly acrylic acid conditioner, both glass ionomer restorative materials (without adding EEP, with 10 % EEP and with 25 % EEP) were mixed and applied on conditioned dentin surface by using tygon tube. Microshear bond strength was evaluated by the universal testing machine.

Results: Two-way ANOVA test revealed that both glass ionomer type and different concentrations of EEP had significant effect on the antibacterial test results and microshear bond strength values (p < 0,05). Glass ionomer restorative material with 25%EEP had the highest antibacterial values whereas glass ionomer restorative material without modifications (control groups) had the lowest values. Resin-modified glass ionomer without any modification (control group) had the highest bond strength while resin-modified glass ionomer with 25%EEP had the lowest bond strength.

Conclusions: Incorporation of ethanolic extract of propolis to glass ionomer restorative material increases the antibacterial effects of both conventional GIC and RMGI. Inspite of this advantage, it seems that it has deleterious effect on microshear bond strength to dentin.

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1. Introduction

Dental caries is a common multi-factorial chronic infectious disease that occurs in the dental hard tissues [1]. It is a localized destruction of dental hard tissues which caused by the acidic by-products from bacterial fermentation of simple carbohydrates [2]. The most common type of bacteria causing dental caries is *Streptococcus mutans*. Traditional caries management strategies depended on the surgical model of treatment via the complete removal of carious lesion followed by modification of the cavity to be convenient for the restoration. The restorative material must be accurately selected to be convenient for the prepared cavity. Type of restorative material might influence oral biofilm accumulation and development of secondary caries. The formation of the biofilm begins when a salivary glycoprotein film called dental pellicle coats a tooth surface [3]. Gram-positive bacteria including *Streptococci* of the *mitis* and *mutans* species are considered the initial colonizers of the biofilm [4]. Accumulation of bacteria, and the risk of development of secondary caries is much lower around glass ionomer cement (GIC) restorations compared to resin based composite restorations [5].

Glass ionomer cement has several advantages such as chemical bonding to enamel and dentin, fluoride release potential, optimal biocompatibility, coefficient of thermal expansion similar to that of tooth structure, ion exchange, aesthetic properties similar to tooth substrate and absence of polymerization shrinkage [6]. Glass ionomer cement has antibacterial effect due to its fluoride release and low PH during the setting reaction however the amount of fluoride in GIC is insufficient for achieving the desired antimicrobial effects. So there is new concern about enhancing the antibacterial property of GIC by adding different antibacterial agents [7].

Antibacterial additives have two effects on glass ionomer, namely slowing down the setting reaction and reducing the mechanical properties of the cement as they interfere with the maturation of the cement, as well as the setting reaction. Addition of propolis to glass-ionomer cements enhance their antibacterial properties. This substance is particularly active against cariogenic bacteria of the *Streptococcus* genus, in particular *S. mutans* and *S. sobrinus*. Though propolis has some adverse effects on the mechanical properties of glass-ionomers, for example reducing the compressive strength slightly, it was shown to improve the micro-hardness and had no adverse effect on micro-leakage [8].

Although a lot of antibacterial agents have been added to GIC to enhance its antibacterial effect, most of these agents are synthetic medicines and have drawbacks [9,10]. Propolis is a biocompatible natural resin product produced by the honeybees with antioxidant, antifungal, antiviral, and antibacterial properties. It is used for treatment of candidiasis, acute necrotizing ulcerative gingivitis, gingivitis, periodontitis, and pulpitis in dentistry [11]. Propolis has the greatest effect on *Streptococcus mutans* among gram-positive and *Shigella* among gram-negative bacteria [12].

Bond strength of restorative materials to tooth substrate is the most important aspect in restorative dentistry as it affects the clinical durability of the restorative material. Glass ionomer cements are brittle materials and assessment of bond strength is very important to evaluate the durability of these materials. Most of the previous studies have focused on the antimicrobial effects of the restorative materials modified by antibacterial agent but its physicomechanical properties have been overlooked. So this research has studied changes in both the antibacterial and adhesion property of the modified conventional GIC and RMGI.

The null hypotheses were that there was no significant difference in.

 i. Antibacterial activity of GIC with or without EEP modification.
 ii. Bond strength of GIC to dentin with or without EEP modification

2. Materials and Methods

2.1. Materials

Conventional GIC (Equia forte- GC Tokyo, Japan), resin-modified glass ionomer (Fuji II LC-GC Tokyo, Japan) and propolis powder (dried extract from honey bees) were used according to the manufacturer's instructions (Table 1).

Table 1
Materials used in the study.

Material	Specification	Manufacture	Composition	Batch number
Equia forte fil	Conventional GIC	GC Co, Tokyo, Japan	Powder: 95 % strontium fluoroAlumino-silicate glass, 5 % polyacrylic acidLiquid: 40 % acquis polyacrylic acid	180126A
Fuji II lc	RMGI	GC Co, Tokyo, Japan	Powder: Aluminosilicate glassLiquid: 20%–22 % polyacrylic acid,30%–40 % HEMA,5 %7 % 2,2,4,trimethylhexamethylenedicarbonate, 4%–6% TEGDMA, 5%–15 % proprietary ingredient	180528A
Propolis	Antibacterial agent	Imtenan, Egypt	50 %–70 % resin, 30 %–50 % oil and wax, amino acids, minerals, sugar, vitamins B,C and E, flavonoids and phenol.	

3. Methods

3.1. Preparation of ethanolic extract of propolis

Twelve and half gm of propolis powder (Imtenan Health Shop, Obour City, Egypt) was dissolved in 125 ml of ethanol 80 % (vol/vol) for 10 % ethanolic extract of propolis (EEP) and 12,5 gm of propolis dissolved in 50 ml of ethanol 80 % for 25 % EEP by using a magnetic stirrer (BioBase hotplate magnetic stirrer BS-2H, China) for 24 h at room temperature. The solution was then filtered using filter paper, centrifuged (Manual Fast Gene High Speed Mini Centrifuge NG003, Germany) at 8800 rpm for 30 min to produce 10 % and 25 % EEP, and stored at 4 °C in incubator (Boxun HPX9082MBE incubator, Shanghai, China) in two separate dark bottles until use. All the procedures were done at the Soil and Tissue pathology lab, Faculty of Agriculture, Mansoura University.

3.2. Antibacterial test assesment

The *Streptoccocus mutans* strain was obtained from the Microbiology Laboratory (Faculty of Medicine, Mansoura University). Blood agar plates were prepared at the microbiological lab by pouring 5 ml layer of blood agar on each plate. Colonies of *Streptococcus mutans* were subcultured on the surface of blood agar plates.

Thirty discs (n = 30) from each glass ionomer restorative material (conventional GI and RMGI) were fabricated. 10 discs from each material used without addition of EEP (control group), 10 discs used with addition of 10 % EEP and the last 10 discs used with 25 % of EEP addition.

Three wells (holes) were made in each agar plate with the help of 5 mm sterile cork borer (Gelsonlab Nickel Cork Borer, China). Ethanolic extact of propolis in two different concentrations were added to GIC using a micropeppite (TopPette, DragonLab, China). Glass ionomer cement were mixed according to manufacture instructions by using dental amalgamator ($^{TAC-400M \; digital \; amalgamator}$, Italy) for 10 s and injected in each well by the dental clicker (Fuji dental applicator gun, GC America) then incubated at 37 °C for 24 h at the laboratory incubator. Agar plate diffusion method was used where the diameter of inhibition zones produced around each disc was measured using digital caliper three times in three different directions, the average diameters were reported for each group.

4. Micro shear bond strength test

4.1. Teeth selection

Freshly extracted sound human permanent molars extracted for periodontal diseases were collected from the Oral Surgery Department, Faculty of Dentistry, Mansoura University. The following criteria were used for teeth selection: no cracks, restorations, surface defects, or caries. After extraction, the teeth were cleaned from any hard calculus deposits and soft tissue remnants using a hand scaler then, they were cleaned using rubber cups, fine pumice in low speed handpiece (Sirona, Germany). All teeth immersed in a 0.5 % chloramine T solution for one day for disinfection. Later, the teeth were examined for the presence of any micro cracks or defects using a stereomicroscope (30× magnification, SZ TP, Olympus, Tokyo, Japan). Finally, 60 M were selected and stored in distilled water at 37 ±1C until they were used. Distilled water was changed periodically every 5 days to avoid dehydration of the teeth and the storage procedure was done under local infection control protocols.

The teeth were prepared with a low-speed diamond saw (PICO 155 precision saw, Pace technologies, Tucson, AZ, USA) by cutting all teeth horizontally to remove the occlusal surface and expose the dentin at the height of contour of all teeth while employing water coolant (Diacut™ Water-based Cutting Fluid Pace technologies, Tucson, AZ, USA) at a concentration of 1:33, lubricant: water during the cutting procedure. In order to standardize the smear layer, the dentin surface was further polished for 60 s on wet #600 grit silicon carbide paper (Microcut™, Buehler, Lake Bluff, IL, USA). All occlusal surfaces were observed under stereomicroscope to be sure that there is no residual enamel or remaining caries on the dentin surface. All specimens were randomly divided into two main groups for both types of GIC.

4.2. Grouping specimens

Sixty permenant molars were used for microshear test (n = 60). All prepared specimens were classified into 2 main groups (n = 30) according to the used GI restorative material. Group A for conventional GIC while group B for RMGI. Each group was subclassified into 3 subgroups (n = 10) according to the addition of two different concentration of EEP. Subgroup 1 (control group) used without any modifications, subgroup 2 with addition of 10 % EEP and subgroup 3 with addition 25 % EEP. All specimens were then placed in a plastic mold and this mold filled with cold-cure acrylic resin approximating the height of normal bone. The acrylic resin was allowed to set.

4.3. Specimens preparation

All specimen bonding surfaces were conditioned using poly acrylic acid conditioner (Detrey conditioner 36 by dentsply, Germany) for 10s according to manufacture instructions then washed with water. Gentle drying for excess water removal was done but avoid to desiccate. Rubber tubes (tygon tube) with an internal diameter of 1 mm and 3 mm length were placed on specimen dentin bonding surfaces and fixed by using a twizer.

Two GIC restorative material were modified by EEP and mixed using dental amalgamator $^{(TAC-400M\ digital\ amalgamator}$, Italy) for 10s according to manufacturer's instructions. Then manipulated using GIC capsule applicator (Fuji dental applicator gun, China), injected into the rubber tube, which was held by a twizer and fixed on the conditioned prepared dentin surface. Any excess material was removed using a microbrush (Micro applicator brush tips, China). Steps were repeated 10 times for each subgroup of both conventional GIC and RMGI.

4.4. Mounting the specimen to universal testing machine

Before shear bond strength testing, cylindrical tubes were cut by a scalpel. The specimens were transferred to a universal testing machine (Model LRX-plus:Lioyd instrument Ltd, Fareham, UK). A loop prepared from an orthodontic wire was wrapped around the bonded specimen to dentin as close as possible to its interface with the dentin surface and aligned with loading axis of the upper movable compartment of the machine. A shearing load was applied till debonding along tooth restoration interface. The load at failure was recorded in Newton and converted to Megapascal (MPa) by dividing the failure load by the cross-sectional area of each specimen which was measured by a digital caliper (Fig. 1).

4.5. Failure mode analysis

All the debonded specimen surfaces were examined using a stereomicroscope (Olympus, SZ-PT, Japan) to analyze the failure mode distribution for fractured specimens with and without EEP modification. Then a representative specimen from each fracture type was sputter-coated with gold and examined by a scanning electron microscope SEM (JEOL JSM 6510 lv, Japan) to verify fracture type.

5. Results

All data were statistically analyzed using SPSS software (SPSS version 20; IBM). The Kolmogorov smirnov test was conducted to check data normality, it showed that all data results followed the normal distribution pattern.

5.1. Two-way ANOVA test

It revealed that both variables (type of glass ionomer and different concentrations of EEP) had significant effect on the antibacterial test results ($p < 0,05$). Post hoc multiple comparison test showed that the conventional GIC modified by 25 % EEP had the highest antibacterial values (7.91 ± 0.49 mm) while the control subgroup had the lowest antibacterial values (5.38 ± 0.96 mm). There was statistically significant difference between the subgroup modified by 25 % EEP and the control subgroup ($p < 0,05$), While there was no statistically significant difference between the control subgroup and the subgroup modified by 10 % EEP ($p > 0,05$). For RMGI, Subgroup modified by 25 % EEP had the highest antibacterial values (7.99 ± 0.85 mm) while the control subgroup had the lowest antibacterial values (4.39 ± 0.34 mm). There was statistically significant difference between the subgroup modified by 25 % EEP and
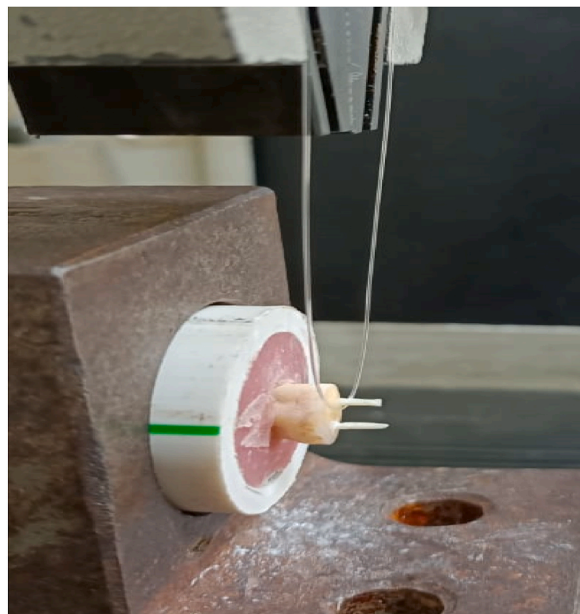

Fig. 1. Specimen mounted to the universal testing machine during µSBST.

the control subgroup (p < 0,05), while there was no statistically significant difference between the control subgroup and the subgroup modified by 10 % EEP (p > 0,05) (Table 2, Fig. 2).

6. Microshear bond strength test results

6.1. Two-way ANOVA test

revealed that the glass ionomer type and EEP concentration had significant effect on microshear bond strength as (p < 0.05). Tukey Post hoc multiple comparison test showed that, For conventional GIC, The control subgroup had the highest bond strength (3.84 ± 0.42 Mpa) while the subgroup with 25%EEP had the lowest bond strength (3.42 ± 0.64 Mpa). There was no statistically significant difference between the control subgroup and the subgroup with 25%EEP (p > 0,05). For RMGI, The control subgroup had the highest bond strength (4.13 ± 0.67 Mpa) while the subgroup with 25%EEP had the lowest bond strength (2.56 ± 0.69 Mpa). There was statistically significant difference between the control subgroup and the subgroup with 25%EEP (p < 0,05) but there was no statistically significant difference between the subgroup with 10%EEP and the subgroup with 25 % EEP (p > 0,05)(Table 3).

7. Results of failure mode analysis

The failure modes were categorized as three types: adhesive failure between dentin and GIC with exposing dentin surfaces, Cohesive failure in GIC or in dentin and mixed failure: partially adhesive failure and partially cohesive failure. The stereoscope and SEM micrographs of failure mode analysis showed that mixed failure mode was dominant for both RMGI and conventional GIC (control and 10 % groups) but the subgroups modified by 25%EEP in both conventional and RMGI were mostly cohesive failure (Fig. 3).

8. Discussion

Glass ionomer restorative materials has gained wide acceptance and popularity in daily dental practice mainly due to its chemical bonding to tooth structure, ease of handling characteristics and fluoride release. However, the amount of fluoride released from GIC is not sufficient to show an antibacterial effect against pathogens causing caries which can give rise to secondary caries. Glass ionomer restorative materials may need to be augmented by adding bactericides to increase its therapeutic benefit [13]. Propolis was selected as it has antibacterial effect against several oral microorganisms especially *Streptococcus mutans* and also it is not toxic and has less side effects than the other synthetic antibacterial agents. *Streptococcus mutans* was selected in this study as it is the principal micro-organism implicated in dental caries [14].

Water plays a critical role in the setting reaction of GIC as it is water-based cement. In the initial phases, it acts as a reaction medium, and in the later stages, it mediates the slow hydration of the cross-linked chains. Water can be easily evaporated during the initial phases due to exposure to air. On the other hand, water contamination during this stage can cause the dissolution of the matrix and the leakage of calcium ions. Thus, uptake of water results in weak, opaque, and more soluble cement [15]. Ethanol was preferred as a solvent more than the water as ethanol may evaporate during the setting reaction, the powder: liquid ratio would not be affected by the incorporation of the ethanolic extract. On the other hand, the water in the aqueous extract of propolis will remain in the cement as loosely bond water [16]. Voids may form during mixing of cement with aqueous extract of propolis, which would negatively affect the mechanical properties [6].

Agar plate diffusion was the method of choice for this study because it allowed both solid (cured) and liquid (uncured) materials to be assayed. Moreover the process is relatively inexpensive and could be performed rapidly and easily with a large numbers of specimens. This method was preferred than other methods such as broth culture which could not allow the testing of uncured material [17]. The application of the modified GIC with EEP immediately after mixing and during the setting (while the material still in liquid phase) in wells created in the agar plate caused growth inhibition zones, while the application of modified GIC and its placement on the agar plate as solid discs and after complete setting did not show any antimicrobial activity. This finding may indicate that release of antibacterial agents from liquids would occur faster and easier than from solid products [18].

Measurement of shear bond strength is a relatively simple, reproducible and widely accepted test [19]. In the current study the wire microshear was used for microshear test instead of using blade. The wire microshear test was easier and more reliable than the blade microshear test due to severe stress concentration caused by chisel as a loading device [20]. The occlusal surfaces of specimens were ground to approximately the same depth midway between the dento-enamel junction and the pulp to represent a site nearly similar to the depth of a typical cavity preparation for testing the shear bond strength [21].

The antibacterial activity of GIC was increased by the addition of high concentrations of propolis, which was shown by an increase

Table 2
Post hoc Tukey test showing mean antibacterial test results ±standard deviation between the studied subgroups.

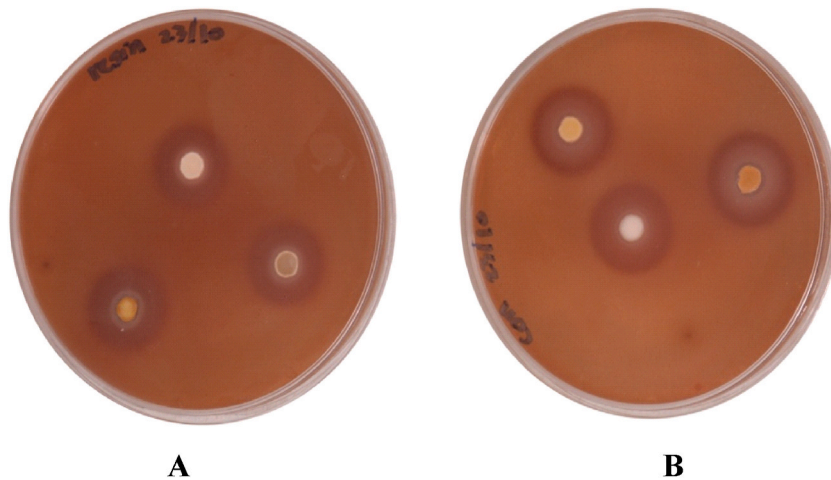| Glass ionomer type EEP Concentration	n	Conventional GIC	RMGI
Control subgroup without EEP	10	5.38 ± 0.96[b,c]	4.39 ± 0.34[a]
With 10%EEP	10	5.94 ± 0.48[c]	4.80 ± 0.22[a,b]
With 25%EEP	10	7.91 ± 0.49[d]	7.99 ± 0.85[d]

Fig. 2. Agar plates showing maximum inhibition zones around 25%EEP modification a; RMGI specimens and b; conventional GIC specimens.

Table 3
Post hoc test showing mean microshear in Mpa ±standard deviation between studied subgroups.

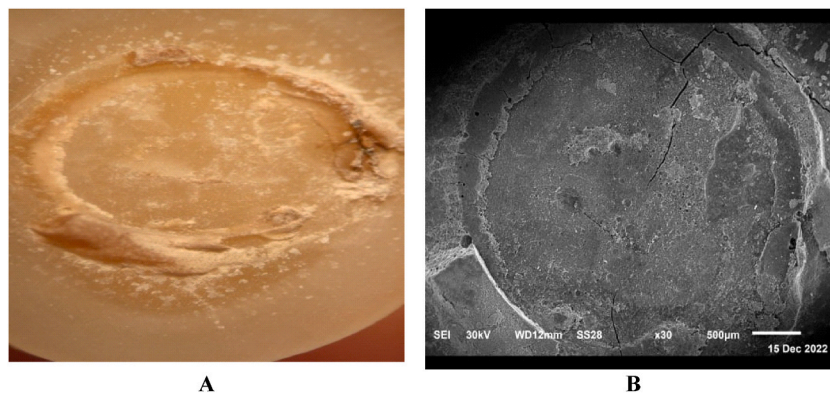| Glass ionomer type EEP concentration	n	Conventional GIC	RMGI
Control subgroup without EEP	10	3.84 ± 0.42^a	4.13 ± 0.67^a
With 10%EEP	10	3.87 ± 0.32^a	3.07 ± 0.44^{bc}
With 25%EEP	10	3.42 ± 0.64^{ab}	2.56 ± 0.69^c

Fig. 3. Cohesive failure in GIC specimen in a; Stereomicroscope b; SEM micrographs.

in the diameter of the growth inhibition zone. The addition of 25 % propolis showed the largest diameter of the inhibition zone, indicating the highest antibacterial activity compared to other treatment groups (10 % propolis and control group). The greater the concentration of propolis, the greater the effect of inhibition produced. This study is in agreement with the studies of Türkün et al. [22] and Deepalakshmi et al. [23] who reported that the antimicrobial efficacy was depended on the concentration of antibacterial agent added to the restorative material. Also Waldner et al. [24] found that propolis may require high concentrations to be developed into an antiseptic agent.

On the other hand, Panahandeh et al. [6] showed that, no growth inhibition zone was noted around the discs of RMGI modified by aquous extract of propolis. However, pure propolis extract caused a growth inhibition zone with 11 mm diameter. Their results were different from the current study, which is probably due to the use of water as a solvent instead of ethanol as type of solvent of propolis can significantly affect its antibacterial activity. Biria et al. [25] disagreed with this study as they observed that although inhibition zones were present around the plate wells containing propolis aqueous extract, there was no inhibition zones around the GIC discs containing propolis, which may be due to the discs were solid and after complete setting.

Ethanolic extract of propolis addition have two effects, namely slowing down the setting reaction and reducing the mechanical properties of the cement. The significant decrease in bond strength value may be attributed to the change in physical properties. Where

the viscosity of GIC liquid decreases by adding EEP that prolongs the working time and interferes with the network formation of GIC systems [25]. Subramaniam et al. [26] conducted a study on the effect of adding propolis on the compressive strength and solubility of GIC. There results demonstrated that adding propolis to the GIC reduced bond strength and increased the solubility of propolis-containing GIC samples, compared to samples without EEP (control group) which agree with this study. Troca et al. [27] agreed with this study as they found that adding EEP has a negative effect on GIC system as the incorporation of propolis decreased the bond strength of glass ionomer cement. This might be attributed to the interference of propolis with the reaction of glass particles and polyacrylic acid, thereby increasing the number of unreacted particles in the structure.

This study disagree with a study conducted by Hatunoglu et al. [16] as they used different method in measuring shear bond strength by cementing stainless steel orthodontic bands with buccal and lingual attachments around the teeth. The force was applied using stainless steel wire attached on the buccal and lingual surface of the bands, the maximum load was applied for debanding. They found that adding propolis enhanced the mechanical properties of GIC, but this effect was not statistically significant.

There was significant decrease in bond strength values which may be attributed to the change in physical properties. Where the viscosity of GIC liquid decreases by adding EEP that prolongs the working time and interferes with the network formation of GIC systems. The high percent of EEP would weaken the scaffold with unfavorable adhesion leading to a negative effect on the physical properties of the mixture [28]. The failure mode analysis supported the results of micro shear bond strength test as cohesive mode of failure was the most common among the groups modified by 25 % EEP. On the other hand, the mixed mode of failure was the most common in the groups without any modifications (control groups).

According to the results of this study, the null hypothesis was rejected regarding the antibacterial test as there was significant difference in antibacterial test results either in relation to the used restorative materials or to the used 2 concentration of EEP. For microshear bond strength test, the null hypothesis was rejected as there was significant difference in microshear bond strength between both types of GIC and there was significant difference in microshear bond strength with the used 2 concentrations of EEP.

Limitations of the current in vitro study are that propolis was used after it had been dissolved in ethanol so it had a negative effect on the restorative material. Propolis can be used as a powder to maintain the mechanical properties of the material. Also the absence of an aging procedure, such as storing the specimens in artificial saliva or thermocycling would allow for a more accurate simulation of real-life conditions. Only the ethanolic extract of propolis was used in this study, other forms of propolis should be incorporated to GIC and studied. *Streptococcus mutans* is the only microorganism studied in this research, more studies needed for other strains in the oral biofilm.

9. Conclusions

Adding ethanolic extract of propolis to glass ionomer restorative material increases the antibacterial effects of both conventional GIC and RMGI. Increase the antibacterial effect with the increase in EEP concentration. However it seems that it has deleterious effect on microshear bond strength.

Funding statement

Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

CRediT authorship contribution statement

Marwa Z. Elmenshawy: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Huda Abed El-Haliem:** Writing – review & editing, Supervision, Conceptualization. **Amr M. Mowafy:** Supervision, Methodology, Conceptualization. **Hamdi H. Hamama:** Writing – review & editing, Supervision, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23710.

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