Studying the Effect of Egyptian Propolis on Antimicrobial Properties of Glass Ionomer Cement

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ABSTRACT

Background: Dental caries is a major concern to the dentist. Many bacterial subspecies are associated with caries, but Streptococcus mutans is still the most important bacterium in the initiation and progress of this disease, the direction toward natural products for medicinal purposes has gained much attention, as these natural products have proven to be effective with less toxic side effects. Studies have also shown other important properties in propolis such as antibacterial, antifungal, antiviral, anti-inflammatory, anesthetic, and ability to promote healing. Objective: To study the effect of propolis ethanolic extract (EEP) on enhancing the antimicrobial activity of glass ionomer cement (GIC). Methods: Two EEP 25% & 50% concentrations were evaluated for antimicrobial activity, in combination with GIC using agar Disk Diffusion test and broth microdilution test. Results: The diameter of the inhibition zone increased with 25% EEP & 50% EEP over the control by 14.4% and 19.6%, respectively. While increasing the concentration from 25% to 50% resulted in only 5% increase in the inhibition zone. MIC calculation for the three groups revealed, the combination of GIC and EEP has reduced the mic against Streptococcus mutans by two folds. Conclusion: The addition of propolis in different concentrations to GIC increased the antibacterial effect. 25% EEP gave the best antibacterial action with the lowest concentration.

Keywords: Glass ionomer cement; Propolis; Dental caries; Streptococcus mutans; Antimicrobial assay.

INTRODUCTION

Dental caries is a major concern to the dentist. Caries is a multi-factorial disease that occurs as a result of interaction between four important factors: host (a susceptible tooth surface), food (fermentable carbohydrates), caries-causing bacteria, and time. Dental plaque forms in an orderly way and has a diverse microbial structure that, in a healthy state, remains relatively stable over time (microbial biofilm)1. Many bacterial subspecies are associated with caries, but Streptococcus mutans is still the most important bacterium in the initiation and progress of this disease.2

Propolis is a natural gummy substance produced by honeybees as a result of the salivary enzymatic reaction of the bees on the plant exudates, it has been used widely in traditional medicine for ages mainly due to its high antioxidant properties3. Studies have also shown other important properties in propolis such as antibacterial, antifungal, antiviral, anti-inflammatory, anesthetic, and ability to promote healing.4,5

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The antimicrobial action of glass ionomer cement (GIC) is attributed to fluoride release; however, this is not sufficient to inhibit the bacteria under an atraumatic restoration. The antibacterial property of GIC might be due to the low pH rather than the fluoride release.\(^7\) \(^8\)

Recently, the direction toward natural products for medicinal purposes has gained much attention, as these natural products have proven to be effective with less toxic side effects, and thus can be used as an alternative source of treatment.\(^9\)

Since GIC has shown a variable antimicrobial activity in many studies in the literature\(^10\)-\(^12\), therefore, to accentuate its antimicrobial effect, the addition of ethanolic Propolis extract (EEP) is the main focus of our investigation.

**MATERIAL AND METHODS**

In the current study, two EEP concentrations 25 % and 50 % in combination with GIC were evaluated for antimicrobial activity.

**Preparation of ethanolic extract of propolis**\(^13\)

Ten grams of propolis (supplied by Imtenan) were dissolved into 20 ml of Ethanol (70% W/V) forming the 50% EEP. Filtering of the extract was done to remove rough particles. 25% dilution was obtained by diluting the 50% concentration 1:1.

**Glass inomer and EEP/ Glass inomer combination preparations**

The GIC was prepared at room temperature by mixing one unit of the powder with one drop of the preparation solution. For the preparation of **GIC with and EEP** combination mixture, 100 µl of desired EEP concentration was added to the mixture, the formed paste was incubated 10-15 minutes at 50 °C to evaporate the alcohol. For MIC calculation, the previously prepared paste was resuspended in 250 µl DMSO. For preparation of the control group, the GIC powder and preparation solution is mixed directly with 250 µl DMSO.

**Antimicrobial evaluation**\(^13\), \(^14\)

The antimicrobial activity of GIC with EEP was tested against *Streptococcus mutans* (ATCC 25175). The strain was obtained from the Microbiological Resources center (Cairo MIRCEN). Agar Diffusion test was used. The study was carried out in 3 groups each group was repeated 7 times. Group a contained the conventional GIC (control group), group b and c contained GIC with two different concentrations 25 %, 50 % of EEP respectively. *Streptococcus mutans* (ATCC 25175) were inoculated on Mueller -Hinton agar, 0.1 ml of 0.5 McFarland of the inoculum was used. Bores were made in the agar with the help of 8 mm sterile cork borer, then a fixed amount of each mixture group paste (0.5 gm) was added to the formed bores, each group was tested seven times. The plates were incubated aerobically in 5% CO₂ atmosphere at 37°C for 24 h. The diameter of inhibition zones produced around specimens was measured using ruler three times in three different directions, the average diameters were calculated for each group.

The Minimum inhibitory concentration (MIC) of the different groups was then evaluated using Broth Microdilution Procedure.\(^15\). In this study, the dilution causing bacterial inhibition was calculated for each group, by which we could compare between the different group’s microbial inhibitory effect.

Two-fold serial dilution of 50 µl tested suspension in DMSO was done using the Mueller Hinton broth Then 50 µl of 1.5*10⁵ *streptococcus mutans* inoculum was added. The plates were incubated aerobically in 5% CO₂ atmosphere at 37°C for 24 h. The lowest dilution showing no growth was taken as the minimum inhibitory concentration (MIC). Each group was repeated three times and average readings were calculated.

**RESULTS**

The inhibition zones of the tested groups were calculated and found as the following. For group 1inhibition zone was 1.6 ± 0.34 cm, while for group 2 (GIC+25% EEP) was 1.87 ± 0.37, group 3 (GIC+50% EEP) was 1.99 ± 0.54 (Table 1 and Figure 1).

MIC calculation for the three groups revealed, the combination of GIC and EEP has reduced the MIC against *Streptococcus mutans* by two folds, which indicates doubling of the antimicrobial activity. On the other hand, no difference between the two EEP concentration used, both groups B and C had the same MIC.

![Figure 1. Mean and standard deviation of bacterial inhibition zone in cm of the study groups.](http://aprh.journals.ekb.eg/337)
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The antibacterial activity of propolis on the oral
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effective against Streptococcus mutans.

The results of the present study showed an increase
in the diameter of the inhibition zone with 25 % EEP and
50 % EEP over the control by 14.4 % and 19.6 %
respectively, this indicates a non-significant increase in
antimicrobial activity of the preparation.

Hatunoğlu et al, 2014 who concluded that the
incorporation of 25% EEP and 50 % EEP to conventional
glass ionomer cement (GIC) used to cement orthodontic
band, activated the inhibition of Streptococcus mutans
growth, this effect did not occur in the control GIC group
or when the concentration of EEP was only 10%.20

Furthermore, Topcuoglu et al in 201221 evaluated
the antibacterial effect of glass-ionomer cement (GIC)
containing EEP in two concentrations 25%, 50% against Streptococcus mutans.
While the calculated MIC had no significant
increase over the control, the MIC of the control group was
at 1:8, and for both EEP groups was 1:16, only a two-folds
decrease in MIC was observed for both 25 % and 50 %
EEP.

On the other hand, Hatunoğlu et al reported four
folds and eight folds decrease in MIC with 25 % and 50 %
EEP, respectively. The difference might be explained by
variation in the volume of EEP in the mixture used, in our
study only 100 µl was of EEP was added to the mixture.
Another factor that may explain the discrepancy in the
results is the type of propolis. In our study we used the
market-available Egyptian propolis.20

EEP anti-caries potential is attributed to the
reduction of the incidence of caries and dental plaque
accumulation in vivo, this effect was explained by two
action mechanisms that have been associated with the anti-
caries/anti-plaque properties of propolis:(i) antibacterial
activity against Streptococcus mutans and Streptococcus
sobrinus the cariogenic bacteria. (ii) inhibition of
glucosyltransferase enzymes (GTFs) activity, which leads
to prevention of dental caries and plaque related disease
through the inhibition of virulence factor. The same study
by Topcuoglu recommended that EEP should be kept as
low as possible, as the EEP does not share in the formation
of the glass ionomer network, and since, high amounts of
EEP would weaken the scaffold and the physical
properties of the antibacterial glass ionomer.20

It was found that sizes of inhibition zones produced
against S. mutans were not conditioned upon the
concentration of EEP according to the disk diffusion test.
Increasing the concentration from 25 % to 50 % resulted
in only a 5% increase in the inhibition zone. This agrees
with MIC results where both EEP concentrations had the
same MIC. The optimum concentration of EEP should be

**DISCUSSION**

In the current study, we aimed at assessing the
antimicrobial effect of GIC alone and in combination
with different EEP concentrations.

GIC has an inherent antimicrobial activity, our
results showed that the inhibition zone of the GIC was
1.60 ±0.34 cm, also the MIC of the preparation was at
1:8 dilution. Several studies agreed with our findings,
Tiwari S et al (16) reported antimicrobial activity of GIC
using the agar diffusion method where the inhibition
zone was 1.31 ± 0.83 cm. Also, Prabhakar et al (17)
reported that the inhibition zone of GIC produced against
Streptococcus mutans was 1.8 cm.

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used in the preparation to enhance the antimicrobial activity without affecting the physical properties of the glass ionomer. Our study showed that EEP 25% is considered the optimum EEP concentration for showing a reasonable antimicrobial activity with the lowest concentration of EEP used. Further studies should be conducted to calculate the best EEP volume that has the lowest effect on the physical properties of GIC.

CONCLUSION

The addition of Propolis in different concentrations to GIC increased the antibacterial effect of conventional GIC. However, the decrease in the MIC was not statistically significant.

A 25 % EEP gave the best antibacterial action with the lowest concentration.

Recommendations

Considering the present data, the following may be recommended:

- Incorporation of 25% EEP to GIC is recommended as Propolis increases the microhardness of GIC to reduce recurrent caries in high-risk patients.
- Further studies are needed to investigate:
  - The Study of shear bond strength of GIC containing EEP.
  - In vivo studies on long-lasting effect of the restorative material.
  - The optimum criteria for the EEP used, including the concentration of the different components, and detection of the adulteration in the used product.

Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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