ISSN: 2357-0547 (Print)
ISSN: 2357-0539 (Online)

Short Communication / JAPR / Sec. E
Elghazouly and Amer, 2021, 5 (3), 336-340

Journal of Advanced Pharmacy Research

Section E: Microbiology & Immunology

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

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Submitted on: 26-04-2021; Revised on: 20-06-2021; Accepted on: 21-06-2021

To cite this article: Elghazouly, Y. M.; Amer, A. N. Studying the Effect of Egyptian Propolis on Antimicrobial Properties of Glass Ionomer Cement. *J. Adv. Pharm. Res.* **2021**, *5* (3), 336-340. DOI: 10.21608/aprh.2021.74157.112

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 properties[3]. Studies have also shown other important properties in propolis such as antibacterial, antifungal, antiviral, anti-inflammatory, anesthetic, and ability to promote healing.[4-6]

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 prepararions

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 is incubated 10-15 minutes at 50 ºC to evaporate the alcohol. For MIC calculation, the previously prepared paste is 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% CO_2 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 $1.5*10^5$ *streptococcus mutans* inoculum was added. The plates were incubated aerobically *in 5% CO^2 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.

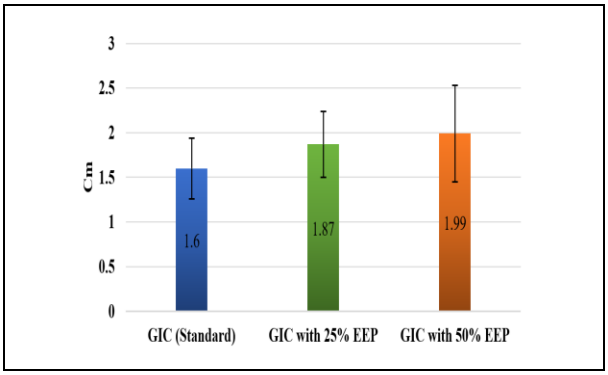

Figure 1. Mean and standard deviation of bacterial inhibition zone in cm of the study groups.

Table 1. inhibition zone in cm of the study groups

Inhibition zone (cm)	GIC (Standard) Group a (n=7)	GIC with 25% EEP Group b (n=7)	GIC with 50% EEP Group c (n=7)
Mean (SD)	1.60 ± 0.34	1.87 ±0.37	1.99 ±0.54
95% CI	1.28, 1.92	1.53, 2.21	1.49, 2.48
***P* value**	0.24		

Table 2. MIC expressed as folds of dilution of the study groups

MIC	GIC(Standard) Group a (n=3)	GIC with 25% EEP Group b (n=3)	GIC with 50% EEP Group c (n=3)
MIC (dilution)	1:8	1:16	1:16

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.

The antibacterial activity of propolis on the oral microorganisms was proven by many studies[18] and now there are many oral products containing propolis in the form of toothpaste and mouth rinses. Airen et al[19] assessed the antibacterial activity of Ethanolic and Water extract of propolis in two concentrations 5% and 20% in vitro. They found that the concentrations of 5% and 20% of Ethanolic Extract of Egyptian propolis EEP were 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 2012[21] 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

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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