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ORIGINAL ARTICLE

Antimicrobial Effects of Propolis Incorporated Dental Composite Resin

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ABSTRACT

Aim/Objective: The aim of this in-vitro study is to assess the antibacterial activity of a propolis-modified experimental dental composite resin in terms of reducing and preventing recurrent caries, usually caused by Streptococcus mutans.

Materials and methods: Control group specimens were made using the 70/30 (filler/resin) method without the use of EPE. Group 1 specimens were made by mixing 12% EPE with 30% resin in experimental dental composite resin. The experimental dental composite was created by adding 16%EPE and 20% EPE to Group 2 and Group 3, respectively. Antibacterial testing was carried out in the inhibition zone using the minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC). Surface roughness was assessed using scanning electron microscopy.

Results: The results were evaluated using one-way ANOVA and Tukey's high significant difference test (HSD). The EPE displayed antibacterial action against S.mutans, according to the findings. The inhibitory zone of 20% EPE integrated dental composite resin was 2.2mm to 2.5mm. Inhibition zones ranging from 1.2mm to 1.8mm were found in 12% and 16% of the samples, respectively. The results of the minimum inhibitory concentration showed that 20% had stronger antibacterial activity than 12 and 16 %. Scanning electron microscopy research revealed that 20% of EPE samples had decreased surface roughness.

Conclusion: The experimental dental composite showed antibacterial activity against S.mutans. Antibacterial activity is increased by increasing the proportion of EPE in the experimental composite. Due to its dark color appearance it is recommended for usage as a lining material under restorative materials.

Keywords: Streptococcus mutans, Secondary Caries, Antibacterial activity, Propolis, Ethanolic extract of Propolis, Dental Composite Resin

INTRODUCTION

Caries is one of the main contributory factors linked with the failure of dental restoration. Multiple narrative and systematic reviews on the clinical performance of dental restorations published over the last few decades proved it[1]. In a regular dental practice about 60% of all the restoration are replaced due to secondary caries[2]. In the human oral microflora, more than 600 microbial species have been reported, with roughly 280 species isolated in culture [3]. The most prevalent cariogenic bacteria among these are Streptococcus mutans species. Kidd et al. found no significant variations in the microbiota makeup in plaque samples taken from sites with primary or recurrent caries in their culture investigations[2]. Streptococcus mutans (S.mutans) has a proclivity for adherence to tooth surfaces and restorations, followed by acidic action, which causes demineralization and caries lesion. Bacterial growth on restorative materials damages the materials and roughens their surfaces. Bacterial reinfection occurs at the interface between the restoration and the tooth as a result of bacterial buildup[4].Dental composite resin is extensively used direct tooth colored restorative material. Plaque formation begins with the colonization of bacteria and their initial adherence to the solid substrate surface of restorative material.[5]. The surface properties of composite resin differ from those of the tooth. Mechanical surface qualities, material components (filler particles and resin matrix), and curing conditions all influence bacterial adhesion and biofilm formation on the surface of composite resin[5]. In vitro and in vivo studies have shown that composite resins acquire more bacteria or plaque than other restorative materials[5]. The diverse surface properties of each type of composite resin[5] can explain differences in the quantity of bacterial adherence. However, dental composite resin, on the other hand, lacks antibacterial qualities and, as a result, biofilm buildup is higher in comparison to other restorative materials[6 7]. A lot of research has been carried out in past to make dental composite resin antibacterial. There are two categories of antibacterial drugs (releasing and non-releasing). Antibacterial agents such as strontium fluoride (SrF2), ytterbium trifluoride (YbF3), silver ions, Ag–silica glass, zinc oxide (ZnO), silver supported fillers, and quaternary ammonium salts have all been used in dental composite resins[8]

Propolis is a resinous compound produced by honey bees and is a natural bee hive product. Propolis is extracted from plant exudates by honey bees and processed using an enzyme found in their salivary glandules. Propolis is a potent antibiotic since it is a rich waxy natural substance with anti-inflammatory and antibacterial characteristics. Flavonoids and terpenoids are responsible for its antibacterial properties. It prevents S.mutans from growing and adhering to the tooth surface. Human plaque buildup and its insoluble exterior polysaccharide content were reduced by propolis, according to Koo.et.al. In an in-vivo investigation, Silvana.et.al evaluated propolis extract against S.mutan[9].

Propolis extract was found to have antibacterial activities against S.mutans in this investigation, suggesting that it could be utilised as an alternative to prevent dental caries. Erdem.et.al investigated the antibacterial properties of propolis added to glass ionomer cement (GIC), finding that GIC containing 25% and 50% EEP (Ethanolic Extract Propolis) activated suppression of S.mutans growth[9].

The aim of this in vitro study is to evaluate the effectiveness of a propolis-modified experimental dental composite resin at reducing and preventing recurrent caries caused by Streptococcus mutans.

MATERIALS AND METHODS

In this in-vitro study the materials and armamentarium enlisted in table no. 1 were purchased.

Fabrication of Stainless steel mold: A split stainless steel mold was fabricated according to the specification no. 66 using CAD-CAM milling machine. It was comprised of two split stainless steel rings with an internal hole of 10mm diameter and 2mm thickness[5].

Extraction of ethanolic propolis extract: First of all crude propolis was frozen for 24 hours at -20^{o}C. It was then converted into powdered form by grinding in coffee milling machine. After grinding propolis 70% of ethanol was taken in conical flask and

twenty-five gram (25g) of ground propolis was added to it. Dissolution of material was done at room temperature for 24 hours with the help of magnetic stirrer. The mixture was then filtered to remove the rough particles. The filtered mixture was centrifuged at 8800rpm for 30 minutes. At the end of centrifugation the propolis extract was suspended on the top of the test tube, while the remaining metabolites were settled down at the bottom. Propolis extract was collected in flask and remaining ethanol evaporation was done using rotary evaporator[10]. (reference)

Table 1:

Materials	Chemical formulation	suppliers
Bis-GMA	2,2-bis(4-(2-hydroxy-3-methacryloxyprop1-oxy)phenyl) propan	Standard scientific suppliers
TEGDMA	triethylene glycol dimethacrylate	Standard scientific suppliers
Silica		Standard scientific suppliers
Tertiary amine	ethyl-4-dimethylaminobenzoate	Standard scientific suppliers
CQ	Camphorquinone	Standard scientific suppliers
DMSO	Dimethyl sulfoxide	Agriculture University
Propolis		Tarnab
Ethanol	C_2H_5OH	Agriculture University

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of EPE solutions: Minimum inhibitory concentration and minimum bactericidal concentration of EPE against S.mutans were calculated by taking 2%,4%,8%,10%,12%.16% and 20% of EPE. The MIC value was 9% and MBC was 12%,, so the minimum concentration of EPE included in this study was 12%. For preparation of 12%, 16% and 20% EPE solutions, 0.12g, 0.16g and .20g of EPE was mixed with 2ml of dimethyl sulfoxide (DMSO).

Sailinization of the organic filler: All the materials were weighed on analytical balance and dispensed in round bottom flask one by one. At first twenty (20) gram (g) of silicone dioxide (Si02) was added to the round bottom flask followed by 0.4g of n-propylamine, 2g of 3 trimethoxysilylpropylmethacrylate and 100ml of cyclohexane. Mixing of ingredients in the flask was done with a glass stirrer, after which the flask was left on table for 30 minutes at room temperature and then placed in water bath for 1 hour at 60^{o}C. Evaporation of the mixture was initially done at at 65^{o}C for 15 minutes and then at 90^{o}C for 1 hour by using rotary evaporator. Lastly silicone dioxide (SiO2) particles were dried for 18hrs in a vacuum oven at 80^{o}C[11].

Specimen preparation: All the components used for experimental composite preparation were weighed on analytical balance. Firstly Silanized fillers were placed on a watch glass. Organic resin matrix containing a mixture of monomers 2,2-bis(4-(2-hydroxy-3-methacryloxyproploxy)phenyl) propane (Bis- GMA) and triethylene glycol dimethacrylate (TEGDMA) were put onto the watch glass, along with 2% Camphoroquinone (CQ) and 4% tertiary amine by weight respectively. For experimental groups 12%, 16% and 20% EPE by weight was incorporated in the resin part of experimental dental composite. All the components were then mixed manually with stainless steel spatula til homogenous paste was obtained. The mixed material was then placed in the stainless steel mold. Before filling the mold it was assembled on a 2mm thick glass slide, which was covered with 0.2mm polyethylene transparent film for the standardization of smooth surface. After that the mixed material was placed into the mold with the help of stainless steel spatula. Both the surfaces of the prepared experimental dental composite were cured with blue visible light for 20 seconds each[11].

Grouping of specimens:

Table 2:

Sr No.	Groups	Percentage of EPE
1	Control group	0%
2	Experimental Group 1	12%
3	Experimental Group 2	16%
4	Experimental Group 3	20%

Agar disk diffusion test: Streptococcus mutans strain was provided by the Department of microbiology, Army medical college Rawalpindi. The antibacterial effects of each specimen was evaluated using agar diffusion method. Brain heart infusion broth was prepared according to manufacturer's instructions. 37g of BHI powder was mixed in 1000ml of distilled water and mixed[4]. The mixed solution was then sterilized at 121oC and 1.5 bar pressure in autoclave. The sterilized culture media was left till cool in laminar flow.

Streptococcus mutans was inoculated in 5mL of BHI broth and was incubated for 24hr at 37^{o}C to form a suspension (inoculum) [12]. Four petri dishes were used for testing the antibacterial specimen. In each sterilized petri dish there was a base layer containing 15mL of blood agar mix. Inoculum was stitched to the blood agar via sterile cotton swab. After solidification the specimens of control and experimental groups were arranged in separate petri dishes. All the test specimens were evaluated for 24 hours and 7 days, of exposure to the bacterial strain. Inhibitory zones (halo) was measured three times by a manual Vernier caliper after each time period[4].

Statistical analysis: Data was analyzed using a statistical software SPSS v.20.Mean and standard deviation were estimated for each study group. To test the mean difference in antimicrobial activity between the groups at each interval (24hrs and 7days), one-way ANOVA was performed separately with $p < 0.05$ as significant level. Post Hoc (Tukey test) was performed to know that the difference exist in between the groups.

RESULT

The antibacterial activity of the experimental composite against the particular microorganism is showed in Figure. The control group which lacked the addition of EPE showed no inhibitory activity. Whereas, the experimental groups containing EPE in various concentrations (12%, 16% and 20%) exhibited dose-dependent antimicrobial activity.

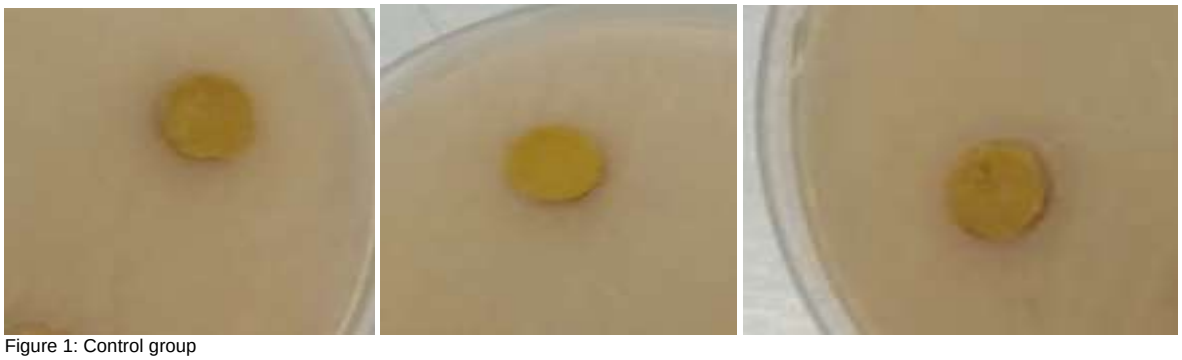


Figure 1: Control group

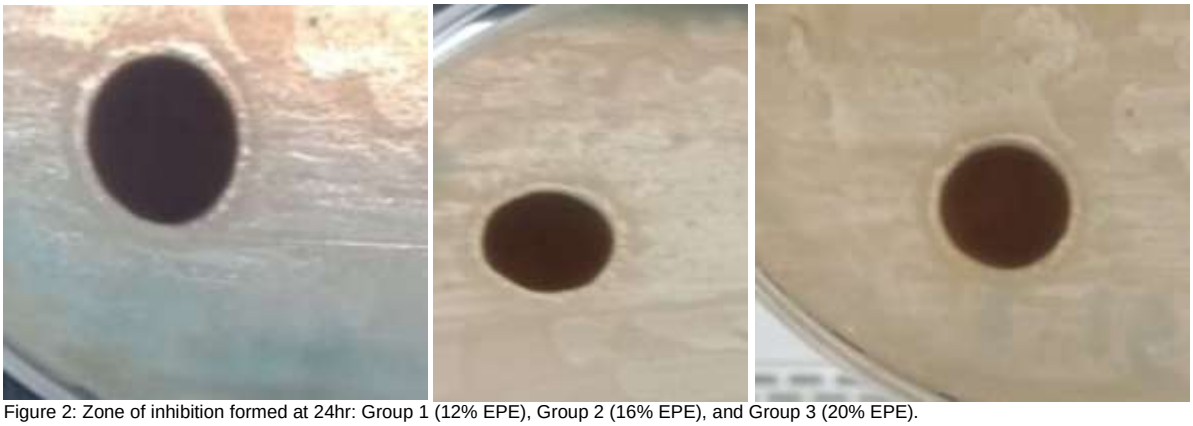


Figure 2: Zone of inhibition formed at 24hr: Group 1 (12% EPE), Group 2 (16% EPE), and Group 3 (20% EPE).

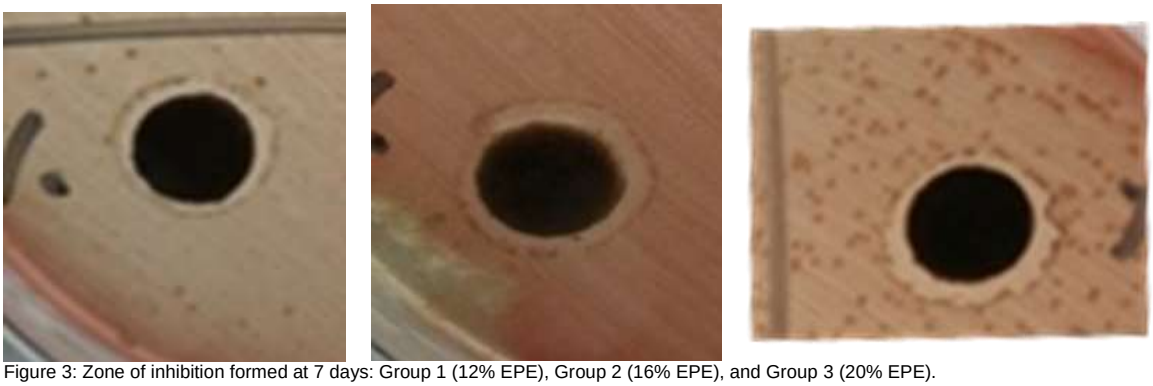


Figure 3: Zone of inhibition formed at 7 days: Group 1 (12% EPE), Group 2 (16% EPE), and Group 3 (20% EPE).

The mean diameter and standard deviation of the zone of inhibition of all the groups against Streptococcus mutans at each time interval (24hrs and 7days) are shown in Table. With increasing percentage of EPE the inhibition levels increased significantly. The comparison of mean values of experimental groups 1, 2 and 3 were done using one-way ANOVA. The results showed that there was significant difference between the experimental groups 1, 2and 3 at 24 hours ($p<0.000$), at 7 days ($p<0.000$) . Furthermore, Post Hoc (Tukey test) was performed to know the difference in between the groups. Among all the groups, experimental group 3 having 20% propolis had the highest inhibition halo at each time interval i.e at 24hrs ($p<0.000$), at 7days ($p<0.000$), which was statistically significant from other groups showing dose-dependent effect.

Table 3: inhibition halos (mm) of experimental composite incorporated with different percentages of propolis at different time intervals

Groups	24 hours	7 days
Group 1	1.2275±0.01258	1.2350±0.01915
Group 2	1.5200±0.01633	1.5675±0.05123
Group 3	2.2200±0.01414	2.3425±0.02986

DISCUSSION

Excellent results have been achieved in the field of restorative dentistry and the advancement is still in progress. One of the biggest challenges being faced is to make the restorative material antimicrobial, because microbes present in the oral flora have the capability to adhere and flourish on the surface of the restorative material thus leading towards recurrent caries.

Dental composite is one of the most widely used restorative material for having its superior esthetic appearance, strength and minimum invasive property[5]. On the other hand, inherently, it lacks antimicrobial activity, so the restoration is prone to secondary caries[6 7]. Secondary caries like other dental caries is initiated by the microorganisms present in dental plaque[2]. When bacterial stagnation occurs on any site of the restored tooth it leads towards the development of secondary caries.[2] Attempts have been made to overcome this problem by incorporating different types of antimicrobial agents (releasing and non-releasing) into the dental composite resin[8]. In this current study, propolis has been added an antibacterial agent because there is also an emerging scientific interest in the antimicrobial prospective of propolis unaccompanied or in combination with certain antibiotics and antifungals[9]

Propolis, Bee's glue has got dominating antimicrobial activity against Gram-positive bacteria such as S.Mutans[13].In this current study, experimental dental composite incorporated with EPE was fabricated. EPE was incorporated in the resin component of the experimental dental composite resin because propolis has got antibacterial activity against S.Mutans[14 15] The reason behind fabrication of experimental dental composite was that if EPE was incorporated in commercially available composite it would have changed the whole formulation of the resin. In this study S.mutans was used as it gets adhered to the restoration strongly and are resistant to chemical or mechanical removal. Attachment of S.Mutans to a biotic substrate is mediated mainly by the non-hydrophobic and electrostatic interactions. The balance between the attraction and repulsion result in the microbial adhesion [16] that was the reason why antibacterial activity of 12% EPE incorporated experimental dental composite was less then 16% and 20% experimental dental composite

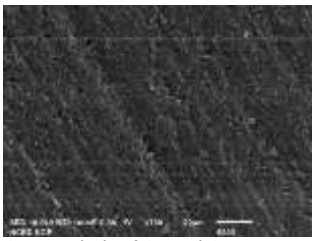
Propolis was added in different concentrations (12%, 16 % and 20 %) in the resin component of the experimental dental composite resin. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests were carried out to calculate the least effective concentration of propolis against S.Mutans. In pilot study, propolis was added in different concentrations starting from minimum 1% to maximum 20%. Propolis showed its antimicrobial effect against S.Mutans at 12% concentration. That's why the concentration of propolis was 12%, 16% and 20% which was in accordance with the study conducted by Martin et al. in 2019. He studied the effect of Red Ethanolic Propolis Extract (REPE) against S.Mutans and calculated its

minimum inhibitory concentration (MIC) and maximum bactericidal concentration (MBC). Our results were in accordance with his study[17].

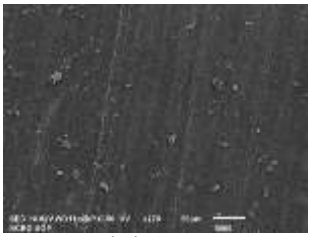
Minimum inhibitory concentration (MIC) revealed that 12% EPE incorporated in experimental dental composite showed effectiveness against S.Mutans. On the other hand, it also demonstrated that by increasing the percentage of EPE incorporation from 12% the antimicrobial activity also increased. The results was is in accordance to the study of Martin et al. 2019, in which it has been shown that that Red Propolis extract exhibited antibacterial activity against the tested strains of S.mutans and reduced the colonization of S. mutans[17] . MBC results showed that there was minimum growth of S.mutans on 20% EPE incorporated experimental dental composite resin specimen as compared to 12% and 16% propolis experimental dental composite resin specimen.

As the concentration of EPE increased from 12% to 16% and 20%, the size of Halo zone also increased accordingly, showing increased antimicrobial activity against S.Mutans. .SEM of one sample from each group including control was done after antibacterial testing. The SEM result showed surface roughness of 12% experimental dental composite when compared to 16% and 20%

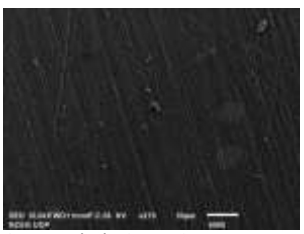
On the other hand, no Halo zone was observed in the control group, confirm the lack of inherent antimicrobial activity of dental resin composite. These results confirm the potent antimicrobial activity against S. mutans of propolis incorporated dental composite resins. It has been revealed that that propolis inhibits the activity of microorganisms by different mechanism. This might include inhibition of cell division, inhibition of bacterial motility, bacteria lysis, collapsing of microbial cytoplasm cell walls & membrance and protein synthesis inhibition[18].On the other hand, it has also been revealed that the polyphenols of propolis interact with microbial proteins by forming hydrogen and ionic bonds, thus altering their three-dimensional structure of a protein and as a consequence of their functionality. High concentration of flavonoids and phenolic compounds present in propolis are responsible for its antimicrobial activity. The ethanolic extract of propolis (EEP) indicates excessive effectiveness towards the lines of bacteroides and Pepto-streptococcus. The other antimicrobial compounds had been found from propolis, in particular consisting of 3,5 di-prenyl-four-hydroxycinnamic acid, 3-prenyl-4- dihdrocinnamoloxycinnamic acid and 22-dimethyl 6-carboxy-e-thenyl-2H-1-bezopyran by Khurshid et al3 in that it has been stated that the preliminary compound displays the maximum interest in opposition to microorganism and is one of the predominant antimicrobial compounds[13].



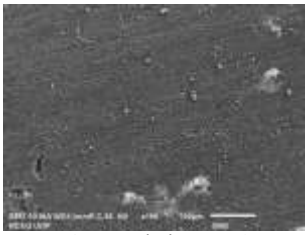
SEM analysis of Control Group



SEM analysis Group 1



SEM analysis Group 2



SEM analysis group 3

CONCLUSION

It was concluded from this study that ethanolic propolis extract (EPE) when incorporated in dental composite resins at different percentage by weight (12% , 16% & 20 %) exhibited a strong antibacterial effect against the S.mutans . Experimental dental composite resin incorporated with (EPE) maintained antibacterial properties for 24 hrs and till 7 days. For composite resin restorations, incorporation of antibacterial propolis extract can prevent biofilm formation and can reduce the or diminish the risk of secondary caries as shown by SEM images of the three groups i.e 12%, 16% and 20% showed less surface roughness when compared with the control group.

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