Analysis of the Role of CPP-ACPF Paste in Combination with Aqueous Extracts of Propolis to Improve Enamel Roughness after Demineralization Challenge

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Abstract
Aims: This study aims to evaluate the combined efficacy of aqueous extracts of propolis and Fluoridated Casein Phosphopeptide–Amorphous Calcium Phosphate (CPP.ACPF) paste or Minimal Intervention (MI) paste plus on improving enamel surface roughness after demineralization challenge. Materials and methods: A total of (75) posterior wisdom teeth were used in the study. Enamel blocks were prepared and divided into five groups randomly, the teeth in all groups were subjected to demineralization cycle and then treated with: Sinjar's aqueous extract of propolis (AEP)-MI paste plus cream n. (15), Sulaymaniah's AEP-MI paste plus cream n. (15), Duhok's AEP-MI paste plus cream group n. (15), control positive group of MI paste plus alone n. (15), and control negative group of artificial saliva alone n. (15). The Roughness of enamel blocks was measured using a profilometer machine at baseline, after demineralization cycle and finally after the treatment protocol. Results: There were statistically high significant differences among study groups after the demineralization cycle and there was an increase in surface roughness in all groups after demineralization, but the least elevation in surface roughness belonged to a mixture of Sulaymaniah's aqueous extract of propolis with MI past plus followed by Duhok's aqueous extract of propolis with MI past plus group after treatment protocol. Statistically, there were high significant reductions in surface roughness in all groups after the remineralization except in an artificial saliva group. Conclusions: Combination CPP-ACPF Paste with Aqueous Extracts of Propolis reducing enamel's roughness and increasing resistance to demineralization.

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References:

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INTRODUCTION

Tooth enamel is one of the four major tissues that make up the human's tooth structure [1]. It is the most mineralized tissue of the body is enamel which forms a very hard, thin, translucent layer of calcified tissue that covers the entire anatomic crown of the tooth [2].

Main process as caries occurs at the outer layer of enamel which is principally in contact with oral environment [3]. The first visible sign of tooth caries, the white-spot lesion, has been defined as "subsurface enamel porosity from carious demineralization" [4]. This subsurface porosity is caused by an imbalance between the dynamic biological processes of de- and remineralization [5].

The principles of minimally invasive dentistry clearly dictate the need for clinically effective measures to remineralize early enamel caries lesions [6]. The CPP-ACP nanocomplexes are derived from bovine milk protein, casein, and calcium and phosphate, preventing demineralization and enhancing remineralization [7]. The combination of CPP–ACP with fluoride caused localization of calcium and phosphate ions with fluoride ions at the enamel surface [8].

The use of natural products by human beings as a therapeutic alternative resembles ancient times [9] as propolis, which is a gummy and balsamic substance, is a honeybee product [10] with beneficial properties like antimicrobial, antiviral, antifungal, and anti-inflammatory [11].

Roughness is a fundamental property of teeth, which affects the attachment of exogenous materials to enamel surfaces and caries progression [12]. Thus, the surface roughness is the irregularity of enamel surface characteristics due to the demineralization process [13]. Enamel surface roughness assessment is a useful means of evaluating the stage and activeness of carious lesions [14].

The aim of the current study is to analyze the synergistic effect of aqueous extracts of propolis on roughness improvement power of fluoridated CPP-ACP paste after demineralization challenge.

MATERIALS AND METHODS

Collection and Extraction of Propolis

The unrefined propolis (Apis mellifera) was obtained from three different regions in the north of Iraq. The first type was from Sinjar's mountains/Ninawa, the second type was from Duhok's mountains and the third one was from Sulaymaniah's mountains. For aqueous extract preparation, Krell method [15] was followed. The extract was clear from any impurities, dark and viscous and its odor is also distinguishable for each type [16].
Formulations of the Fluoridated CPP–ACP Propolis Extracts Tooth Coating Complex

Both ingredients were combined into a cream formulation made from a base of starch and other suitable binding material (glycerin). The fluoridated CPP-ACP paste and distilled water were adjusted at a specific ratio and mixed with the base solution, then propolis extracts 30% were added after complete dissolving in ethanol, shake well via vortex tube stirrer then placed in the lyophilizer till complete evaporation of the solvent and homogenous cream was obtained for the three types of propolis as shown in Figure (1).

Sample Collection

The sample in this study consisted of (75) human permanent third molars extracted for impaction reasons. After extraction, the teeth were cleaned with tap water and examined with 10X magnifying lens, the selection of the teeth followed specific criteria; the teeth must be sound, free from enamel defects, decay, stain, cracks, hypoplasia, and fluorosis and unaltered by extraction procedure. The teeth were stored in 0.1% thymol solution at 4°C to avoid dehydration and prevent bacterial growth until their use within three months.

Preparation of Enamel Blocks

Sound extracted third molars were cleansed accurately before using, they were scrubbed with non-fluoridated pumice and white rubber prophylactic cup using a low speed hand piece, wiped free of soft tissue debris and sanitized in tap water then the crowns separated from the roots via a diamond disc bur in the high speed hand piece cooled with water, after that the crowns were mounted in
cylindrical plastic tubes (16mm diameter×14mm depth) with cold cure acrylic resin with the outer buccal enamel surface exposed. The buccal surface of each sample was polished using 240, 400, 600, and 1200 grit silicon carbide abrasive papers under flooding water to obtain standardized flat enamel surface (18). Then smoothed by using the universal polisher machine. Each specimen was then coated under the digital stereomicroscope (X 40) with two layers of an acid-resistant nail varnish, leaving 3×3mm² window on the middle third of the enamel surface to define the experimental area (19, 20) as shown in Figure (2).

**Figure (2):** Preparation of Enamel Blocks, (A) Cylindrical Plastic Tubes, (B) Separation Of The Crowns From The Roots, (C) Crowns After Cutting, (D) Cold Cure Acrylic Mold &Varnish Application.

**MATERIALS AND METHODS**

Approval of study was from the Scientific Research Committee / Department of Pedo. Ortho. Preventive Dentistry / College of Dentistry / University of Mosul. Commercially available topical cream with bioavailable calcium and phosphate (GC America, Recaldent, Alsip, USA), which contains 10% by weight of CPP–ACP in addition to Sodium fluoride 0.20%, (MI Paste Plus) was used in remineralization of one group of the samples in addition to previously formulated fluoridated CPP–ACP-propolis aqueous extracts tooth coating complex of three types (Sinjar, Sulaymaniah and Duhok) for remineralization of other groups in the study.

**Design of Study and Methods of Application**

The total number of teeth samples in the study was (75) samples, randomly divided into five groups, (15) samples in each group as follows:

**Group 1: control negative group** (N. =15), after immersion of the teeth samples in demineralization solution, they were immersed in artificial saliva only that it is changed daily for 14 days.

**Group 2: control positive group** (N. =15), after immersion of the samples in demineralization solution, the enamel
surfaces were coated by a fine brush with a thin layer of MI paste plus and left for 30 minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

**Group 3: Sinjar's AEP-MI plus paste complex group** (N. =15), after immersion of the samples in demineralization solution, the enamel surfaces were coated by a fine brush with a thin layer of Sinjar's AEP-MI plus paste complex and left for 30 minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

**Group 4: Sulaymaniah's AEP-MI plus paste complex group** (N. =15), after immersion of the samples in demineralization solution, the enamel surfaces were coated by a fine brush with a thin layer of Sulaymaniah's AEP-MI plus paste complex and left for 30 minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

**Group 5: Duhok's AEP-MI plus paste complex group** (N. =15), after immersion of the samples in demineralization solution, the enamel surfaces were coated by a fine brush with a thin layer of Duhok's AEP-MI plus paste complex and left for 30 minute then washed with deionized water and kept in artificial saliva that it is changed daily. This procedure was repeated twice daily for 14 days.

**Demineralization Procedure**

Before application of treatment protocol, each group was individually suspended in demineralizing solution for 5 days at temperature of 37°C to create artificial caries like lesions. The demineralizing solution contained 2.2 mmol/L CaCl₂, 2.2 mmol/L NaH₂PO₄ and 50 mmol/L acetic acid adjusted to pH 4.5 with NaOH at 37°C. The pH values of the demineralization solution were checked every day using a pH meter and the solution was changed every day (19).

**Surface Roughness Measurement**

In order to evaluate the changes in the surface texture of the teeth, the enamel surface roughness was chosen. The procedure includes measurement of surface roughness for sound enamel at baseline, before and after demineralization-cycling regime and after treatment regime in each tested group. Such evaluation was conducted by a profilometer machine with a magnification of x50 as shown in figure (3). It is the maximum of all peak-to-valley values (21) and is measured in micrometers (μm) (22). Each specimen's surface was placed parallel to the horizontal plane and perpendicular to the tip of the profilometer which measured the roughness of the specimen along a 3-mm path. The distance between the peaks and valleys of the sampled line was gauged in the y direction. The cut-off value for surface roughness was 0.8 mm (23). All readings
were carried out by the same examiner using the same calibrated machine.

**Figure (3):** Surface Roughness Measurement Using Profilometer Machine with Magnification Of X50.

**RESULTS**

The data were analyzed using SPSS program (version 19). Table (1) delineates one way analysis of variance (ANOVA) test for comparison of mean roughness values between the groups at baseline, after the demineralization cycle and after treatment scheme. Results showed that there was highly significant difference at $p \leq 0.01$ of mean roughness values among tested groups in the three stages of the study.

Table (1): Analysis of Variance (ANOVA) Test of Mean Roughness Values for Comparison Between Aqueous Extracts for Sinjar, Sulaymaniah & Duhok's Propolis Groups & Controls at Every Stage in The Study

<table>
<thead>
<tr>
<th>Time</th>
<th>Source of variance</th>
<th>Sum of Squares</th>
<th>DF</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Baseline data</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between Groups</td>
<td>1.009</td>
<td>4</td>
<td>.252</td>
<td>20.367</td>
<td>.000*</td>
</tr>
<tr>
<td></td>
<td>Within Groups</td>
<td>.867</td>
<td>70</td>
<td>.012*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>1.877</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>After demineralization</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between Groups</td>
<td>.081</td>
<td>4</td>
<td>.020</td>
<td>3.441</td>
<td>.013</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>.410</td>
<td>70</td>
<td>.006*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>.490</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>After treatment</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Between Groups</td>
<td>8.040</td>
<td>4</td>
<td>2.010</td>
<td>341.197</td>
<td>.000*</td>
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<tr>
<td></td>
<td>Within Groups</td>
<td>.412</td>
<td>70</td>
<td>.006*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>8.452</td>
<td>74</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Highly significant difference at $p \leq 0.01$.

Table (2) formulates means, number, standard deviation and Duncan's multiple range tests of roughness mean values of the enamel blocks of the tested groups. Statistically, the results of the mean roughness values were significantly different for the groups at all stages but after treatment, the least elevation in
surface roughness mean value belonged to mixture of Sulaymaniah's aqueous extract of propolis with MI past plus followed by Duhok's aqueous extract of propolis with MI past plus group then Sinjar's AEP-MI plus paste complex group followed by control positive group (MI plus paste alone) while the highest value of roughness was found in control negative group that was preserved in artificial saliva only. It is obvious that all of the remineralizing treatment pastes decreased the surface roughness values above the baseline means except for artificial saliva.

Table (2): Mean Values, Standard Deviation and Duncan's Multiple Range Test of Surface Roughness for Comparison Between Aqueous Extracts for Sinjar, Sulaymaniah & Duhok's Propolis Groups & Controls In The Three Stages Of Experiment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variables</th>
<th>Baseline data</th>
<th>After demineralization</th>
<th>After treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>N</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Control -</td>
<td>.831 a</td>
<td>.966 ab</td>
<td>1.273 a</td>
<td>.04627</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>.08025</td>
<td>.09612</td>
<td>.009877</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>.791 a</td>
<td>2.006 ab</td>
<td>.623 b</td>
</tr>
<tr>
<td>Control +</td>
<td>N</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Sinjar aqueous</td>
<td>Mean</td>
<td>.664 b</td>
<td>1.975 ab</td>
<td>.560 b</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>.13500</td>
<td>.10747</td>
<td>.09275</td>
</tr>
<tr>
<td>Sulaymaniah aqueous</td>
<td>Mean</td>
<td>.632 b</td>
<td>1.929 b</td>
<td>.355 c</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>.17013</td>
<td>.03583</td>
<td>.05375</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>.508 c</td>
<td>2.023 a</td>
<td>.422 c</td>
</tr>
<tr>
<td>Duhok aqueous</td>
<td>N</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Std. Deviation</td>
<td>.05375</td>
<td>.05321</td>
<td>.01676</td>
</tr>
</tbody>
</table>

*Duncan's Multiple Range Tests: Means with different letters are statically significant vertically (within the same column).
*Group with letter (a) has the highest value of roughness.

**DISCUSSION**

Statistical analysis revealed the mean roughness values of enamel specimens in all groups were increased compared with the baseline values after demineralization cycle, next, roughness of all groups decreased after treatment protocol compared to the surface roughness values measured after demineralization. Differences in the decreasing value of enamel surface roughness of each group occurred due to differences in mechanism and effectiveness of remineralized materials used. The effectiveness of the remineralization material is influenced by numerous factors such as the degree of acidity, the concentration and solubility of the remineralization material, complex forming reaction, temperature, and position of the balance point and the
chemical formulation of the remineralization material (24).

The casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) substances cause increases the density of the hydroxyapatite crystals and also the density of the enamel prism. This increase in enamel prism density can result in decrease surface roughness (25).

Propolis displayed a slight ability to prevent demineralization, though, they were better than the other groups. This shows that those natural products release several remineralizing agents, however with slight impact on inhibition of demineralization (26). Propolis also decreased the accumulation of dental plaque and its insoluble external polysaccharide content (27). It is a non-toxic material and its antimicrobial activity is due to the presence of flavonoids and terpenoids (28).

The existing study showed that the mean roughness values for groups with different letters are significantly different from each other at \( p \leq 0.05 \). Also, showed that there was highly significant difference at \( p \leq 0.01 \) of mean roughness values among tested groups in the three stages.

Among all extracts types investigated, aqueous extract of propolis (AEP) had the lowest number of active substances and weakest biological effects. The solubility of propolis compounds increased by the addition of co-solvent Pg (Polyethylene glycol) which also enhance penetration of active substances into cells however has no effect on the biocompatibility of the product. As a result, such type of extract could be suggested to be used not only for biological research but as well for design of pharmaceutical products (29).

Sometimes, the compounds in propolis have been considered to arise from three sources: plant exudates gathered by honey-bees, substances secreted from bee metabolism and substances that are introduced during the elaboration of propolis (30).

Honeybees gather propolis from practically any abundant plant source in the neighborhood of the hive. The chemical profile of propolis that is made by the same species is not always the same although different species of honeybee prefer different plants (31). For that reason, the alternative chemical composition of propolis depends on the bees’ preferences of botanical sources and the species and varieties of bees. Moreover, the method would not recognize the plant neither less if it had the same chemical constituents as those from propolis. The other is that the chemical constituents may differ with each part of the plant or even the growth of the plant or season of sampling (32).

The chemical composition of propolis differs greatly depending on, plant vegetation, geographical location, type of bees, season and time of collections, and the concentration and nature of the solvents used for the extraction (33) so the chemical composition
of propolis is unsteady and varies according to hive, season and region \(^{(10)}\). Daugsch et al. in 2008 \(^{(31)}\) concluded that the botanical origin and its abundance are necessary for the production of the type of propolis when the bees' gathered resins from various plants to yield propolis.

**CONCLUSION**

Combination CPP-ACPF Paste with Aqueous Extracts of Propolis from the three regions reducing enamel's roughness obviously and all have superior results to MI paste Plus alone.

**Limitations of the study:**

The chief limitation of the current study is that it is an in vitro study in which the demineralization cycle was achieved by using chemical products and did not occur due to the presence of Streptococcus mutans bacteria and it is acid byproducts. Also, surface roughness in vitro may be different when compared to the natural dynamic conditions in the oral cavity in vivo.

**REFERENCES**


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