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MICROBIOLOGICAL, HISTOLOGICAL AND MICROHARDNESS EVALUATION OF DENTIN AFTER CARIES REMOVAL WITH PAPACÁRIE AND CONVENTIONAL METHODS IN PRIMARY TEETH

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ABSTRACT

Aim of the work: This in vitro study was performed to assess the efficacy of Papacárie in comparison with the conventional rotary instruments to remove carious lesion in primary teeth. Microbiological study, histological picture and microhardness of dentin were evaluated after caries removal with both methods.

Materials and Methods: Twenty primary carious molars were selected for this study. Ten molars were prepared for the microbiological and histological assessment and the other ten were prepared for the microhardness assessment. After caries removal with the conventional method and Papacárie, the samples were subjected to microbiological analysis with culture test, histological analysis and to Vicker's hardness test.

Results: The conventional method and Papacárie are efficient in caries removal. The culture test has revealed that both methods have reduced *Streptococcus mutans* count significantly with a percentage of reduction of 84.686% & 85.06% respectively. Also, the conventional method and Papacárie have reduced *Lactobacilli* count significantly with a percentage of reduction 83.227% & 83.872% respectively. Light microscopic analysis showed that the Papacárie preserves the dentin structure more than the conventional method and the bacterial deposits left in the dentinal tubules were less in case of Papacárie. The microhardness test revealed that the mean hardness of dentin after caries removal with the conventional method was 75.49 Kg/mm^2 and after caries removal with Papacárie was 76.51 Kg/mm^2 with no statistically significant difference between both methods.

Conclusion: It was concluded that Papacárie is efficient in caries removal, has antibacterial action on *Streptococcus mutans* and *Lactobacilli*, and preserves the dentin structure and microhardness.

KEY WORDS: Chemo-mechanical, Papacárie, Cariogenic bacteria, Microhardness.

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INTRODUCTION

Modern dentistry has evolved into minimally invasive approach. Natural human enamel and dentin are still the best dental materials in existence and thus "minimally invasive procedures" that conserve a great part of the original, healthy tooth structure, are being focused on.[1] So, Restorative dentistry has suggested alternative methods for the removal of carious tissue. Due to the disadvantages of using the conventional rotary techniques such as heat generation, pressure, vibration, and pain, restorative dentistry has observed an increased interest in the development of alternative methods for the removal of carious tissue with tooth preservation.[2,3]

Chemomechanical caries removal (CMCR) has been introduced as an alternative method of caries removal. It is indicated as an option for minimally invasive treatments.[4] Chemomechanical caries removal method was first introduced in 1975 by Habib et al,[5] using sodium hypochlorite, followed by introduction of Carisolv,[6] and Caridex system.[3] These agents have many disadvantages like short shelf life, high corrosiveness, requirement of specialized instruments and high cost.[7]

In 2003, a new chemomechanical agent called Papacárie has been marketed. Papacárie is a bio-compatible gel that eliminates the need for anesthesia during the restorative procedures as it completely removes the decayed tooth structure with no trauma. Moreover, it has antibacterial properties without affecting healthy tissues.[8,9-13] Papacárie is composed of; Papain enzyme, an antioxidant (D-α tochoperol acetate), humectants (glycerine), emulsifier (amylopectin), thickener (carbopol), preservative (propyl-p-hydroxybenzoate), coloring agent (green apple), chloramines, toluidine blue, and distilled water as a vehicle.[8,14,15]

The papain enzyme is the main active ingredient of Papacárie gel. It is a cysteine protease of broad proteolytic activity. It is extracted from carica papaya fruit and is similar to human pepsin.[11,16] It was reported that the papain enzyme acts by exclusively breaking down the partially degraded collagen molecules by dissolving hydrogen bonds and thus facilitating tissue removal.[17,18] It contributes to the degradation and elimination of the fibrin "mantle" formed by the carious process without damaging the intact collagen fibrils. Also it acts synergically with chloramines, which have the potential of dissolving carious dentin by means of chlorination of the partially degraded collagen.[8,10,12,14,19]

Motta et al[20] have revealed that Papacarie is an excellent option for the minimally invasive removal of carious tissue, achieving significant reductions in total bacteria, total Streptococcus and S. mutans with the same effectiveness as that observed in the traditional caries removal method, while offering the advantage of less destructive effects on sound dental tissue. Reddy et al[21] have studied Efficacy of antimicrobial property of Carisolv and Papacarie. The authors have concluded that both agents softened dentinal caries for effective mechanical removal criteria. From bacteriological point of view, these strategies additionally removed cariogenic bacterium to a good extent. Chowdhry et al[22] have concluded that Carisolv and Papacarie removed caries effectively and with high patient acceptance and, therefore, they can be considered as viable alternatives to painful caries removal technique like Airotor in the management of dental caries, especially in pediatric patients.

Caries removal methods may lead to differences in the characteristics of dentine surface. Dentine ultrastructure generally affects the bonding of adhesive materials commonly used in restorative dentistry.[23] Dental cavities cleaned by the Papacarie method demonstrated no evidence of smear layer that may hinder the adhesive properties of some restorative materials.[13] Hamama et al have concluded that Papacárie method of caries removal has provided shorter excavation time and potentially enhanced morphological features of residual dentine

for subsequent bonding.[24] Papacarie has no adverse effect on adhesion of RMGIC adhesives to sound and caries-affected dentine.[25]

Dentin is the tissue forming the bulk of the tooth. It is a hydrated biological complex with different regional characteristics that may be altered by physiological processes, age and diseases.[26] With respect to dentin caries, histopathologically, two zones can usually be identified; infected dentin and affected dentin. The infected dentin is the superficial layer of caries which is wet and soft. It is highly infiltrated by bacteria, the collagen is irreversibly damaged and cannot be re-mineralized. On the other hand, the affected dentin is located deeper to the superficial layer. It is hard and leathery in consistency with no bacterial penetration. It is partially demineralized, the collagen fibers are intact and can be re-mineralized.[10,13]

Currently, the physical parameter used most commonly by dental practitioners to guide clinical excavation of infected, carious dentin is the hardness of the carious tissue. Hardness has been associated with the level of infection of carious dentin, helping the dentist distinguish between either heavily infected (soft) or minimally infected (hard) dentin.[27,28]

Few studies have been reported about the effect of Papacárie method on the microbiology, microhardness of dentin structure after removal of caries and on the integrity of the dentin structure histologically.[7,29,30] Therefore, this study was conducted to investigate the efficacy of Papacárie method in caries removal as well as its influence on the microhardness and the histology of dentin structure after removal of caries. Our null hypotheses were: (i) there is no difference between the Papacárie and conventional method in caries removal. (ii) there is no difference in the histological picture of the dentin structure between the two methods. (iii) there is no difference between the two methods as regard to the dentin microhardness.

MATERIALS AND METHODS

Twenty freshly extracted primary molars with occlusal caries involving the dentin, selected from cases that were formerly planned for serial extraction or near their exfoliation time as detected by clinical examination and by radiograph. Cases were selected from the outpatients of Pedodontics clinic, Faculty of Dentistry, Tanta University. After extraction, all molars were cleaned from debris and blood stains then stored in distilled water at 4°C, for no more than half an hour until processed.[31] The extracted molars were divided into two groups, ten molars each. One group was prepared for microbiological and histological investigation and the other group for microhardness investigation. Each cavitated molar was sectioned through the lesion in the mesio-distal longitudinal plane into two sections that contain equal amount of dental caries using a diamond wheel (ISO.104 345) rotating at low speed with water cooling to maintain the integrity of the dentin structure.[7]

I) Microbiological investigation (Culture test)

Baseline dentin sample

Before sampling, the upper most layer of carious dentin was removed with a sharp excavator to avoid the contaminated layer with plaque bacteria. An initial sample of carious dentin was removed using a sharp sterile excavator of the same size for all the samples for standardization and immediately transferred into a sterile vial containing 1 ml of saline.[11,32]

Caries removal

For microbiological and histological investigation, ten extracted molars were sectioned as described before producing twenty sections. Dental caries of one section of each tooth was removed with the conventional method and the other section with Papacárie.

In the conventional method group, the carious dentin was removed by means of a low speed round

bur with water cooling. Caries removal was verified according to the color and hardness of the lesion; by checking the hardness of the dentin with a dental explorer until a leather hard texture was reached or hearing a sharp scratching sound.

In Papacárie group, the carious dentin was removed with Papacárie gel according to the manufacture's instructions. Papacárie was kept out of the refrigerator half an hour before use, then Papacárie gel was applied on the carious dentin by dispensing it from its syringe and left on the lesion for 30 sec. The infected dentin was removed by scraping with blunt hand excavators. Reapplication of the gel and scraping of infected dentin was continued until no signs of softened dentin remained.

Second dentin sample

A second dentin sample was taken from the cavity floor (residual dentin) using a sterile sharp excavator and immediately transferred into a sterile vial containing 1 ml of saline for immediate processing.[11]

***Streptococcus mutans* media preparation**

Mitis Salivarious agar (Product from Difco), a selective medium for *Streptococcus mutans* isolation was prepared according to the manufacturer's instructions as follows: Ninety grams of the medium were suspended in one liter of purified water. Then the suspension was heated with frequent agitation and boiled for one minute to completely dissolve the medium. After that the medium was autoclaved at 121°C for 15 minutes for sterilization, cooled to 50 - 60°C and aseptically added 1 ml of Tellurite Supplement (1%) Chapman (Potassium Tellurite, 100 mg) to the medium.

***Lactobacilli* media preparation**

Rogosa (Product from Oxford) selective agar, a selective medium for *Lactobacilli* isolation, was prepared as follows: Eighty two grams of the medium were dissolved in one liter of purified water and boiled to dissolve completely, 1.32 ml glacial acetic acid was added and mixed thoroughly. Then the medium was heated to 90-100 for 2-3 minutes with frequent agitation.

Cultivation and incubation

The dentin samples were processed in the microbiology laboratory of Faculty of Medicine, Tanta University within half an hour of collection. Each sample was vortexed for about 30 seconds in order to dislodge the bacteria from the dentin. The samples were then diluted to produce concentrations of 1:10, 1:100, 1:1000, and 1:10000 in sterile physiologic saline. Aliquots of 1 μL of the diluted samples were plated on the Mitis Salivarious agar plates for the *Streptococcus mutans* and on the Rogosa agar for the *Lactobacilli*.[11]

- Mitis Salivarious agar plates were incubated in candle jar which provided an atmosphere of 5% Co_2.
- Ragosa agar plates were incubated in anaerobic atmosphere by the presence of gas generating pack. The jars with plates were incubated in an electric incubator adjusted to 37C° for 72 hours.

Identification of *Streptococcus mutans* and *Lactobacilli* colonies

Identification was based on colony morphology. The colonies that appeared as raised, convex and pale-blue colonies that are granular (i.e., "frosted glass") in appearance were identified as *Streptococcus Mutans* colonies. While the colonies that appeared as large round smooth white highly convex and soft in consistency were identified as *Lactobacilli* colonies.

Colony counting

The number of colonies was counted using the colony counter and expressed as colony forming unit (CFU) per sample then the colonies per milliliter were calculated by dividing the number of colonies

on the dilution factor multiplied by the amount of specimen added to the liquefied agar.

$$\text{Number of bacterial colonies/ml} = \frac{\text{Number of colonies (CFUs)}}{\text{Dilution factor X Amount plated}}$$

II) Histological investigation

The histological processing was performed in the Histological Laboratory of the Faculty of Dentistry, Tanta University, according to Jawa et al.[7] The teeth sections of the conventional and Papacárie groups were immediately processed for histological examination after caries removal and taking the dentin samples for the culture. The samples were put in 10% neutral formalin (HT5011) for 24 hours, then decalcified in 11.9% (w/v) Ethylene Diamine Tetra Acetic acid (EDTA) (Chemicals, E0068000). Decalcified tissues were dehydrated and embedded in paraffin blocks. Sections of 5 μm thickness were prepared then mounted on glass slides, deparaffinised, dehydrated and stained with Haematoxylin and Eosin (H&E) stains, for observation under light microscope (Olympus–Bx50) at X200 magnification. Each slide was examined for the presence of bacteria in dentin.

III) Microhardness investigation

For microhardness investigation, ten extracted molars were sectioned as described before producing twenty sections. Dental caries of one section of each tooth was removed with the conventional method and the other section with Papacárie. Specimens were embedded in a chemically cured acrylic resin (Acrostone cold cure denture base material cross linked) to facilitate handling, keeping the surface to be examined exposed. At the dough stage of the polymerization process, the resin blocks were socked in distilled water to compensate for temperature rise as a result of the auto-polymerization.

The surface to be examined was flattened and smoothed using a universal metallurgical rotary polisher (Metaserv metallurgical services, betchworth surrey, England) with P1000, P1200 and P2000-grit SiC abrasive paper. Polishing was conducted using sandpaper and diamond paste (Ultradent® Diamond Polish Mint) of 1μm and ¼ μm. Smoothing and polishing of specimens were continued until a shiny surface with no scratches can be seen by the light microscope incorporated. Care was taken to keep the specimen surface perfectly parallel with the base of the resin block to be able to be correctly tested. Then specimens were stored in distilled water at room temperature for 24 h before testing.

Vicker's microhardness tester (Shimadzu DUH-211S, Dynamic Ultra Micro Hardness Tester, Japan) was used in this study. Hardness testing was performed using a diamond pyramid indenter, which has a square base with a 136º angle. A load of 0.025 Kgm was applied on the dentin surface of the specimen for 15 second which produced a square indentation on the surface. Load and time were constant for all specimens throughout the study

Vicker's hardness number (VHN) was measured at three points of each specimen. The mean and the standard deviation of the three values were obtained. The criteria for accepting an indentation were sharpness of diagonal edges, uniformity of diagonal shape and free of irregularities in the testing area. Measurement was taken using a microscope of X100 magnification by a microscopic ruler incorporated in the tester since the indentation was too small to be seen and measured with the naked eye. The VHN was calculated from the following equation:

$$VH = \frac{1.854 \times F}{d^2}$$

Where *VH* is the hardness, *F* is the load by Kgm and *d* is the diagonal of the square in dentin in microns. The load used was 0.025 Kgm for 15 seconds and 1.854 is a standard of the Vickers' hardness tester used in this study supplied by the manufacture.

Statistical analysis

The results obtained were recorded, tabulated and statistically analyzed. The mean values and standard deviation were calculated for each group. The difference between the two means was statistically analyzed with unpaired *t*- test at 5% level of significance.

RESULTS

Microbiological evaluation (Culture results)

Colonies of *Streptococcus mutans* on Mitis Salivarious agar before caries removal and after caries removal with the conventional method and Papacárie are shown in Fig. (1). Colonies of *Lactobacilli* on Rogosa agar before and after caries removal are shown in Fig. (2). The mean values and standard deviation of *Streptococcus mutans* and *Lactobacilli* count CFU/ ml $x10^4$ before and after caries removal with both methods are listed in Table (1). The mean value of *Streptococcus mutans* count has decreased from 3.5248 CFU/ ml $x10^4$ in the caries samples, to 0.5398 CFU/ ml $x10^4$ in case of the conventional method and 0.5266 CFU/ ml $x10^4$ in case of Papacárie, with a percentage of reduction of 82.604% & 84.188% respectively. Also, the mean value of *Lactobacilli* count has decreased from 3.567 CFU/ ml $x10^4$ in the caries sample, to 0. 5983 CFU/ ml $x10^4$ after caries removal with the conventional method and to 0.5753 CFU/ ml $x10^4$ after caries removal with Papacárie with a percentage of reduction 83.231% & 83.891% respectively.

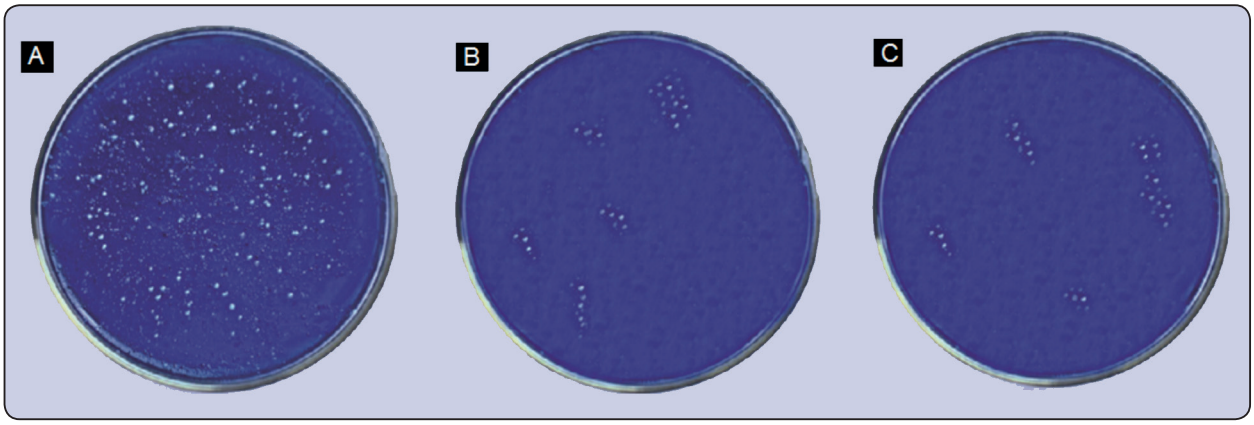

Fig. (1) Colonies of Streptococcus mutans on Mitis Salivarious agar: (A) Before caries removal. (B) After caries removal with the conventional method. (C) After caries removal with Papacárie

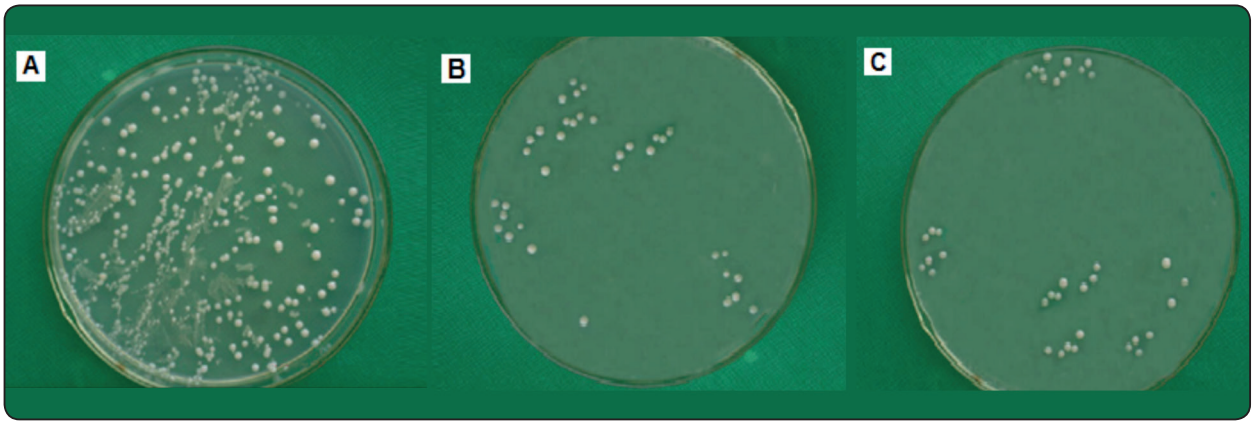

Fig. (2) Colonies of Lactobacilli on Rogosa agar : (A) Before caries removal. (B) After caries removal with the conventional method. (C) After caries removal with Papacárie

TABLE (1) Mean values and standard deviation (SD) values for *Streptococcus mutans* and *Lactobacilli* count CFU/ ml x10^4 before and after caries removal by conventional method and Papacárie.

Bacterial type	***Streptococcus mutans***			***Lactobacilli***		
Caries removal	**Before caries removal**	**Method of caries removal**		**Before caries removal**	**Method of caries removal**	
		Conven.	**Papacárie**		**Conven.**	**Papacárie**
Mean ± SD	3.5248 ± 0.29	0.5398 ± 0.03^A	0.5266 ± 0.03^A	3.567 ± 0.13	0.5983 ± 0.06^B	0.5753± 0.06^B
***t*- test**		P<0.0001*	P<0.0001*		P<0.0001*	P<0.0001*
% of bacterial reduction		84.686%	85.06%		83.227%	83.872%

Values have significant differences (P<0.0001). Values with the same uppercase letter have no significant differences (P> 0.05) after t-test

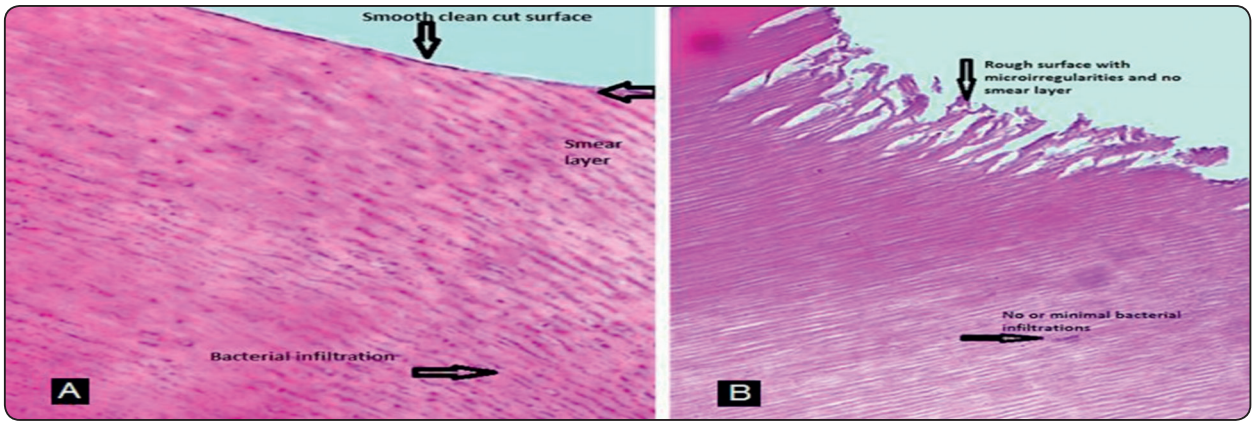

Fig. (3) Photomicrographs (H&E X 200) of dentin after caries removal with: (A) Conventional method. (B) Papacárie

There was a statistically significant reduction of *Streptococcus mutans* and *Lactobacilli* count after caries removal with the conventional method and Papacárie. However, there was no statistically significant difference in bacterial reduction between the conventional method and Papacárie.

Histological evaluation

Histopathologically, in the conventional method, there was bacterial penetration in the deep layers of dentin. The dentinal tubules appeared swollen and beaded. Some tubules were destructed as shown by the light microscope in Fig. (3A). Clean-cut surface with evidence of smear layer was obtained. On the other hand, in Papacárie group, there was almost normal arrangement of dentinal tubules, with no or minimal bacterial infiltrations as indicated by arrows in Fig. (3B). Rough dentin surface with micro-irregularities and no or minimal smear layer was obtained.

Microhardness evaluation

The mean values (Kgm\mm^2) of Vicker's hardness number (VHN) of dentin after caries removal with both the conventional and Papacárie methods are shown in Fig.(4). The mean of VHN and standard deviation (±SD) of dentin after caries removal with the conventional method and Papacárie is 75.49±12.02 and 76.51±10.88 respectively. Unpaired *t*- test has revealed no statistically significant difference between the two groups at 5% level of significance (P-Value 0.846) .

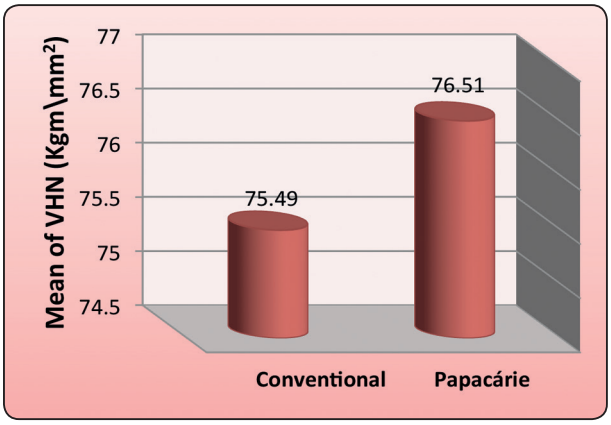

Fig.(4) Mean values of VHN of dentin after caries removal with conventional and Papacárie

DISCUSSION

Considering the improvement of biomaterials that facilitate atraumatic restorative techniques in dentistry, a papain-based gel called Papacárie have been used in the chemomechanical removal of decayed dental tissue.[33]

The present study was performed to assess the efficacy of the Papacárie in comparison with the conventional rotary instruments to remove carious lesion in primary teeth. Microbiological study, histological picture and microhardness of dentin were evaluated after caries removal with both methods.

Removal of bacteria was used to judge the caries removal as bacteria is one of the main etiological factors in caries occurrence.[34] Culture techniques have provided information regarding the microbiota associated with caries. Culture studies showed that *Streptococcus mutans* is the chief pathogen associated with caries, in addition to *Lactobacillus* spp. and *Actinomyces* spp.[35,36]

Microhardness analysis has been considered as a method to assess loss and reincorporation of minerals to the dental tissue because the reduction in the numerical hardness value presents a linear relation to mineral loss.[30,37] The dentin hardness has been reported as an indicator for caries removal because it is associated with the relative infectivity of carious dentin, helping the dentist to distinguish between either heavily infected (soft) or minimally affected (hard) dentin.[9] Similarly, the histo-pathological picture reflects the existence and extent of carious process in the dental tissues.[29,7]

In the present study, the extracted molars were selected with open cavitated lesions; as Papacárie is effective for removal of carious lesions when not covered with undermined enamel. The molars were kept in distilled water for no more than half an hour until processed to avoid dehydration and further bacterial growth.[31] Also the molars were sectioned longitudinally in the center of the carious lesion with water-cooling to obtain two equal caries halves that have similar properties and characters to avoid bias, this is in accordance with Jawa et al.[7]

In the current study, a selective media specific for *Streptococcus mutans* and *Lactobacilli* was used to avoid other bacterial or fungal growth, and to obtain accurate details about percentage of reduction of *Streptococcus mutans* and *Lactobacilli* after caries removal by the conventional method and Papacárie. While the light microscopic analysis of the same samples was used to see the condition of the deep layers of dentin and whether there is remaining bacterial deposits in the dentinal tubules.

The EDTA system was adopted for decalcification as it has a milder effect on gram positive staining bacteria than nitric acid and formic acid. Wijnbergen and Van Mullem[38] have tested the effect of EDTA, nitric acid and formic acid, on gram positive staining bacteria and found that the EDTA is the less affecting on their viability.

Microbiological findings

The results of the present study showed that conventional method and Papacárie have decreased significantly the mean values of *Streptococcus mutans* and *Lactobacilli* count. However, there was no statistical significant difference between the conventional method and Papacárie, both methods have

removed caries effectively. So, the first null hypothesis was accepted.

The effect of Papacárie on both types of bacteria was in agreement with the studies of Motta[20] and Matsumoto[39] which inferred Papacarie as an excellent option for minimally invasive removal of caries tissue, achieving significant reduction in total bacteria and Streptococcus mutans. These results also coincided with the study of El-Tekeya et al [40] which has evaluated the effectiveness of Carisolv, Papacárie and traditional hand excavation on the *Streptococcus mutans*, and *Lactobacilli* counts in the dentin of primary teeth. The authors have found that the Carisolv, Papacárie and traditional hand excavation significantly reduced the *Streptococcus mutans*, and *Lactobacilli* counts and the best results were given by Papacárie. Reddy et al[21] have studied the efficacy of antimicrobial property of Carisolv and Papacarie. The authors have concluded that both methods softened dentinal caries for effective mechanical removal criteria. From bacteriological point of view, these methods additionally removed cariogenic bacterium to a good extent.

The antibacterial efficiency of Papacárie may be attributed to the papain enzyme which has bactericidal and bacteriostatic properties which inhibits the growth of gram positive and gram negative organisms. Also the Papacárie contains chloramines which has bactericidal and disinfectant properties and are broadly used to chemically soften the carious dentin, and the toluidine blue a photosensitive pigment that is highly effective against *Streptococcus mutans* by fixing to the bacterial membrane.[7,11]

Histological Findings

The histological investigation has revealed a difference in the picture of the dentin structure between the two methods. So, the second null hypothesis was rejected. The conventional method of caries removal resulted in a cavity with nearly smooth clean-cut surface covered with smear layer (Fig.3A). On the other hand, Papacárie method gave a cavity with roughened surface with micro-irregularities (Fig.3B). Moreover, with Papacárie method, there was no evidence of smear layer, which could be counted an advantageous point over the conventional method.

Obviously, smear layer has been considered as a hindering factor in the mechanical bonding capacity of the adhesive materials to the cut dentin surface. As the Papacárie method exhibited high effectiveness in removing the smear layer, one can expect higher bonding results after cleaning the decayed tooth materials with this method. In addition, the Papacárie method left a rough surface with micro-irregularities (Fig.3B) in which the adhesive material can penetrate to form resin tags, thus improving the bonding between the tooth surface and the restorative filling materials. This result was in agreement with Hamama et al.[24,25] Moreover, SEM analysis conducted by Botelho et al has showed no interference of papain-based gel in the formation of hybrid layer.[41]

Leaving an irregular surface without smear layer and minimal bacterial infiltrations can be explained on the basis that the Papacárie method is a chemo-mechanical approach of caries removal which contains papain enzyme and chloramines in its formula. Papain, has antibacterial and anti-inflammatory properties[42] and also acts as a debris-removing agent. It does not harm healthy tissues and promotes tissue healing and acts only on caries tissue, which lacks alpha-1-antitrypsin plasmatic protease inhibitor that inhibits proteolysis in healthy tissues. So, its proteolytic action is inhibited on healthy tissue as healthy tissue contains this substance.[43] Chloramines also have the potential of dissolving caries dentin by means of chlorination of partially degraded collagen. This helps in disruption of collagen structure, dissolves hydrogen bonds and helps in tissue removal.[44]

The results of the current study showed also that the Papacárie preserved the anatomical structure of

dentinal tubules; the dentinal tubules appeared continuous and compacted. This may be attributed to the low viscosity of the Papacárie gel which enables its particles to penetrate deep in the dentinal tubules also the manufacture's instruction recommended to keep the Papacárie out of the refrigerator half an hour before use to change the Papacárie form from the gel state to a more liquid state. Whereas the conventional method has affected the anatomical structure of dentinal tubules; which appeared swollen and beaded, this may be attributed to the generated heat, that has a drastic effect on the dentinal tubules even with cooling system. This result was in agreement with the study of Jawa et al[7] which has reported that the Papacárie preserved the tubular structure of dentin in a perfect condition, in contrast to the conventional method which destructed the dentinal tubules.

The difference between the results of the culture and that of the histological analysis may be attributed to the difference in the methodology as the culture showed only the external surface while the microscopic analysis showed the whole depth of dentin.

Microhardness Findings

The results of the present study showed that the hardness of dentin remaining after caries removal with Papacárie did not show statistically significant difference from the hardness of the dentin remaining after caries removal with the conventional method (p=0.8464). Therefore, the third hypothesis was accepted and hence caries removal with Papacárie can keep the dentin hardness. This outcome was in agreement with that reported by Correa et al[30] where there was no difference in the dentin hardness remained after cleaning the decayed tooth materials by both methods. Also, Ramamoorthi et al[45] have demonstrated that Carie-cáre, a gel based on papain and containing chloramines, similar to Papacárie has no adverse side effects on dentin microhardness.

The results of a study conducted by Bittencourt et al[46] could add to the reliability of our findings. The authors quantified the mineral content removed from the primary teeth after using the Papacárie gel method by atomic absorption spectrophotometry technique. They found that the amount of calcium removed with the Papacárie affects only the carious component of teeth without affecting the hardness of dentin.

The results of the present study were not in accordance with the study of Mollica et al[47], which has reported that the remaining dentin microhardness after caries removal with Papacárie was higher than the remaining dentin microhardness after caries removal with other conventional methods, such as hand-excavation method. Based on the chemo-mechanical action of the Papacarie method, no evidence of precipitating minerals into the remaining dentinal tissues that could increase their hardness, thus, it is difficult to trust this finding. Moreover, it was hypothesized[48,4] that the Papacárie can decrease the dentin hardness more than the conventional method because of its mechanism of pepsin digestion and chlorine chlorination.

CONCLUSION

From the present findings, we can conclude that:

1. Papacárie is highly effective in caries removal and has antibacterial action on *Streptococcus mutans* and *Lactobacilli*.
2. Papacárie can preserve the integrity of the dentin structure.
3. Papacárie has no adverse effects on dentin microhardness.

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