Clinical and Microbiological Evaluation of the Carious Dentin Before and After Application of Papacarie Gel

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Objective: *To evaluate clinically and microbiologically the efficacy of Papacarie® in the removal of carious dentin in both permanent and primary teeth.* ***Study design:*** *Thirty permanent and primary molars with dentinal carious lesions were excavated and subjected to clinical and microbiological assessment before and after application of Papacarie®. The gel was further tested for in vitro antimicrobial efficacy against standard cariogenic micro-organisms using agar diffusion assay.* ***Results:*** *Papacarie® was able to differentiate between infected and affected dentin clinically along with high patient comfort during caries excavation. The mean time taken for caries removal and restoration was observed to be 4.17 ± 0.40 min. and 8.57 ± 0.45 min. for permanent teeth and 4.21 ± 0.36 min. and 9.24 ± 0.58 min. for primary teeth. There was a significant reduction in the total viable colony forming units from the dentin samples before and after application of Papacarie®. It was also observed that Papacarie® had no inhibitory effect on standard cariogenic microorganisms in the agar diffusion assay.* ***Conclusions:*** *Papacarie® is an effective caries removal method clinically in both permanent and primary teeth. The number of viable microorganisms after complete caries excavation using Papacarie® still appears to be high and this bacterial count should be tackled by a suitable restorative material with potent antimicrobial activity.*

Keywords: *Chemo-Mechanical, Caries, Papacarie, children.*

INTRODUCTION

Dental Caries continues to affect a significant portion of the world's population and treatment of the dental decay is associated with pain in many patients, so painless dentistry, minimal intervention are thus giving comfort, relief, solace and to instil positive attitude towards the dental treatment, are some of the factors justifying the specialty of Pediatric Dentistry. Although the rotary method of caries removal is a very speedy technique, it elicits pain, discomfort, and increases the amount of sound tooth tissue loss. Other newer methods like Lasers, Air abrasion, Air polishing, Ultrasonics, and Sono-abrasion have advantages like less amount of tissue removal and less patient discomfort and time saving also. But these techniques require costly equipment making their use more expensive. The chemomechanical method of caries removal (CMCR) satisfies most of the criteria needed for an ideal caries removal technique. CMCR is a non-invasive technique which eliminates infected tissues, preserving healthy dental structures, avoiding pulp irritation and patient discomfort.[1]

Since viable bacteria can persist in the tooth cavities regardless of the technique used for caries removal, bacteria that persist in the dentin immediately after complete removal of carious tissue remain viable and are able to proliferate. However, only small numbers of *Mutans streptococci* and *Lactobacillus spp*. were detected after sealing. The increased frequency of *Streptococcus spp*. and total microbial count after sealing in some samples suggests recolonization of the cavity. Thus an agent is needed with potent antimicrobial property for complete removal of the bacteria before sealing the cavity with a biocompatible restorative material.[2]

In 2003, a new CMCR agent called Papacarie® (Fórmulae Ação, São Paulo, Brazil) has been introduced and marketed worldwide. It claims to preserve healthy dental tissues at the same time being antimicrobial and anti-inflammatory in nature. The advantages include easy application and no requirement of special instruments.[3] Many studies had been carried out on these recently available CMCR agents but with some of the clinical parameters and only few of the studies[4,5] existing in the literature have evaluated microbiologically the efficacy of Papacarie® gel in the removal of carious dentin. The present study was, therefore, conducted with the following objectives:

I. To evaluate the efficacy of Papacarie® gel in the removal of carious dentin in the primary and permanent teeth

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II. To evaluate the time taken for caries removal and restoration using Papacarie® gel

III. To evaluate pain perception of the treatment by the child patients during caries excavation using Papacarie® gel with the help of Visual Analog Scale[6] and Wong Baker's Faces Rating Scale[7]

IV. To assess the level of total viable colony forming units (CFU) per carious dentin sample, before and after application of Papacarie® gel and,

V. To evaluate *in vitro* antimicrobial efficacy of Papacarie® gel against pure culture of standard cariogenic microorganisms using Agar Diffusion Assay

MATERIALS AND METHOD

The research protocol was reviewed and approved by the institutional ethical committee. Prior to the initiation of this study, parents or legally responsible persons received detailed information about the study and signed a free informed consent form, permitting the participation of their children. Sixty teeth with primary carious lesion involving dentin (i.e. thirty carious permanent teeth designated as Group I and thirty carious deciduous teeth designated as Group II) were chosen from twenty seven children, who attended the Out Patient Department of Pedodontics and Preventive Dentistry with the following criteria;

1. Patients, whose parents/guardians gave consent to their child for participation
2. Medically fit patients and with at least one tooth with primary carious lesion involving dentine
3. Cooperative patients

Inclusion Criteria[4]

1. Presence of primary occlusal carious lesions involving dentin and all the four walls should be intact, with the buccal-lingual opening measuring at least 2 mm
2. Radiographically the carious lesion should be present on the superficial aspects of the dentin

Exclusion Criteria

1. Teeth with a history of spontaneous or nocturnal pain
2. Teeth which are tender on percussion or palpation
3. Presence of abscess/soft tissue swelling in relation to the involved tooth
4. On radiographic examination where there is involvement of the pulp or caries approximating the pulp

For each tooth, two samples of dentin were collected in order to assess the level of total viable colony forming units (CFU).

Sample "A" – carious dentin prior to excavation in permanent teeth.

Sample "C" – carious dentin prior to excavation in primary teeth.

Sample "B" – dentin sample collected when the color of the Papacarie® gel remained unchanged in permanent teeth.

Sample "D" – dentin sample collected when the color of the Papacarie® gel remained unchanged in primary teeth.

Efficacy of papacarie® gel in the removal of carious dentin

Clinically the efficacy of caries removal was evaluated by the visual and tactile criteria as described by Ericson *et al* [4] The visual criteria included the presence or absence of any discoloured dentin (infected or affected dentin) after the application of Papacarie® gel and caries excavation. The tactile criteria included the smooth passage of the explorer and presence or absence of a catch or a "tug-back" sensation. Caries was considered removed and the remaining dentin was affected in nature when the explorer did not stick in dentin and did not give a tug-back sensation.

Time taken for caries removal and restoration

The time taken for the removal of carious dentin, beginning from the application of the gel until complete restoration of the teeth was evaluated using a digital stopwatch.

Evaluation of pain perception of the treatment

The pain perception of the treatment by each patient was evaluated using "Visual Analog Scale[6] and "Wong-Baker Faces Pain Rating Scale.[7]

Wong-Baker Faces Pain Rating Scale or Faces Rating Scale was developed primarily for use in young children. However, this scale can also be used in adults also who have difficulty using the numbers on the visual/verbal analog scales. The scale is comprised of 6 facial expression scores:

0 – No Hurt

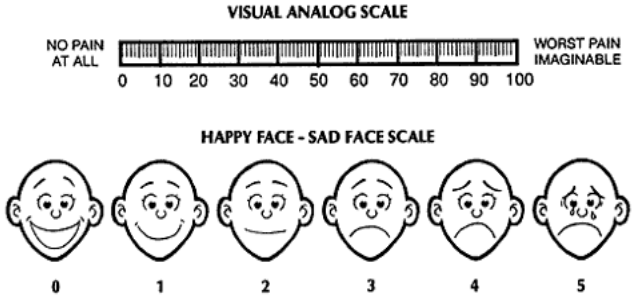
1 – Hurts Little Bit

2 – Hurts Little More

3 – Hurts Even More

4 – Hurts Whole Lot

5 – Hurts Worst



The happiest face is with the smile, the saddest face is with the tears, and the intermediate faces show varying degrees of happiness and sadness. The scale was presented to patients with the following question: "If you were this face right now, which one would you be?" The child would then point to the corresponding face that best represented their degree of pain or discomfort.

Assessing the level of total viable colony forming units (cfu) per dentin sample

The methodology in assessing the total viable colony forming units per dentin sample was in accordance with Zacharia *et al* [8] and Munshi *et al* [4] Each selected tooth was isolated with the help of the rubber dam; the surrounding area of the tooth and the dam was disinfected

using 2% chlorhexidine solution.[2] To standardize the amount of dentin sample obtained, small headed sharp spoon excavators (approx 1mm in size) was utilized for all the teeth. A sample site of carious dentin was selected and excavated in a pendulum movement. The excavated sample was then placed in transport liquid medium contained in a sterile tube and labeled as – "Sample A" for permanent teeth and "Sample C" for primary teeth. After this the stop watch was turned on to record the time, and the carious tooth was filled with Papacarie® (PAPAC 3, NCM 3006.40.12, Fórmulae Ação, São Paulo, Brazil), and the gel was allowed to work for 60 seconds as *per* the manufacturer's instructions. The gel was gently swapped with moist cotton and this procedure was repeated until the gel appeared clear and reached an unchanged light color. Lastly, the gel was removed using a moist cotton swab, cavity was irrigated and the second dentin sample was collected and stored in transport liquid medium contained in a sterile tube and labeled as – "Sample B" for permanent teeth and "Sample D" for primary teeth. The whole remaining soft dentine was completely excavated and the time taken till the complete excavation was recorded. The cavity was then evaluated using visual and tactile criteria as described earlier.[4] The cavity was restored using composite restorative material (Valux™ Plus, 3M ESPE, U.S.A), followed by finishing and polishing of the restoration. At this point the time taken to complete the restoration was also recorded.

The collected dentin samples were transported within 2 hours to the laboratory, where they were vortexed with glass beads for about 30 seconds in order to dislodge the bacteria from the dentin sample. The total viable bacterial counts in all the dentine samples were estimated using Miles and Mishra's Method.[9] A serial dilution of 10^{-4} (1/10, 1/100, 1/1000, 1/10000) of the homogenized suspension in sterile saline were prepared in sterile test-tubes. Using a calibrated pipette, 0.01 ml volume of the homogenized suspension was allowed to fall from a height of 2.5 cm onto a Schaedler agar plate, spreading it over an area of 1.5 to 2 cm in diameter. The plates were incubated anaerobically using Gas Pak System at 37ºC for 48 hours. After incubation the number of colonies in the drop areas showing the largest number without confluence was counted. The mean of the three plates of same dilution gave the variable count for 0.01ml of diluted bacterial suspension. The viable colony forming units per millilitre of the homogenized suspension was expressed as total colony forming units (CFU) per millilitre and calculated using the formula.

$$\text{CFU} = \frac{\text{No. of Colonies X Dilution Factor}}{\text{Volume Inoculated}}$$

All the values of CFU were converted to LOG_{10} [8] for the ease of comparison and were carried out using Microsoft excel sheet.

Agar diffusion assay 10. The revival of the microorganisms [i.e. Streptococcus mutans (MTCC NO. 497), Lactobacillus casei (MTCC NO. 1423) and Actinomyces viscosus (MTCC NO. 7345) obtained from Microbial type culture collection and gene bank (MTCC), Institute of Microbial Testing and Technology, Chandigarh, India] was carried out as per the instructions from Microbial type culture collection and gene bank (MTCC), Chandigarh, India.

A Barium Sulphate ($BaSO_4$) turbidity standard,[11] equivalent to 0.5 McFarland was used in this study for standardizing the inoculums for agar diffusion assay. Within 15 minutes after adjusting the turbidity of the inoculum suspensions (S. mutans, A. viscosus, L. casei), a sterile cotton swab was dipped into the adjusted suspension and the swab was rotated several times and pressed firmly on the inside wall of the test tube above the fluid level to remove the excess inoculum from the swab. The dried surface of Brain Heart Infusion (BHI) agar plate (for S. mutans), Pikovskaya agar (for A. viscosus) and Lactobacillus de Man, Rogosa and Sharpe (MRS) agar (for L. casei) which was of 4mm in height was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60º each time to ensure an even distribution of the inoculum. Three wells of 10 mm in diameter were punched at three equidistant points using an agar well puncher on three inoculated agar plates for each standard micro-organism. The Papacarie® gel was gently filled into these wells and the plates were incubated at 37º C for 24 hours for *S mutans* and *A viscosus* and 48 hours for L. casei under anaerobic conditions. The zones of inhibition around each well were measured using an antibiotic zone reader by three different examiners at three different points. Each zone was measured three times by the single examiner and the mean was calculated out of the nine readings.

Statistical analysis

The data thus obtained was subjected to statistical analysis which was performed using SPSS (Statistical Package for Social Science) version 17.0 for windows.

- Paired "t" test was used to compare the pain perception of the treatment by the child patient using VAS/FRS within the Groups.
- Paired "t" test was used to evaluate any difference between the CFU of "Sample A" and "Sample B" for Group I (Permanent teeth) and "Sample C" and "Sample D" for Group II (Primary teeth) respectively.
- One way ANOVA was used to compare the mean zones of inhibition between S. mutans, L. casei and A. viscosus.

The significance level for all the statistical tests utilized in this study was set at $p < 0.05$.

RESULTS

The efficacy of Papacarie® gel after caries excavation using visual and tactile criteria in both the groups are shown in Table I which shows presence of only affected dentin in all the teeth. The time taken for caries excavation and restoration of the teeth in both the groups are shown in Table II and the mean time taken for caries removal and restoration was observed to be 4.17 ± 0.40 min. and 8.57 ± 0.45 min. for permanent teeth and 4.21 ± 0.36 min. and 9.24 ± 0.58 min. for primary teeth. Table III shows pain perception of the treatment using VAS and FRS in Group I (permanent teeth) and Group II (primary teeth). The microbial load (CFU) in LOG_{10} of Sample 'A' and Sample 'C' (Before Application) and in LOG_{10} of Sample 'B' and Sample 'D' (After Application) of the gel in Group I (Permanent Teeth) and Group II (Primary Teeth) are shown in Table IV. A statistically significant difference in the microbial load before and after Papacarie® gel application was observed in both Group I and Group II ($p < 0.05$) indicating decrease in the mean viable bacterial count after the treatment with Papacarie® gel. The mean zones of inhibition observed for each of the tested microorganisms against Papacarie® gel was '0' mm indicating no antibacterial activity against standard cariogenic microorganisms in the agar diffusion assay.

Table 1. Efficacy of Papacarie® gel after caries excavation using visual and tactile criteria in both groups

Groups	n	Visual Criteria Presence or absence of any discoloured dentin after caries excavation in all the teeth with Papacarie®.	Tactile Criteria Presence or Absence of any catch or a tug back sensation after caries excavation in all the teeth with Papacarie®.	Presence or Absence of Infected and Affected Dentin	
				Infected Dentin	Affected Dentin
Group I (Permanent Teeth)	30	Absent	Absent	Absent	Present
Group II (Primary Teeth)	30	Absent	Absent	Absent	Present

Table 2. Time taken for caries excavation and restoration of the teeth in both groups

Groups		n	Mean	S.D
Group I (Permanent Teeth)	Till Complete Excavation	30	4.17 min.	0.40
	Till Complete Restoration	30	8.57 min.	0.45
Group II (Primary Teeth)	Till Complete Excavation	30	4.21 min.	0.36
	Till Complete Restoration	30	9.24 min.	0.58

DISCUSSION

The current odontological era has shifted from the principle of "extension for prevention" in the operative treatment of the carious lesion, towards practicing preventive dentistry and adopting more conservative and tooth preserving approach.[12] The chemo mechanical caries removal system has found to be easy, simple and economical, as well as effective also. In 2000, Banerjee *et al* [13] suggested that an ideal caries removal technique should satisfy both operator and child – comfort and ease of use in the clinical environment. It should have the ability to discriminate and remove diseased tissue only, painless, silent, requiring only minimal pressure for optimal removal of the carious dentin, not generating vibration or heat during the periods of operation and should be affordable.

As the chemomechanical caries removal solution is effective on only the denuded collagen fibres in the demineralized dentin, painful removal of and damage to the sound dentin is avoided thus reduces the chances of pulpal exposure during caries removal.[14] According to Kent *et al* [15] many of the school going children are afraid of dentist and may consequently avoid dental care. Consequently, fearful dental patients often do not receive optional and regular dental care. The negative behaviour is often linked to early traumatic experiences and negative attitudes in the patient's family.[16] Studies have shown that dental patients both attenders and non-attenders have emphasized the importance of fear associated with anticipated pain during treatment.[17] Therefore, a chemomechanical caries removal method is more preferable for paediatric patients.

In this study all the 60 treated teeth were found to be clinically caries-free after the application of the Papacarie® gel based on visual and tactile criteria.[8] Abdelnur *et al* [18] described three alternate techniques for caries removal (Atraumatic restorative treatment, Carisolv, and Papacarie) and concluded that these three methods are alternatives to the conventional method. Mohamed *et al*,[19] Jawa *et al* [20] and Kochhar *et al* [21] found similar results when Papacarie® gel was compared with conventional method and with Carisolv.

In the present study, the visual and tactile criteria[4] were followed to evaluate the efficacy of Papacarie gel in the removal of carious dentin. Kidd *et al* [22] reported satisfactory results when this method was used to assess the caries-free status of the lesion where as Fusayama[23] reported that this method of caries detection may not be reliable and caries detector dye may help in confirming the cavity caries-free. A new diagnostic aid to differentiate the infected and affected dentin after complete caries removal was not utilized in this study so as to prevent any introduction of a bias at this stage. The mean time taken for caries excavation by Papacarie® gel in this study was 4.17 min. for permanent teeth and for primary teeth it was 4.21 min. One such study by Ferrari *et al* [24] has shown that when Papacarie was compared with conventional method, caries removal took 22 seconds for slow speed hand piece and it was 119.9 sec for Papacarie. This indicates that the chemomechanical caries removal takes a longer time than drilling. Banerjee *et al* [13] evaluated 5 alternative methods (conventional hand excavation, bur, air-abrasion, sono-abrasion and Carisolv gel) of the carious dentin excavation and found that the airotor was the quickest method and CMCR method was the slowest out of the 5 methods. According to Kakaboura *et al* [25] the reason for increased time taken might be because of the multiple applications of the gel for complete caries removal. While Chourio *et al* [26] stated that the variation in the time may be related to the differences in type and size of the cavities, type of teeth and age of the patient. Carrillo *et al* [27] in their study concluded that caries removal using Papacarie® gel took 8 minutes per tooth in disabled

Table 3. Evaluation of pain perception of the treatment using VAS and FRS in Group I (permanent teeth) and Group II (primary teeth)

	Groups	n	Mean of Scores
Pain perception of the treatment using VAS	Group I (Permanent Teeth)	30	0 (No Pain At All)
	Group II (Primary Teeth)	30	0 (No Pain At All)
Pain perception of the treatment using FRS	Group I (Permanent Teeth)	30	0 (Happiest Face)
	Group II (Primary Teeth)	30	0 (Happiest Face)

Table 4. Microbial load (CFU) in LOG_{10} of Sample 'A' and Sample 'C' (Before Application) and in LOG_{10} of Sample 'B' and Sample 'D' (After Application) of the gel in the Group I (Permanent Teeth) and Group II (Primary Teeth).

	Sample (LOG_{10})	n	Mean	S.D	t –test	p-value	NS/S
Group I (Permanent Teeth)	LOG_{10} A	30	8.54	0.87	3.302	0.003	S
	LOG_{10}B	30	7.19	2.59			

	Sample (LOG_{10})	n	Mean	S.D	t -test	p-value	NS/S
Group II (Primary Teeth)	LOG_{10} C	30	7.32	0.90	3.679	0.001	S
	LOG_{10} D	30	5.94	2.22			

NS = Not Significant S = Significant

LOG_{10} A – Before Application LOG_{10} B – After Application LOG_{10} C – Before Application LOG_{10} D – After Application

patients. This longer operation time may be due to the use of gel in disabled patients and uncooperative behavior of the child.

Kochhar *et al* [21] compared Papacarie caries removal method with Carisolv and found that the mean time taken for caries removal using Papacarie was less as compared to Carisolv caries removal method. Thus indicating in all the CMCR methods Papacarie took lesser time in caries excavation which is important in managing the child patient on the dental chair. In pediatric dentistry, where the duration of the treatment sessions is often limited by the child's inability to sustain the prolonged cooperation this can be disadvantageous hence it seems important to consider strategies to prevent paediatric patients from becoming restless and negative towards the use of CMCR, as its advantages of being less invasive, lesser fear and anxiety in the child patients overweighs the time factor.[24] Pain during removal of dentinal caries is commonly reported phenomenon when using rotary instruments and it is claimed that chemomechanical systems eliminates the painful symptomatology.[26] In this study, all the patients were comfortable with this method of caries excavation. None of the patients complained of pain or discomfort during the excavation process, assessed by using Visual Analogue Scale and Faces Rating Scale. According to Bergman *et al*,[28] both the children and dentist reported reduced anxiety levels and lower degree of pain with CMCR method than the traditional method. But, contradictory to the above studies Inglehart *et al* [29] found that the subject's fear of the dentist increased in the CMCR group, while it is slightly decreased in the traditional method group. They have attributed this finding to the longer treatment time required for CMCR method.

In this study, the dentin samples were obtained carefully with a sterile spoon excavator for microbial evaluation after the use of Papacarie gel on the selected carious teeth. Similar method was also followed by Zacharia *et al* [8] with caries detector dye, Azrak *et al,*[30] Subramaniam *et al* [5] with Carisolv and El-Tekeya *et al* [31] with Papacarie. Kidd *et al* [32] utilized a round bur of a defined size for obtaining samples from the residual dentine, and established the reproducibility of this method. In this study, a sterile sharp excavator was chosen because it is atraumatic and reduces the risk of accidental pulpal exposure, especially when sampling hard dentine for obtaining the second dentin sample. In addition, loss of sample is not expected, unlike the bur method, which may spread the dentin particles during its rotation. Moreover, the dentin particles were easily visualized on the excavator rather than the bur's blades.

In this study, the mean LOG_{10} for total bacterial count of Sample 'A' (Before Application) was observed to be 8.54 ± 0.87 and for Sample 'B' (After Application) it was 7.19 ± 2.59 for permanent teeth and the mean LOG_{10} for Sample 'C' (Before Application) was observed to be 7.32 ± 0.90 and for Sample 'D' (After Application) it was 5.94 ± 2.22 for primary teeth with Papacarie®. The microbiological results revealed that in all the 60 teeth (30 Primary and 30 Permanent) there was a significant reduction in the total viable bacterial count after the application of Papacarie® gel even though the count was still high in the dentin sample (i.e. Sample 'B' and 'D') obtained after the gel application and its action.

Over the years several researchers have addressed the problem of what caries-free dentin is or how many microorganisms can be left in the cavity that will not promote further disease progress.[33] When excavating a lesion, the bulk of micro-organisms are removed along with most of the necrotic dentine. This does not render the prepared cavity bacteria-free, and the rationale behind removal of carious dentine is still uncertain and based on rather blunt clinical criteria.[34] Kidd *et al* [35] reviewed this problem and they concluded that "it is not possible to remove all the infected dentin". Several investigations had shown that often a low number of residual microorganisms (10^1-10^3 CFU) that remains behind in clinically sound hard dentine in spite of a significant reduction in the bacterial count. However, this low level of bacteria is considered to be clinically acceptable by several authors.[32] Heinrich *et al* [36] and Kidd *et al* [35] studied the relationship of the clinical appearance of carious dentine and the number of bacteria, and they found the values were below 10^2 CFU's for the streptococci and lactobacilli counts in hard dentin. In order to judge these results one needs to keep in mind that residual bacteria cannot be held solely responsible for occurrence of dental caries, since individual factors like oral hygiene and dietary habits of the patients, may also greatly influence caries progression. The clinical impact of bacterial persistence on caries free dentine is not clear but some authors agree that elevated bacterial counts remaining after caries removal procedure can be considered clinically significant because they cause further disease progression[32] but with the new adhesive restorations providing completely sealed margins and with the recently introduced antimicrobial cavity cleaners, this small amount of bacteria would seem to have a trifling effect on producing any further demineralization.[37] The antimicrobial potential of Papacarie Gel appears to be small but this technique of caries removal is more advantageous especially in Paediatric Dentistry when compared to conventional method.

With regard to the zones of inhibition against tested standard microorganisms, there were no zones of inhibition observed for *S mutans, L casei* and *A viscosus* with the Papacarie gel. The possible reason for no antibacterial activity of the Papacarie gel in agar

diffusion assay may be linked to the variation in the behavior of clinical bacterial strains and the standard tested microorganisms. It is also well known that the microbiology of the carious lesions is mixed in nature with complex floral interactions. The effect of the test gel against a single bacterial strain may vary against a mixed variety of species.[38] Hence, it is difficult to draw conclusions based on *in vitro* evaluation of antimicrobial activity with isolated bacteria.

To the best of our knowledge there is no study available in the literature regarding the antimicrobial activity of Papacarie gel using agar diffusion assay. The use of artificial media also plays an important role in determining the results. The solution form can diffuse at a faster rate than the gel form in the agar media. It is possible that different results might have obtained if other methods of testing antimicrobial activity i.e. Agar dilution method, Direct contact test etc., were employed.

CONCLUSIONS

Papacarie® is an effective caries removal method and was able to differentiate the infected dentin from the affected dentin clinically in both primary and permanent teeth. The mean time taken for caries removal and restoration was observed to be 4.17 ± 0.40 min. and 8.57 ± 0.45 min. for permanent teeth and in primary teeth it was 4.21 ± 0.36 min. and 9.24 ± 0.58 min. respectively. A high patient comfort during the caries excavation procedure using this gel was documented. The carious dentin sample collected after Papacarie® gel application demonstrated significant reduction in the total viable bacterial colony count. Although the number of viable microorganisms after complete caries excavation still appears to be high and this count should be tackled by a suitable restorative material with potent antimicrobial activity. Papacarie® gel had no inhibitory effect on the tested standard cariogenic microorganisms in the agar diffusion assay.

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