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CODEN: IJRSFP (USA)

International Journal of Recent Scientific Research Vol. 8, Issue, 10, pp. 20511-20516, October, 2017 International Journal of Recent Scientific Rerearch

DOI: 10.24327/IJRSR

APACARIES GEL – A ONE MAN ARMY-"TO COMPARE AND EVALUATE THE EFFICACY AND EFFICIENCY OF CHEMO-MECHANICAL CARIES REMOVAL AGENT WITH CONVENTIONAL METHOD- AN IN - VITRO STUDY"

Research Article

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DOI: http://dx.doi.org/10.24327/ijrsr.2017.0810.0906

ARTICLE INFO	ABSTRACT						
Article History: Received 17 th July, 2017 Received in revised form 21 st August, 2017 Accepted 05 th September, 2017 Published online 28 th October, 2017	The recent odontologic era demands the eradication of dental caries which is still challenging						
	besides having ample treatment modalities like air-abrasion, ultrasonic instrumentation, laser and chemomechanical method. Chemomechanical method of caries removal being non-invasive, cost-effective always remains the choice of treatment but if some caries preventive measures could be added in them then it might render the remaining dentin more resistant to future caries attack. Newly introduced Apacaries Gel is one such material. Thus, this is an in vitro study to comparatively evaluate the efficacy and efficiency of chemo-mechanical caries removal agent i.e., Apacaries Gel with conventional method via rotary instrument. 80 exfoliated deciduous molars with caries						
<i>Key Words:</i> Dental caries, conventional rotary instrument, chemomechanical caries removal agent, Apacaries Gel.							
	involving dentin upto varying degree were selected for the study. The molars were divided into two groups: Group A; to assess the microhardness of remaining dentineand Group B; for bacteriological evaluation. After removal of the caries with the respective methods in both the groups, the efficacy and efficiency were evaluated by Vicker's Hardness Tester and Light Microscope respectively. Though complete removal of caries was achieved in both the methods with no-significant difference, but there was less marked destruction of dentinal tubules in chemomechanical caries removal method as compare to conventional method. There was also non-significant difference found between both the groups regarding remaining dentine microhardness. Thus, concluding that Apacaries Gel can be recommended as an efficient, easy to perform, comfortable, hasselfree and less destructive solution for the patient seeking an alternative to the conventional method especially in children.						

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INTRODUCTION

A "mindful practice" is the objective that continuously monitors and re-evaluates the results and the techniques over with the introspection. The human tooth which is a marvel of nature needs to be protected otherwise it will be lost mainly due to dental caries. Thus, dental caries is one of the common occurring oral conditions whose impedance is required for the welfare of mankind especially in children. As various methods were used since antiquity from the hand instruments to recently introduce newly rotary instruments but still the removal of dental caries is a task. This may be due to various drawbacks like pain, annoying sounds, vibrationetc, which eventually leads to fear and anxiety and act as barrier to the receipt of treatment and thus leads to further deterioration of teeth.¹ As a famous quote stated that "the beginning of ANXIETY is the end of faith, and the beginning of true faith is the end of ANXIETY". Thereby, instilling the positive behaviour in children should be the first step towards adentiphobic future.

Thus, chemomechanical removal of caries has been developed as an alternative to overcome these lapses. These agents used

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for dental caries removal have been developed, starting with the use of 5% sodium hypochlorite (Naocl), by *Habib et al* followed by GK-101 or n-monochloroglycine by *Goldman et al* (1972), Caridex approved by FDI in 1984 in USA, "Carisolv" by Swedish researchers, "Papacarie®" developed in Brazil in 2003 by *Bassadori et al*² and Carie-Care in India. Besides having various advantages, these chemomechanical agents also have several disadvantages less shelf life, generally used on large extensive carious lesions only leaving affected dentine and have no preventive properties.

Hence, it brings us to a novel chemomechanical caries removal agen t i.e., Apacaries gel.⁴ Along with the various advantages of chemomechanical agent it also has the added advantage of being preventive which leaves the remaining affected dentine more resistant to future caries attack.

Therefore, the aim of the present study was to compare the efficacy and efficiency of chemo-mechanical caries removal agent i.e., Apacaries Gel with conventional method via rotary instrument in terms of bacteriological and microhardness evaluation. During the course of this study, time factor was also evaluated for both the method.

MATERIALS AND METHODS

80 exfoliated deciduous molars with caries involving dentin upto varying degree were selected for the study. The stored teeth were autoclaved and cleaned using ultrasonic scaler to remove the debris. Sampled teeth were stored in 0.1% thymol solution at room temperature to avoid dehydration and further sectioned through the centre of the lesion mesio-distally by using rotating disk bur making the sample size of 160 [Figures 1]. These sampled teeth were further randomly divided into two groups i.e., Group A- microhardness evaluation & Group Bbacteriological evaluation (n=80 each). The carious lesions on sample of both the groups were removed by conventional method (A₁ & B₁) and chemomechanical caries removal method (A₂ & B₂). (Table 1)



Figure 1 Mesio-distal sectioning of Samples

Removal of caries

Conventional method of caries removal

The one part of the carious lesion of each samples i.e., Group A_1 & B_1 was removed using round bur with slow and intermittent speed micromotor (Figure 2).



Figure 2 Caries removed by conventionalmethod

Chemo- mechanical caries removal method

Whereas the other half of carious tissue of samples i.e., Group $A_2\&$ B_2 was removed by using Apacaries Gel (Figure 4). Before applying the gel, it was removed from the refrigerator half an hour before to thaw. It was applied with the help of an applicator tip on the carious lesion and left for 30-40 seconds. The time of application was measured by using stopwatch. The softened dentine was removed using the spoon excavator in a pendulum motion without applying pressure. The remaining gel was removed with cotton pellet soaked in saline. This procedure was repeated 1 to 4 times according to the extent and depth of caries.

The complete removal of caries by both the methods of caries removal was checked according to Erikson criteria {i.e., optical (colour) and tactile (hardness)}. The colour was checked visually and hardness of the lesion was checked by the dental explorer until a leather-hard texture and sharp scratching sound was heard. The preparation time for each caries removal technique was evaluated by using stopwatch (Figure 3).



Figure 3 Caries removed by Apacaries Gel Preparation of samples for measuring microhardness

Samples of both the groups (group A_1 - caries removed by conventional method and A_2 - caries removed by chemomechanical caries removal method) were smoothened sequentially with 400, 500 and 600 grit sandpapers. Standardized blocks were prepared by filling the plastic pipe of 15mm diameter with acrylic resin. Care was taken to mount the prepared samples on it before acrylic resin gets harden. The samples were mounted in such a way that the occlusal surface was exposed to external environment. These blocks were again smoothed sequentially with sandpaper of 400, 500 and 600 grit. The blocks were then stored in a container having distilled water with few crystals of thymol for maintaining hydration. (Figure 4)



Figure 4 Testing of samples by Digital Vickers Microhardness Tester



Figure 4 Decalcification of sample in 10% nitric acid

Preparation of samples for bacteriological evaluation

Samples of both groups (group B_1 - caries removed by conventional method and B_2 - caries removed by chemomechanical caries removal method) were suspended in glass beakers having distilled water with the help of thread such that the tooth was completely immersed in it and then approximately 5ml of decalcifying agent was (10% nitric acid) poured into it. (Figure 5) The samples were kept in decalcifying solution till the time they were completely decalcified. Decalcification of samples was checked by piercing them with fine needle or pinching through dental probe or explorer. Decalcification was confirmed if the needle or probe goes through the samples implying that the samples are completely soft.



Figure 5 Mounting the samples in paraffin blocks for sectioning

After confirming the completion of decalcification, the samples were washed thoroughly under running tap water for minimum of 12 hours to remove the remaining decalcified fluid. The tissue dehydration was carried out by passing it through ascending degrees of ethanol (50%, 60%, 70%, and 90%) and cleared in xylene for one hour. These samples were then embedded in paraffin wax and blocks were prepared. (Figure 6) Serial sections of 5 μ m were cut from the block using rotary microtome.

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Figure 6 Stained GlassSlide (n=40)

The samples were then mounted on glass slides and stained with eosin and hematoxylin to evaluate the presence of bacterial deposits. (Figure 7)

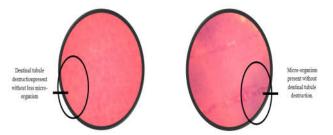
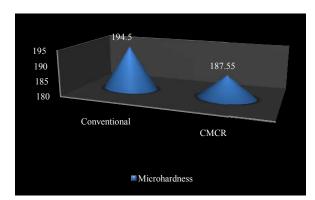


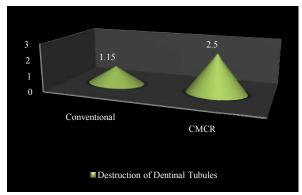
Figure 7 Microscopic view of the slide showing absence of microbes after caries removal using Conventional method of carires removal and Chemomechanical caries removal method (Apacaries Gel)

Bacteriological evaluation

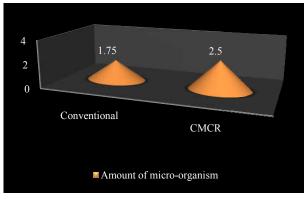
The microscopic slides with dye stained specimens were observed under the light microscope (40x) and were assessed for the presence/absence of microorganisms on remaining dentine. The presence of microorganisms was denoted with a positive sign (+) and the absence was depicted with a negative sign (-) by the observers. Images were captured and analysed by using B540 software. (Figure 8)



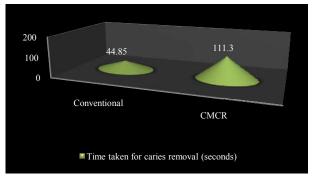
Graph 1 Graphical representation of mean of microhardness of both the groups



Graph 2 Graphical representation of mean of destruction of dentinal tubules of both the groups



Graph 3 Graphical representation of mean of amount of microorganism of both the groups



Graph 4 Graphical representation of mean of time taken for caries removal of both the groups

Time assessment

The preparation time for each caries-removal technique in all the samples (n=80) was evaluated using a stopwatch. The total time taken for caries removal was calculated from the beginning of removing caries till its complete removal in seconds in both the methods.

RESULTS

Microhardness evaluation (Table 2)

Table 2 showed the mean values of microhardness in Group A_1 (conventional method of caries removal) and Group A_2 (chemo-mechanical method of caries removal). The mean value of microhardness was found to be 194.55±6.41 for group A_1 and 187.5± 6.10 for group A_2 which was high in group A_1 (conventional method of caries removal) followed by group A_2 (chemo-mechanical method of caries removal). After applying Mann-Whitney test (or U-test), it was revealed that there was non-significant difference found between Group A_1 and Group A_2 with p value of 0.001.

Table 2 Mean values of microhardness in Group A_1 and Group A_2

Group*	N Mean		Mann- Whitney U	p Value		
A ₁	20	194.55	6.41	20.50	200.00	1.000****
A_2	20	187.5	6.10	20.50	200.00	

Destruction of dentinal tubules (Table 3)

Table 3 revealed the mean value of destruction of dentinal tubules to be 1.15 ± 0.36 for group B₁ (conventional method of caries removal) and 2 ± 0.0 for group B₂ (chemo-mechanical method of caries removal). It was noted that 18 samples out of 20 showed destruction in conventional caries removal group as compared to no destruction in any of the samples of chemomechanical caries removal group. After applying Mann-Whitney test (or U-test), it was revealed that there was significant difference between Group B₁ and Group B₂ with p value of .001.

Table 3 Mean values of destruction of dentinal tubules in Group B_1 and Group B_2

Group*	N	Mean	Std. Deviatio	n Mean Rank	Mann- Whitney U	p Value	
B ₁	20	1.15	0.36	12.00	30.000	.000**	
B_2	20	2	0.0	29.00	30.000		

Bacteriological evaluation (Table 4)

The mean value of amount of micro-organism left after caries removal was found to be 1.75 ± 0.444 for group B₁ and 1.6 ± 0.502 for group B₂. The presence of microbes after caries removal by both methods was evaluated under light microscope. It was found that microbes were present in 5 samples out of 20 in conventional caries removal group as compared to 8 samples out of 20 in chemomechanical caries removal group and had non-significant difference between them with p value of .429. (Table 4)

Table 4 Mean values of amount of micro-organism in Group B_1 and Group B_2

Group*	N	Mean	Std. Deviation	Mean Rank	Mann- Whitney U	p Value
B_1	20	1.75	0.444	22.00	170.000	.429***
B_2	20	1.6	0.502	19.00	170.000	.429

Time assessment (Table 5)

Table 5 showed that the mean value of time taken for caries removal was found to be 0.74 ± 0.27 minute for conventional method (Group A₁ and Group B₁) and 1.85 ± 0.77 for chemomechanical method (Group A₂ and Group B₂). It was noted that time taken for caries removal was more in chemo-mechanical method (Group A₂ and Group B₂) than conventional method(Group A₁ and Group B₁) which was significant.

Table 5 Mean values of time taken for caries removal in conventional method (Group A_1 and Group B_1) and by chemomechanical method (Group A_2 and Group B_2)

Group*	N	Mean (in seconds)	Std. Deviation	Mean Rank	Mann- Whitney U	p Value
Group A ₁ & Group B ₁	40	44.85	16.413	21.40	36.00	0.000**
Group A ₂ & Group B ₂	40	111.3	46.559	59.60	30.00	0.000**

DISCUSSION

Fear and anxiety which are known barriers to the receptivity of dental treatments were the main rationale behind the advent of the chemomechanical caries removal agent. Since, the emergence of the chemomechanical caries removal agent in 1975 by Habib *et al* there were enormous furtherance undergone to achieve the archetypal chemomechanical caries removal agent. Despites of the various chemomechanical agents like carie care, papacaries etc, no literature is found on the their preventive aspect.³ To overcome this flaw of chemomechanical caries removal agent- Apacaries Gel was introduced in 2012, by ApaJuntavee which is composed of polyphenol in mangosteen extracts and papain in a gel preparation and also has the added advantage of being preventive which makes the remaining affected dentine more resistant to future caries attack.⁴

The newly introduced "Apacaries gel" was composed of polyphenol in mangosteen extracts and papain in a gel preparation.⁴ The mangosteen extract was obtained by mangosteen pericarp powder which mainly consist of amangostin component and Garcinia mangostana L which -a potent inhibitor of acid production by S. mutans. These component were active against membrane enzymes, including and the phosphoenolpyruvate F(H+)-ATPase sugar phosphotransferase system and also inhibited the glycolytic enzymes aldolase, glyceraldehyde-3-phosphate dehydrogenase and lactic dehydrogenase'. Other targets of α -mangostin included inhibition of malolactic fermentation by S. mutans by increasing alkali production from malate to neutralizes the acid production of S.mutansand inhibits NADH oxidase (major respiratory enzyme in S.mutans).^{4,5} The mangosteen is also anti-inflammatory, anti- histamine, anti-oxidant, anti-bacterial.

The papain can cleave the bonds of the amino acids of Gramnegative bacteria and, result in perturbing the membrane permeability. Dawkins *et al* stated that Papain is bactericidal and bacteriostatic properties¹ which inhibit the growth of gram positive and gram negative organism. Thereby conjointly, both Papain as well as mangosteen extract have synergistic effect on removing as well as inhibiting further progress of caries and leaving the remaining affected dentine more resistant to further caries attack. Papain present removes the infected dentine followed by the reduction in S.mutans due to the inhibitory effect of mangosteen extract. ApaJuntavee et al (2014) also stated the inhibition zone of mangosteen extract and papain mixture in gel preparation was larger than the zones for the separate components individually.⁴ So, Apacaries Gel is a newly developed chemomechanical caries removal agent which is one of its own kind. An in vitro study for Apacaries Gel shows that 1 mg/ml mangosteen extract mixed with papain in Apacaries gel can effectively inhibit S. mutanswithin 2 minutes.5

A split-tooth methodology was used in the present study for standardization purposes which allows the comparisons to be made between different, paired excavation methods as stated by Magalhães CS et al^{6} , Singh D J et al^{7} . All samples were sectioned into two halves mesio-distally by using rotating disk bur in micromotor to avoid desiccation of the sample due to heat produced. Out of two samples obtained by splitting, the carious lesion of one sample was removed with conventional method using round bur with slow and intermittent speed micromotor whereas the other sample was prepared with chemomechanical method of the caries removal. Correa FNP, Filho LER, Rodrigues CRMD⁸ prepared standardized blocks which were smoothened sequentially with 400, 500 and 600 grit sandpapers for measuring the remaining dentine microhardness as flat surface was needed for accurate measurements. Vickers hardness tester was used as the square shape which was prepared by Vickers indentor is close to the outer surface and the DEJ.⁵

For bacteriological evaluation, the samples were decalcified using 10% nitric acid because of its rapid action. The decalcified samples were then washed in water, dehydrated in ascending degrees of ethanol (70-100%) and were cleared in xylene and later embedded in paraffin. The teeth were then sectioned into 5 μ m thickness and stained with eosin and hematoxylin. This was done to check the presence of bacterial deposits microscopically and dentinal tubule destruction using conventional light microscope (40x) by a single operator to reduce bias.^{7,10}

The time taken for caries removal was recorded using a stop watch in both conventional as well as chemomechanical method of caries removal. The time taken for complete caries removal was calculated, from the start of the procedure to the completion of caries removal. There was non-significant difference between both the groups in remaining dentine microhardness. As in chemomechanical method there was selective removal of caries-infected dentine only, which has lower hardness values as compared to sound dentine. Whereas, in conventional method, some of the affected dentine was removed along with infected dentine which has higher hardness values as compared to infected dentine. But the percentage of this removal was minimal in the present study because there was a conscious and cautious effort made to remove the infected dentin only, leaving behind the affected dentine thereby resulting in comparable hardness in both the groups.⁶

The similar results was found in the study conducted by Correa FNP *et al* (2007), Qasim AS and Suliman AA (2008), Flückiger L(2008) and Jaya S *et al* (2011) who concluded that the hardness of the remaining dentin after carious tissue removal was lower than that obtained on healthy dentin, without significant difference between the different means of carious tissue removal.^{8, 9, 11, 12}

The complete elimination of bacteria from the carious lesion is not possible microscopically either by conventional or chemomechanical method of caries removal as in conventional method, affected dentine too has slight microbial invasion which has not undergone demineralization as infected dentine.² Whereas, in chemomechanical method of caries removal, the caries is removed by scrapping with spoon excavator which may leave some of the infected dentine behind where microbes are present more than affected dentine. But the percentage of leaving infected dentine behind is very minimal leading to comparable amount of bacteria remaining in both the methods. ^{7, 13, 14} The conventional method showed more destruction as compared to chemomechanical method of caries removal as the burs which are generally used in conventional method tends to be a fast and rather non-conservative with negative rake angle which leads to a less controlled movement of the instrument. On contrary, chemomechanical preserves remineralizable tissue and prevent over-excavation of the cavity.¹⁵ Similar study was reported by Jawa D et al (2010), Divya G et al (2015), Sahana S et al (2016) who compared the effectiveness of chemomechanical caries removal agents in primary molars.^{7, 10,} ¹⁵ Chemomechanical method took significantly more time because of multiple applications of the gel for complete caries removal. Moreover, the extended time may be due to prolonged time for excavation with spoon excavator.¹⁵

CONCLUSION

In the present study it can be concluded that the chemomechanical method of caries removal involving dentin in cavitated primary molars by means of Apacaries Gel was comparable to that of the conventional drilling method in terms of efficacy and efficiency. This shows that it could be a suitable non-invasive, economic, less painful, has selfree alternative method for caries removal along with the preventive aspect of further resist the caries attack and has great potential for use in pediatric operative dentistry. We recommend further studies to authenticate these results.

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