Original Article

DOI: 10.7860/JCDR/2019/37606.12512

Dentistry Section

Comparing the Efficacy of Berberine against Sodium Hypochlorite and Chlorhexidine Cetrimide as a Chairside Disinfectant of Gutta Percha Cones

NAMITH RAI[1], SHOBANA SHETTY[2], RAVI GUPTA[3], AHANA SHETTY[4], ASHISH SHETTY[5], BENOY JACOB[6]

ABSTRACT

Introduction: The primary objective in Endodontics has been to disinfect the root canal space, by both mechanical and chemical means. Clinicians rarely give a thought to the fact that contamination can happen from all sorts of instruments, materials and the by clinical setup itself. When disinfection of the root canal is been spoken, studied and researched so extensively, gutta percha cones which are the most commonly used root canal filing material for over 100 years, thanks to its malleability and biocompatibility. They may be contaminated right from the manufacturing process, packaging and use.

Aim: This study intended to compare the efficacy of berberine as a more biocompatible alternative to sodium hypochlorite and chlorhexidine cetrimide as a chairside disinfectant for gutta percha cones.

Materials and Methods: 180 gutta percha cones collected from freshly opened boxes were contaminated with Microbial suspensions of *E. Faecalis* (ATCC29212) and *S. aureus* (ATCC 6538) of approximately 10^8 CFU/mL artificially. Split into three groups with three different disinfecting agents (6% NaOCl, vista dental products, USA, berberine chloride 2 mg/mL, Chlorhexidine 2% and cetrimide 0.2%) further subdivision was done based on exposure time (one minute, three minutes, five minutes) respectively, After disinfection in chemical agents, the cones were washed in 10 mL of detergent solution (3% Tween 80 and 5% sodium thiosulfate) for five minutes. The cones were rinsed in 10 mL of sterile distilled water, transportation to sterile test tubes containing 10 mL of thioglycollate media and incubated at 37°C for seven days, then were transferred from the 10 mL thioglycollate media to a petridish containing brain heart infusion agar, incubating for 48 hours aerobically at 37°C and the colony forming units were graded under light microscope.

Results: Statistical analysis was done with Tukeys HSD test. The mean bacterial count of *E. Faecalis* or *S. aureus* was found to be significantly lesser after treating the cones with 6% sodium hypochlorite when compared with other disinfecting solutions for all time intervals tested. Berberine was found to be mildly effective against *S. aureus* but not effective against *E. Faecalis* even at the five minute stage.

Conclusion: Within the limitations of the study, Sodium Hypochlorite is still the disinfectant to beat, further research is needed with berberine along with concentration modifications and duration.

Keywords: Berberine, Gutta percha disinfection, Herbal disinfectant

INTRODUCTION

Since the inception of Endodontics as a specialisation, the primary objective has been to disinfect the root canal space, by both mechanical and chemical means. Clinicians rarely give a thought to the fact that contamination can happen from all sorts of instruments, materials and the by clinical setup itself. When disinfection of the root canal is been spoken, studied and researched so extensively, gutta percha cones which are the most commonly used root canal filling material for over 100 years, thanks to its malleability and biocompatibility, may be contaminated right from the manufacturing process, packaging and use. Gutta percha is a vegetable substance extracted in the form of latex, and forms portion of about 19 to 20% of the composition of cones, together with 59% to 75% of zinc oxide and 1.5% a 15% of radio-opacifying agents, waxes, coloring agents, antioxidants and metal salts [1]. In an observation done by Pang NS et al., gutta percha cones stored in clinical conditions for more than three months, they reported a 19.4% contamination rate in hospital-based Endodontic clinics [2]. Contrary to Pang et al., Montgomery [3], Namazikhah [4] et al., and Gomes et al., [5] reported in their studies that even freshly opened boxes were corrupted with microorganisms from 8 to 25%. Therefore, the following observation constantly inveterate the fact that, gutta percha cones even when used from sealed packages cannot entirely guarantee that they are one hundred percent sterile even though they are packed and processed in sterile aseptic conditions, gutta percha cones must be free of microorganisms irrelevant of whether they can cause an infection by themselves or not, but the alterable levels exhibit minimal amounts of contamination as the different disinfection investigations were only relevant for the identification of a subset of quiescent microbes. Although contaminants isolated in these studies may be non-oral species and may not be considered pathogenic, it is decisive to know that most are capable of being opportunistic infections if conditions are appropriate. Studies on the microbiology of endodontic infections have documented the breadth, capability and complexity of potential pathogens, including bacterial and fungal species that may typically be unassociated with the human oral cavity. Cones can be contaminated by aerosols, improper storage and also once exposed to the dental office environment or even by clinical manipulation by the usage of cotton tweezers or any other instrument being used to carry gutta percha cones from the box which can contaminate the other cones in the box, some clinicians place the gutta percha accessory cones back into the box instead of discarding them which can be infested by any number of microorganisms. Gutta percha are the rigid natural latex produced from the sap of trees, due to their physical and chemical properties made up of polyisoprene chains. Disinfection

is not possible by moist heat or dry heat as they decompose when heated, they cannot be sterilised by humid or dry heat, which is the cause for concern since the maintenance of the aseptic chain is essential to prevent new microorganisms from being introduced into the root canal systems. Clinicians are occasionally faced with the problem of re-infection after endodontic treatment, re introduction of new species of microorganisms may be an addition to the existing microbial environment, migration of certain species of bacteria into the blood stream of immune and systemically compromised patients should be prevented because of the risk of infective endocarditis [6]. Gutta percha cones become accessible to potential contamination by variety of organisms such as coccus, rods, and yeasts present in the air or contacting objects once the box of gutta percha points are exposed to dental chair side clinical environment [7,8]. Supplementary decontamination of gutta percha cones is judicial, preferred method for decontamination of gutta percha cones is cold sterilisation, primarily done by immersing the cones in various proposed chemical disinfectants like sodium hypochlorite (NaOCl), glutaraldehyde, alcohol based solutions, iodine compounds and hydrogen peroxide. For optimum result from an Endodontic therapy, every instrument and material placed within the canal should be sterile.

This study intended to check the efficacy of more biocompatible plant extract berberine against the more routinely used sodium hypochlorite and a mixture of chlorhexidine and cetrimide.

Availability of Berberine and Preparation of the Sample

Berberine-containing plants including goldenseal, barberry, Oregon grape and goldthread, has been extensively studied in both experimental and clinical settings for its antibiotic activity [9].

Extracts can be of dried root or as infusion (tea), 2 to 4 g, tincture (1:5), 6 to 12 mL (1.5 to 3 teaspoons), fluid extract (1:1), 2 to 4 mL (0.5 to 1 teaspoon), solid (powdered dry) extract (4:1 or 8% to 12% alkaloid content), 250 to 500 mg [9].

Fresh root of *Berberis vulgaris* L. was collected from parts of Northern India, cleansed with running tap water, cut into small pieces. They were dried for five days and then ground to powder. The aqueous extract was prepared by cold maceration of powdered root 10 mg/mL in equal proportion of distilled water for 48 hours. Followed by drying for the water content to evaporate, working solution is prepared by the use of 1:1 of any of these solutions

1. Ethyl alcohol
2. Methanol
3. Honey or edible oil, Aloe Vera
4. The mixture of distilled water and methanol

MATERIALS AND METHODS

180 (ISO size 60, 2%, (Dentsply Maillefer, USA) gutta percha cones, divided into 90 for *E. Faecalis* and 90 for *S. aureus* and sub grouped into groups I, II, III (6% NaOCl, vista dental products, USA, berberine chloride 2 mg/mL, chlorhexidine 2% and cetrimide 0.2%) and they were further divided into Ia, Ib, Ic and IIa, IIb, IIc and IIIa, IIIb, IIIc (based on exposure time one minute, three minutes, five minutes) respectively [Table/Fig-1]. Each of the subgroups had 10 specimens. Artificial contamination was done with The Microbial suspension of (-80°C) *E. Faecalis* (ATCC29212) and *S. aureus* (ATCC 6538), of approximately 10^8 CFU/mL. Gutta percha cones were taken from freshly opened boxes and arranged in three groups of 30 each, immersed in 20 mL microbial suspension for one hour. Cones were then transported to sterile paper pads in petri dishes and allowed to air dry for 10 minutes at room temperature. Followed by disinfection of the cones in different solutions of sodium hypochlorite, berberine and chlorhexidine solutions for time intervals of one minute, three minute and five minutes. Groups I, II, III were evaluated for the efficacy of the disinfecting solutions. Groups I, II and III were further divided into a, b and c according to the disinfecting time which was one minute, three minutes, five minutes). Following immersion in disinfecting solutions, cones were cleansed in 10 mL of detergent solution (3% Tween 80 and 5% sodium thiosulfate) for five minutes. The residual effects of the test agents were neutralised by rinsing with 10 mL of sterile distilled water. The cones were then individually transferred to sterile test tubes of 10 mL of thioglycollate media and incubated at 37°C for seven days. After which the media with the growth were smeared to a petridish containing Brain Heart Infusion agar, culture medium used commonly for the growth of microorganism based on turbidity [10]. The plates were then nursed by incubating for 48 hours aerobically at 37°C and the colony forming units were graded. Microbial growth was also confirmed with Gram staining, colony morphology, and with a microbial growth identification kit under the microscope by an experienced microbiologist. Statistical analysis was done with Tukeys HSD test [11] SPSS version 16.

Group I a	Ten contaminated gutta percha cones immersed in 6% sodium hypochlorite for one minute
Group I b	Ten contaminated gutta percha cones immersed in 6% sodium hypochlorite for three minute
Group I c	Ten contaminated gutta percha cones immersed in 6% sodium hypochlorite for five minutes
Group II a	Ten contaminated gutta percha cones immersed in berberine for one minute
Group II b	Ten contaminated gutta percha cones immersed in berberine for three minute
Group II c	Ten contaminated gutta percha cones immersed in berberine for five minutes
Group III a	Ten contaminated gutta percha cones immersed in Chlorhexidine+cetrimide for one minute
Group III b	Ten contaminated gutta percha cones immersed in Chlorhexidine+cetrimide for three minute
Group III c	Ten contaminated gutta percha cones immersed in Chlorhexidine+cetrimide for five minutes

[Table/Fig-1]: Depicts the methodology.
(90 cones artificially contaminated with *E. Faecalis* and 90 for *S. aureus* with the same methodology as mentioned above)

[Table/Fig-2] depicts the action of solutions against *E. feacalis* at different time intervals with the p-value <0.001, [Table/Fig-3-5] exhibits the multiple comparisons of all the three agents against *E. feacalis*. [Table/Fig-6] illustrates the action of solutions against *S.aureus* at different time intervals with the p-value <0.001, multiple comparisons between the three solutions against *S.aureus* is depicted in [Table/Fig-7-9].

E. faecalis						
Time		**n**	**Mean**	**Std. deviation**	**H**	**p-value**
One minute	6% NaOCl	10	1.250	0.019	26.28	<0.001 vhs
	Berberine	10	8.000	0.000		
	CHLO+CETRI	10	1.787	0.044		
Three minutes	6% NaOCl	10	0.000	0.000	27.46	<0.001 vhs
	Berberine	10	8.000	0.000		
	CHLO+CETRI	10	0.981	0.101		
Five minutes	6% NaOCl	10	0.000	0.000	29	<0.001 vhs
	Berberine	10	8.000	0.000		
	CHLO+CETRI	10	0.000	0.000		

[Table/Fig-2]: Evaluation of mean and standard deviation of the disinfecting solutions at different time intervals against *E. Faecalis*, level of significance <0.001.

RESULTS

The mean bacterial count of *E. feacalis* or *S.aureus* was found to be lesser after treating the cones with 6% sodium hypochlorite when compared with other disinfecting solutions for all time intervals tested. [Table/Fig-10,11].

Multiple comparisons					
Tukey HSD					
Time	Dependent variable	(I) group	(J) group	Mean difference (I-J)	Sig.
One minute	*E.faecalis*	6% NaOCl	Berberine	-6.750	0.000
			CHLO+CETRI	-0.537	0.000
		Berberine	CHLO+CETRI	6.213	0.000
Three minutes	*E.faecalis*	6% NaOCl	Berberine	-8.000	0.000
			CHLO+CETRI	-0.981	0.000
		Berberine	CHLO+CETRI	7.019	0.000

[Table/Fig-3]: Tukeys Honest Significance test formultiple comparisons against *E Faecalis*.

Enterococcus faecalis						
Group		n	Mean	Std. deviation	H	p-value
6% NaOCl	1 minute	10	1.2500	0.01913	27.67	<0.001 vhs
	3 minutes	10	0.0000	0.00000		
	5 minutes	10	0.0000	0.00000		
Berberine	1 minute	10	8.0000	0.00000		
	3 minutes	10	8.0000	0.00000		
	5 minutes	10	8.0000	0.00000		
CHLO+CETRI	1 minute	10	1.7869	0.04415	27.01	<0.001 vhs
	3 minutes	10	0.9810	0.10110		
	5 minutes	10	0.0000	0.00000		

[Table/Fig-4]: Depicts mean value that are significantly different from each other.

Multiple comparisons					
Tukey HSD					
Group	Dependent variable	(I) time	(J) time	Mean difference (I-J)	Sig.
6% NaOCl	*E. faecalis*	1 minute	3 minutes	1.25004	0.000
			5 minutes	1.25004	.0000
		3 minutes	5 minutes	0.00000	1.000
Beberine	*E. faecalis*	1 minute	3 minutes	0.00000	0.486
			5 minutes	0.00000	0.069
		3 minutes	5 minutes	0.00000	0.486
CHLO+CETRI	*E. faecalis*	1 minute	3 minutes	0.80589	0.000
			5 minutes	1.78685	0.000
		3 minutes	5 minutes	0.98096	0.000

[Table/Fig-5]: Depicts comparison within the time groups against *E. Faecalis*.

Staphylococcus aureus						
Time		n	Mean	Std. deviation	H	p-value
One minute	6% NaOCl	10	0.0000	0.00000	27.65	<0.001 vhs
	Berberine	10	4.6977	0.03560		
	CHLO+CETRI	10	0.0000	0.00000		
Three minutes	6% NaOCl	10	0.0000	0.00000	27.71	<0.001 vhs
	Berberine	10	4.5949	0.04056		
	CHLO+CETRI	10	0.0000	0.00000		
Five minutes	6% NaOCl	10	.0000	0.00000	27.61	<0.001 vhs
	Berberine	10	4.2897	0.04985		
	CHLO+CETRI	10	0.0000	0.00000		

[Table/Fig-6]: Evaluation of mean, standard deviation of the disinfecting solutions at different time intervals against *S. aureus*, Level of significance <0.001.

Chlorhexidine cetrimide combination was found to be the second most effective disinfecting solution while the herbal irrigant berberine was the least effective among the solutions tested [Table/Fig-10,11]. Berberine was unable to eliminate *E. feacalis* even after exposure time of 5 minutes, but proved effective against *S.aureus*. (*E. feacalis* and *S.aureus* group, statistically evaluated separately)

Multiplecomparisons					
Tukey HSD					
Time	Dependent variable	(I) group	(J) group	Mean difference (I-J)	Sig.
One minute	*S. aureus*	6% NaOCl	Berberine	-4.698	0.000
			CHLO+CETRI	0.000	1.000
		3% H_2O_2	CHLO+CETRI	4.698	0.000
Three minutes	*S. aureus*	6% NaOCl	Berberine	-4.595	0.000
			CHLO+CETRI	0.000	1.000
		3% H_2O_2	CHLO+CETRI	4.595	0.000
Five minutes	*S. aureus*	6% NaOCl	Berberine	-4.290	0.000
			CHLO+CETRI	0.000	1.000
		3% H_2O_2	CHLO+CETRI	4.290	0.000

[Table/Fig-7]: Tukeys honest significance test for multiple comparisons against *S. aureus*.

Staphylococcus aureus						
Group		n	Mean	Std. deviation	H	p-value
5.25% NaOCl	1 minute	10	0.0000	0.00000		
	3 minutes	10	0.0000	0.00000		
	5 minutes	10	0.0000	0.00000		
Beberine	1 minute	10	4.6977	0.03560	25.64	<0.001 vhs
	3 minutes	10	4.5949	0.04056		
	5 minutes	10	4.2897	0.04985		
CHLO+CETRI	1 minute	10	0.0000	0.00000		
	3 minutes	10	0.0000	0.00000		
	5 minutes	10	0.0000	0.00000		

[Table/Fig-8]: Depicts mean value that are significantly different from each other against *S. aureus*.

Multiple comparisons					
Tukey HSD					
Group	Dependent variable	(I) time	(J) time	Mean difference (I-J)	Sig.
Beberine	*S. aureus*	1 minute	3 minutes	0.10277	0.000
			5 minutes	0.40799	0.000
		3 minutes	5 minutes	0.30522	0.000

[Table/Fig-9]: Tukeys honest significance test against *S. aureus*.

Enterococcus faecalis			
	6% Sodium hypochlorite	Berberine	Chlorhexidine and cetrimide
1 minute	18	10^8	60
3 minutes	No growth	10^8	10
5 minutes	No growth	10^8	No growth

[Table/Fig-10]: Efficacy of different solutions against *E. faecalis*.

Staphylococcus aureus			
	6% Sodium hypochlorite	Berberine	Chlorhexidine and cetrimide
1 minute	No growth	5×10^4	No growth
3 minutes	No growth	4×10^4	No growth
5 minutes	No growth	2×10^4	No growth

[Table/Fig-11]: Efficacy of different solutions against *Staphylococcus aureus*.

- The mean bacterial count of *E. Faecalis* or *S.aureus* was found to be significantly lesser (p<0.001) after treating the cones with 6% sodium hypochlorite when compared with other disinfecting solutions for all time intervals tested. [Table/Fig-2-4].
- Chlorhexidine cetrimide combination was found to be the second most effective disinfecting solution while berberine extract was the least effective among the solutions tested. [Table/Fig-3,7].

- Berberine was significantly (p<0.001) inferior to both chlorhexidine and sodium hypochlorite in eliminating the microorganism [Table/Fig-2,7,9].

DISCUSSION

The causative role of microorganisms in the pathogenesis of pulp and periapcial diseases has been demonstrated time and again. The elimination of these organisms from infected root canal systems is a diverse and an intricate proceeding involving the use of complex instrumentation techniques, wide range of irrigating solutions and regimens along with judicious use of intracanal medicaments [12]. When endodontic treatment is performed under aseptic conditions and according to accepted clinical principles, the success rate is generally high [13]. Obturation is the final stage of the endodontic regimen, three dimensional hermetic seal is achieved by a sealer along with a core root-filling material. Gutta percha is the most universally used material for this purpose. Core-filling materials, such as GP and resilon cones are thermolabile and therefore ideally should be sterilised by the manufacturer. If not sterilised or if contaminated, they should be disinfected chairside through chemical procedures before their usage [14]. As their sterilisation is questionable, and they can be easily contaminated by handling [15]. Studies have shown that freshly opened packages of gutta percha cones were domiciling microorganisms, further contamination was seen with clinical use [16]. Chemical sterilisation is mandatory for effective sterilisation of gutta percha points [17].

In the present study, we artificially contaminated the cones. The rationale behind it was that *Staphylococcus* is found to be the most common microorganism present in freshly opened boxes, Pang et al., recovered 19.4% of *Staphylococcus* genus. Gomes et al., 5.5% *Staphylococcus* genus [5].

S. aureus is a facultative anaerobic coccus bacterium also known as "*golden staph*" and is found commonly in the saliva and skin. *S. aureus* is one of the most common microorganism contaminating gutta percha boxes after handling with gloves. Faulty handling of gutta percha cones during treatment or during evaluation of the fit of the master cone before final rinse may lead to the contamination of the gutta percha with *E. faecalis*, which is known for its matchless virulence factors and considered as a gold standard to represent the other possible microorganisms that may contaminate gutta percha cones. *Enterococcus faecalis* is an organism that is found to be the most resistant intra canal pathogen in failed root canals, it is known that this strain can survive in dentinal tubules for long periods even when there is severe nutrition depletion [18] *E. faecalis* concentration was seen to be about nine times more in re-infection cases than primary infections [19] *E. faecalis* has been the gold standard bacterium for endodontic microbiology research for sometime now and it is the most frequently found endodontic pathogen in re-infection cases with the prevalence score ranging from 30% to 90% of the cases [20], although contamination from *E. faecalis* may not be a realistic possibility during packing of boxes but a clinical scenario could infest the gutta percha cones with *E. faecalis.* In a study done by Senia et al., where they assessed the microbial contamination by turbidity method concluded that if clinicians are using bare hands to manipulate cones, there was contamination with microorganism, and there were no viable microorganism in newly opened boxes, so it enlightens on the fact that as clinicians we must be concerned not only with endogenous oral microbial flora, but with exogenous bacterial contamination as well [21]. Studies have reported that *Enterococcus faecalis* is the most common bacteria associated with post-treatment infection of the root canal system [19,20,22,23,24,25]. If *E. faecalis* is the villain, sodium hypochlorite is its demise. Sodium hypochlorite (NaOCl) is the recommended root canal irrigant and used by the majority of dentists because of its important properties of antimicrobial effect and tissue dissolution capacity with acceptable biologic compatibility [26] Gomes et al., found that 5.25% NaOCl is a competent agent for a rapid disinfection of gutta percha cones [5]. Sodium hypochlorite has been a gold standard irrigant and a top disinfective in Endodontics justifying its use for this study but could be responsible for serious complications which can result from inadvertent use due to its cytotoxic features. Most of the complications are the result of accidental extrusion of the solution from the apical foramen or accessory canals or perforations into the periapical area and periodontium. Being an acid harmful effects on the skin are seen if it escapes into tissue spaces [27], also the deposition of cuboidal crystal like structures on the gutta percha after disinfection with sodium hypochlorite is proven [2], Chlorhexidine after sodium hypochlorite is the second most commonly used irrigant. In fact most researchers suggesting alternative use of both for optimum disnfection [8]. In this study we used a combination of 2% chlorhexidine and 0.2% cetrimide, CHX has been widely used in periodontics and is known to kill vegetative bacteria by disrupting the membrane integrity and bringing about the precipitation of the cytoplasm. In endodontics, CHX is used as an irrigant because of its substantivity, sporicidal activity and antibacterial efficacy [5,28]. Cetrimide with combination of chlorhexidine is known to give a wider range of antibacterial effect [16,29]. Berberine which a more biocompatible and milder solution available from naturally occurring in a plant named beriberi been in used in Chinese medicine around 3000 BC. Studies have been initiated into possible use of berberine against methicillin-resistant *Staphylococcus aureus* infection. Berberine has been studied against endodontic biofilms by Qian Xie et al., [30] they found berberine to have comparable antibacterial efficacy when used along with sodium hypochlorite. Berberine in combination with chlorhexidine was found to effective against *E. faecalis* in a study done by Moussa NMA et al., [31] for the evaluation of efficacy of these three disinfecting solutions, the cones after being treated with the disinfecting solutions were cultured in thioglycollate media for seven days and it was sub-cultured in BHI agar using culture plate method. Earlier studies by Senia E et al., [21] showed that 1-minute immersion of gutta percha cones in 5.25% NaOCl eliminated microorganisms, whereas chlorhexidine was efficient only at five minutes. In our results we found sodium hypochlorite 6% to be the most effective solution for disinfection of gutta percha cones followed by a combination of chlorhexidine and cetrimide. Berberine was not effective against *E. faecalis* at any time interval tested. Whereas its action against staphylococcus aureus was mild but not enough to totally eliminate it. Berberine had exhibited a broad spectrum of antibiotic activity against streptococcus species in a study done by Peng L et al., [32].

On the other hand both sodium hypochlorite and chlorhexidine cetrimide were very effective against *S. aureus* at all-time intervals.

Against *E. faecalis*, sodium hypochlorite was effective at three minutes interval itself whereas chlorhexidine cetrimide was effective only after five minutes. Unlike our results, Cardoso CL et al., found that 2% CHX effectively disinfected gutta percha cones contaminated with *E. faecalis* after a 1-minute treatment [33]. Similarly, Gomes et al demonstrated that 1% and 2% CHX effectively eliminated *E. faecalis* and *C. albicans* in 15 seconds [5]. But in the present study, only two species of microorganisms were used. Therefore, the range of microorganisms tested and the antimicrobial agents were less than the previous studies. Valois CRA et al., observed aggressive deteriorative effects on gutta percha cone elasticity for 5.25% NaOCl at one minute [34]. Some of the other more biocompatible solutions tried are paracetic acid povidone iodine also showing various results. Despite the good performance of paracetic acid for the disinfection of gutta percha cones, Bounoure F et al., reported that repeated exposure to paracetic acid is toxic [35] because of the toxicity of various chemicals used presently for disinfection of gutta percha cones having a more biocompatible herbal disinfectant is the need of the hour. Even though berberine showed poor results against *E. faecalis*, it was significantly effective against staphylococcus genus at longer time duration. The aim of the present study was not successful even though we have a lot of hope with berberine

in future, extensive research with better delivery systems should yield better results. The effect of berberine as an antibacterial agent against candida in chronic diarrhoea cases has been very effective. Berberine has also been used in prevention of dental caries to some success in Chinese population [36,37].

It's a well-known fact that the delivery system and the concentration of the antibacterial agent at which it will effective is an important factor which can't be ignored.

LIMITATION

The concentration of berberine used, is not yet a determined amount, varying the concentration could have different results and also the duration.

CONCLUSION

Sodium hypochlorite still is the mainstay when it comes to disinfecting microorganism. More research with berberine should be encouraged along with possible combinations with other disinfectants, different concentrations and longer time durations. More biocompatible and less cytotoxic irrigant should be the future of chemical disinfection.

REFERENCES

[1] Vidotto APM, Kamchi JT, Bueno CE da S, Ribeiro MC, Bernardi SM. Contaminaçãobacteriana dos cones de guta-perchautilizadosnasclínicasodo ntológicas da Faculdade de Odontologia da PontifíciaUniversidadeCatólica de Campinas. Rev CiêncMédicas. 2012;15(1).

[2] Pang NS, Jung IY, Bae KS, Baek SH, Lee WC, Kum KY. Effects of short-term chemical disinfection of gutta-percha cones: identification of affected microbes and alterations in surface texture and physical properties. J Endod. 2007;33(5):594-8.

[3] Montgomery S. Chemical decontamination of gutta-percha cones with polyvinylpyrrolidone-iodine. Oral Surg Oral Med Oral Pathol. 1971;31(2):258-66.

[4] Namazikhah MS, Sullivan DM, Trnavsky GL. Gutta-percha: a look at the need for sterilization. J Calif Dent Assoc. 2000;28:427-32.

[5] Gomes BPF de A, Vianna ME, Matsumoto CU, Rossi V de P e S, Zaia AA, Ferraz CCR, et al. Disinfection of gutta-percha cones with chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2005;100(4):512-7.

[6] Dumani A, Yoldas O, SehnazIsci A, Köksal F, Kayar B, Polat E. Disinfection of artificially contaminated Resilon cones with chlorhexidine and sodium hypochlorite at different time exposures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2007;103:e82-5.

[7] Linke HA, Chohayeb AA. Effective surface sterilization of gutta-percha points. Oral Surg Oral Med Oral Pathol. 1983;55(1):73-7.

[8] Böttcher DE, Sehnem NT, Montagner F, FatturiParolo CC, Grecca FS. Evaluation of the Effect of Enterococcus faecalis Biofilm on the 2% Chlorhexidine Substantivity: An In Vitro Study. J Endod. 2015;41(8):1364-70.

[9] Murray MT. Chronic Candidiasis: Your Natural Guide to Healing with Diet, Vitamins, Minerals, Herbs, Exercise, and Other Natural Methods. Harmony; 2016 Dec 14.

[10] Van Eldik DA, Zilm PS, Rogers AH, Marin PD. Microbiological evaluation of endodontic files after cleaning and steam sterilization procedures. Aust Dent J. 2004;49(3):122-7.

[11] Keppel G, Wickens TD. Simultaneous comparisons and the control of type I errors. Des Anal Res Handb. 2004; 111-30.

[12] Mohammadi Z, Abbott PV. The properties and applications of chlorhexidine in endodontics. Int Endod J. 2009;42(4):288-302.

[13] Zamany A, Safavi K, Spångberg LSW. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2003;96(5):578-81.

[14] Seabra Pereira OL, Siqueira JF. Contamination of gutta-percha and Resilon cones taken directly from the manufacturer. Clin Oral Investig. 2010;14(3):327-30.

[15] da Motta PG, de Figueiredo CB, Maltos SM, Nicoli JR, RibeiroSobrinho AP, Maltos KL, et al. Efficacy of chemical sterilization and storage conditions of gutta-percha cones. Int Endod J. 2001;34(6):435-9.

[16] Portenier I, Waltimo T, Ørstavik D, Haapasalo M. Killing of Enterococcus faecalis by MTAD and chlorhexidinedigluconate with or without cetrimide in the presence or absence of dentine powder or BSA. J Endod. 2006;32(2):138-41.

[17] Senia ES, Marraro RV, Mitchell JL, Lewis AG, Thomas L. Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite. J Endod. 1975;1(4):136-40.

[18] Portenier I, Haapasalo H, Rye A, Waltimo T, Ørstavik D, Haapasalo M. Inactivation of root canal medicaments by dentine, hydroxylapatite and bovine serum albumin. Int Endod J. 2001;34(3):184-8.

[19] Rôças IN, Siqueira JF, Santos KRN. Association of Enterococcus faecalis with different forms of periradicular diseases. J Endod. 2004;30(5):315-20.

[20] Molander A, Reit C, Dahlén G, Kvist T. Microbiological status of root-filled teeth with apical periodontitis. Int Endod J. 1998;31(1):1-7.

[21] Steve Senia E, Marraro RV, Mitchell JL. Cold sterilization of gutta-percha cones with formocresol. J Am Dent Assoc 1939. 1977;94:887-90.

[22] Haapasalo M, Udnæs T, Endal U. Persistent, recurrent, and acquired infection of the root canal system post-treatment. Endod Top. 2003;6(1):29-56.

[23] Portenier I, Waltimo TMT, Haapasalo M. Enterococcus faecalis- the root canal survivor and 'star' in post-treatment disease. Endod Top. 2003 Nov 1;6(1):135-59.

[24] Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod. 2006;32(2):93-8.

[25] Sirén EK, Haapasalo MPP, Waltimo TMT, Ørstavik D. In vitro antibacterial effect of calcium hydroxide combined with chlorhexidine or iodine potassium iodide on Enterococcus faecalis. Eur J Oral Sci. 2004;112(4):326-31.

[26] Estrela C, Estrela CRA, Barbin EL, Spanó JCE, Marchesan MA, Pécora JD. Mechanism of action of sodium hypochlorite. Braz Dent J. 2002;13(2):113-7.

[27] Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod. 2001;27(7):452-5.

[28] Haapasalo M, Endal U, Zandi H, Coil JM. Eradication of endodontic infection by instrumentation and irrigation solutions. Endodontic Topics. 2005;10;77-102.

[29] Arias-Moliz MT, Ferrer-Luque CM, González-Rodríguez MP, Valderrama MJ, Baca P. Eradication of Enterococcus faecalis biofilms by cetrimide and chlorhexidine. J Endod. 2010;36(1):87-90.

[30] Xie Q, Johnson BR, Wenckus CS, Fayad MI, Wu CD. Efficacy of berberine, an antimicrobial plant alkaloid, as an endodontic irrigant against a mixed-culture biofilm in an in vitro tooth model. J Endod. 2012;38(8):1114-7.

[31] Moussa NMA, Xie Q, Begole E, Johnson B, Wenckus C, Wu C. The antimicrobial efficacy of Berberine versus sodium hypochlorite and chlorhexidine against E. faecalis. J Endod. 2010;36.

[32] Peng L, Kang S, Yin Z, Jia R, Song X, Li L, et al. Antibacterial activity and mechanism of berberine against Streptococcus agalactiae. Int J Clin Exp Pathol. 2015;8(5):5217-23.

[33] Cardoso CL, Redmerski R, Bittencourt N de LR, Kotaka CR. Effectiveness of different chemical agents in rapid decontamination of gutta-percha cones. Braz J Microbiol. 2000;31(1):67-71.

[34] Valois CRA, Silva LP, Azevedo RB. Structural effects of sodium hypochlorite solutions on gutta-percha cones: atomic force microscopy study. J Endod. 2005;31(10):749-51.

[35] Bounoure F, Fiquet H, Arnaud P. Comparison of hydrogen peroxide and peracetic acid as isolator sterilization agents in a hospital pharmacy. Am J Health-Syst Pharm AJHP Off J Am Soc Health-Syst Pharm. 2006;63(5):451-5.

[36] Namba T, Tsunezuka M, Hattori M. Dental caries prevention by traditional Chinese medicines. Part II. Potent antibacterial action of Magnoliae cortex extracts against Streptococcus mutans. Planta Med. 1982;44(2):100-6.

[37] Iwasa K, Nanba H, Lee DU, Kang SI. Structure-activity relationships of protoberberines having antimicrobial activity. Planta Med. 1998;64(8):748-51.

PARTICULARS OF CONTRIBUTORS:

1. Assistant Professor, Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences, Manipal, Karnataka, India.
2. Postgraduate, Department of Pedodontics, AJ Institute of Dental Sciences, Mangalore, Karnataka, India.
3. Assistant Professor, Department of Faculty of Dentistry, Melaka Manipal Medical College, Manipal, Karnataka, India.
4. Assistant Professor, Department of Conservative Dentistry and Endodontics, AJ Institute of Dental Sciences, Mangalore, Karnataka, India.
5. Postgraduate, Department of Pedodontics, AJ Institute of Dental Sciences, Mangalore, Karnataka, India.
6. Postgraduate, Department of Conservative Dentistry and Endodontics, Saveetha Dental College, Chennai, Tamil Nadu, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Namith Rai,
Assistant Professor, Department of Conservative Dentistry and Endodontics, Manipal College of Dental Sciences,
Manipal-576104, Karnataka, India.
E-mail: drnamithrai@gmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Jun 21, 2018**
Date of Peer Review: **Aug 02, 2018**
Date of Acceptance: **Sep 27, 2018**
Date of Publishing: **Jan 01, 2019**