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AN IN-VITRO COMPARISON OF EFFICACY OF 1 % PERACETIC ACID, 2% CHLORHEXIDINE GLUCONATE AND PANCHA TULSI IN DISINFECTION OF GUTTA-PERCHA CONES.

Dental Science

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ABSTRACT

Aim: Aim of this study was to compare the effectiveness of 1% Peracetic acid, 2% Chlorhexidine Gluconate and Pancha Tulsi solutions to disinfect gutta-percha cones.

Methodology: 110 gutta-percha cones of size 70 were used and divided depending upon the type of disinfecting agents utilized. Cones used in the study were contaminated with E. faecalis and then immersed in 1% Peracetic acid, 2% Chlorhexidine Gluconate and Pancha Tulsi for 1 and 5 minutes. For analysing surface topography, cones were immersed in disinfecting solutions and observed under scanning electron microscope.

Results: 1% Peracetic acid showed best results for both 1 and 5 minutes' disinfection and there was statistically significant difference ($p<0.01$) between the test groups for 1 minute. Scanning Electron Microscope analysis showed surface changes in all the groups.

Conclusion: All the solutions tested were effective in disinfection of the cones. However, 1% Peracetic acid showed rapid disinfection potential when compared with the above test groups.

KEYWORDS

gutta-percha, 1% Peracetic acid, 2% Chlorhexidine Gluconate, Pancha Tulsi.

INTRODUCTION

Disinfection of root canal system is an important aspect of successful endodontic treatment. This can be achieved by following aseptic chain from the beginning of access opening, during cleaning and shaping and obturation of the root canal system. Various materials such as gutta-percha and resilon are used for obturation of the root canals. Although gutta-percha cones are manufactured under aseptic conditions and have potential antimicrobial properties especially owing to zinc oxide component[1], they can be contaminated by handling, by aerosols and by physical sources during storage process[2]. Hence, there is a need to disinfect the obturating material before introducing them into the root canal[3].

As gutta-percha cones are thermoplastic, conventional heating methods such as moist and dry heat cannot be used. Therefore, cold sterilization is the only means for sterilization.

Various studies have shown efficacy of different chemical and herbal disinfectants in eliminating microbes from the root canal system. Studies have shown the effectiveness of 5.25% sodium hypochlorite in eliminating most gram-positive, gram-negative and spore forming microorganisms[3]. However, in all concentrations, crystal deposition on the surface of gutta-percha cones is reported, hampering the bond of the sealers with gutta-percha cones, leading to micro leakage[4].

2% Chlorhexidine Gluconate is a routinely used irrigation solution due to its broad antimicrobial spectrum and substantivity. A study observed that 2% Chlorhexidine Gluconate solution was effective in decontaminating gutta-percha after 10 minutes of exposure[5].

The effectiveness of a disinfectant depends on adequate length of treatment time and adequate concentration of the disinfectant.

Peracetic acid since its introduction in the market is a powerful oxidant against microbes like bacteria, fungi, viruses and spores. Peracetic acid ($C_2H_4O_3$) is a combination of acetic acid (CH_3COOH) and hydrogen peroxide (H_2O_2) in a watery solution. When peracetic acid dissolves in water, it breaks up to hydrogen peroxide and acetic acid, which will fall apart to water, oxygen and carbon dioxide. One percent of peracetic acid has been evaluated for calcium hydroxide removal from the root canals[6].

Pancha Tulsi is an herbal disinfectant effective against different bacterial & viral infections. The popularity of herbal medications has increased due to the search for cheaper, more accessible and natural form of alternatives[4]. However, details regarding their application as disinfection agents in endodontic practice is inadequate.

The physical changes that occur in the cones after chemical sterilization have been reported. 5.25% sodium hypochlorite, being a strong oxidizing agent, is able to cause extreme topographic alterations in the cones, which results in aggressive deterioration. However, 2% Chlorhexidine Gluconate did not change gutta-percha cone properties following exposure for up to 30 minutes, suggesting that this substance is less detrimental to the structure of gutta-percha[7]. A recent study observed that disinfection with 1% Peracetic acid showed no alterations in the surface topography of the gutta-percha cones when treated for 1 and 5 minutes respectively[8].

However, there are no recent studies being done regarding the surface changes in gutta-percha cones after disinfection with Pancha Tulsi. Also, there is no study which has compared these three disinfectants for gutta-percha disinfection.

Therefore, the objective of this study was to compare the effectiveness of 1% Peracetic acid, 2% Chlorhexidine Gluconate and Pancha Tulsi solutions to disinfect the gutta-percha cones contaminated by *Enterococcus faecalis*, after the immersing in these solutions for 1 and 5 minutes respectively.

Methodology

In the present study, one hundred and ten gutta-percha cones of size 70 (Dentsply, Maillefer) were used and divided into 3 different groups depending upon the type of disinfecting agents utilized.

The disinfecting solutions tested were 2% Chlorhexidine Gluconate (V Consept, Vishal Dentocare Pvt. Limited, Ahmedabad, Gujrat, India), 1% Peracetic acid (Leo Chemicals, Bengaluru, Karnataka, India) and Pancha Tulsi (Deltas Pharma, India).

The gutta-percha cones were taken from freshly opened boxes under sterile conditions, bent and damaged cones were discarded.

The gutta-percha cones were artificially contaminated with 30 ml of microbial suspension of *E. faecalis* (ATCC2912) of approximately 107 CFU/ml in Brain Heart Infusion (BHI) Broth for 30 minutes.

Fig. 1: Materials used

After contamination, the gutta-percha cones were immersed in respective disinfectant solutions for 1 minute and 5 minutes separately. The experimental groups were as follows:

Group 1A: 11 gutta-percha cones treated with 2% Chlorhexidine Gluconate for 1 minute.

Group 1B: 11 gutta-percha cones treated with 2% Chlorhexidine Gluconate for 5 minutes.

Group 2A: 11 gutta-percha cones treated with 1% Peracetic acid for 1 minute.

Group 2B: 11 gutta-percha cones treated with 1% Peracetic acid for 5 minutes.

Group 3A: 11 gutta-percha cones treated with Pancha Tulsi for 1 minute.

Group 3B: 11 gutta-percha cones treated with Pancha Tulsi for 5 minutes.

The positive control group consisted of 22 gutta-percha cones contaminated with *E. faecalis* and then immersed in sterile water for 1 and 5minutes respectively. The negative control group comprised of 22 uncontaminated gutta-percha cones also treated with respective disinfecting solutions.

After disinfection, the cones were dried using a sterile filter paper and inserted individually into sterile Eppendorf Microcentrifuge tubes containing Brain Heart Infusion (BHI) Broth and incubated at 37^0 Celsius for seven days. All the tubes were checked for turbidity, indicating for bacterial growth, by holding the samples to light at 24-hour intervals. The samples showing bacterial growth was further evaluated by comparing the turbidity of the test suspension with McFarland Standards Set (Himedia Laboratories).

The samples showing bacterial growth was analysed using one-way analysis of variance(ANOVA), followed by post-hoc-Bonferroni test to analyse the data.

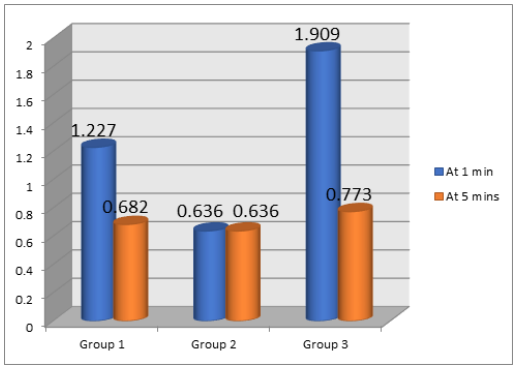
For evaluating the outcome of the different disinfectants on the gutta-percha cone surface, they were immersed in each of the disinfectant solutions for 1 and 5 minutes respectively, vacuum dried for 12 hours, gold coated for conduction and analysed by scanning electron microscope (B.A.R.C, Mumbai) for surface topography at 500X and 1000X magnification.

RESULTS

The positive control group showed heavy turbidity in all the samples and negative control group showed no growth. There was a statistically significant difference ($p<0.01$) between the test groups for 1 minute. (Table 1) (Graph 1) (Table 2).

Table 1 - Mean distribution of the samples based on turbidity score.

Groups	Sub-groups	N	Minimum	Maximum	Mean	Std. Deviation
Group 1	At 1 min	11	0.5	4.0	1.227	1.1697
	At 5 min	11	0.5	1.0	0.682	0.2523
Group 2	At 1 min	11	0.5	1.0	0.636	0.2335
	At 5 min	11	0.5	1.0	0.636	0.2335
Group 3	At 1 min	11	0.5	4.0	1.909	1.5463
	At 5 min	11	0.5	1.0	0.773	0.2611



Graph 1 - Mean distribution of the samples based on turbidity score.

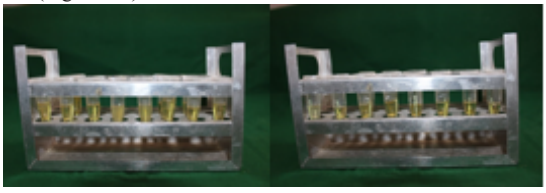
Table 2-Comparison of the mean number of bacterial colonies among the disinfecting solutions against E. faecalis for 1minute and 5 minutes using ANOVA and Post-hoc tests.

	F VALUE	p value
At 1 min	3.510	0.043
At 5 min	0.854	0.436

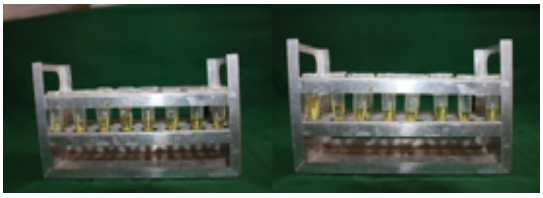
Dependent Variable	Group	Groups	Mean Difference	p value
At 1 min	Group 1	Group 2	0.5909	0.686
		Group 3	-0.6818	0.499
	Group 2	Group 3	-1.27	0.038*
At 5 min	Group 1	Group 2	0.0455	1.000
		Group 3	-0.0909	1.000
	Group 2	Group 3	-0.1364	0.628

*indicates statistically significant difference between the experimental groups.

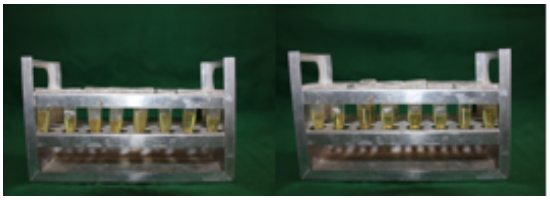
The samples disinfected with 2% Chlorhexidine Gluconate for 1 minute showed turbidity in 2 of the 11 tubes. None of the samples disinfected with 1% Peracetic acid showed turbidity for 1 and 5 minutes respectively. Among the experimental groups, cones treated with Pancha Tulsi for 1 minute showed turbidity in 4 of the 11 tubes. In this study 1% Peracetic acid showed best results for both 1 minute and 5minutes disinfection followed by 2 % Chlorhexidine Gluconate and Pancha Tulsi was least effective in disinfection among the solutions tested. (Fig.2a to h)



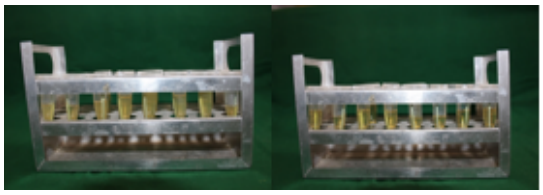
a) Group 1A **b) Group 1B**



c) Group 2A **d) Group 2B**



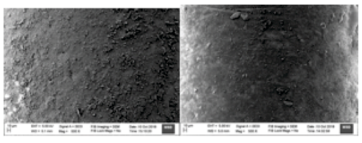
e) Group 3A f) Group 3B



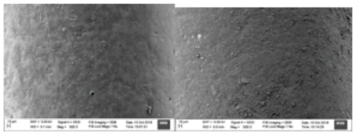
g) Positive control h) Negative control

Fig.2a to h: Gutta-percha cones immersed in different solutions.

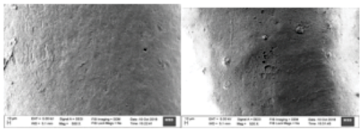
Scanning Electron Microscope analyses showed surface changes and irregularities of the cones after the immersion in 2% Chlorhexidine Gluconate and 1% Peracetic acid solutions. 2% Chlorhexidine Gluconate disinfection had shown surface deposits after 5 minutes. Gutta-percha cones disinfected with 1% Peracetic acid had granular crystals after 1 and 5 minutes respectively. Pancha Tulsi disinfection showed no surface deposits, though surface asymmetry was evident. 1% Peracetic acid showed increased amount of granular deposits after 1 and 5 minutes when compared with 2% Chlorhexidine Gluconate. (Fig.3a to l)



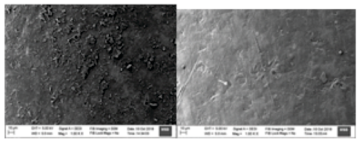
a) Group 1A b) Group 1B



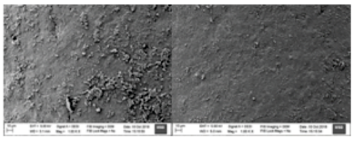
c) Group 2A d) Group 2B



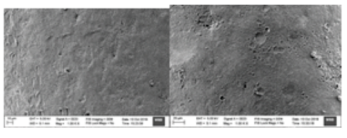
e) Group 3A f) Group 3B



g) Group 1A h) Group 1B



i) Group 2A j) Group 2B



k) Group 3A l) Group 3B

Fig.3a to l): Scanning electron microscope images of gutta-percha cones after immersion in different disinfectants at 500X magnification: a) Group 1A: 2% Chlorhexidine Gluconate for 1 minute. b) Group 1B: 2% Chlorhexidine Gluconate for 5 minutes. c) Group 2A: 1% Peracetic acid for 1 minute. d) Group 2B: 1% Peracetic acid for 5 minutes. e) Group 3A: Pancha Tulsi for 1 minute. f) Group 3B: Pancha Tulsi for 5 minutes. Scanning electron microscope images of gutta-percha points after immersion in different disinfectants at 1000X magnification: g) Group 1A: 2% Chlorhexidine Gluconate for 1 minute. h) Group 1B: 2% Chlorhexidine Gluconate for 5 minutes. i) Group 2A: 1% Peracetic acid for 1 minute. j) Group 2B: 1% Peracetic acid for 5 minutes. k) Group 3A: Pancha Tulsi for 1 minute. l) Group 3B: Pancha Tulsi for 5 minutes.

DISCUSSION

The presence and persistence of microorganisms in root canals is the main reason of failure in the endodontic treatment. Poor permanent restoration, inadequate cleaning and shaping, unsatisfactory filling of the canal as well as the use of contaminated materials for these procedures, could be a possible explanation for this problem[9,10]. The inefficiency to sterilise gutta-percha cones at higher temperatures leads to the need of chemical agents for disinfection. Also it should be an economical and rapid method which can be routinely used in dental clinics.

There is always a risk of contamination of the gutta-percha cones despite being produced under aseptic conditions and sold in sealed packages. Gutta-percha cones have been the ideal material of choice for obturation of the canals due to its characteristics such as biocompatibility, radiopacity, thermoplasticity and dimensional stability. The presence of zinc oxide in their composition might provide antimicrobial properties[11,12,13,14], but this action is uncertain[15]. The inefficiency to sterilise gutta-percha cones at higher temperatures leads to the need of chemical agents for disinfection.

Various chemicals such as zephiran, thimerosal, alcohol, povidone iodine, formaldehyde gas, $NaOCl$[3] and various herbal solutions such as Amla, Aloe-Vera juice, Neem[16] have been studied for the disinfection of gutta-percha cones.

In this study, efficacy of two chemical and one herbal disinfectant against *E. faecalis* was evaluated. *E. faecalis*, is a facultative gram-positive cocci shaped bacteria that were chosen as the test bacterial contaminant because it is one of the common micro-organisms found in infected root canals and it is the most common bacteria associated with the post-treatment infection of the root canal system[17,18].

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In our study 2% Chlorhexidine Gluconate was effective in eliminating *E. faecalis* from the gutta-percha cones after immersion for 5 minutes. Gomes et al. stated that 2% Chlorhexidine Gluconate liquid took <30 seconds to completely eliminate *E. faecalis* from contaminated gutta-percha cones[12].

Peracetic acid is utilised mainly in the food industry, where it is applied as a cleanser and as a disinfectant. Nowadays, peracetic acid is used for the disinfection of medical supplies and to prevent bio-film formation in pulp industries. It can be applied for the deactivation of a large variety of pathogenic micro-organisms including bacteria, fungi, viruses and spores. It releases free oxygen and hydroxyl radicals decomposing in oxygen, water, and acetic acid[19]. In a study, 2% Peracetic acid was found to be effective against the biofilms of microbes including *Bacillus subtilis* spores on gutta-percha cones at 1 minute of exposure time[20] whereas, in other study it was revealed that 1% Peracetic acid was effective against *E. faecalis* for both 5 and 10 minutes[8]. In the present study, 1% Peracetic acid was effective in disinfecting the cones at 1 and 5 minutes respectively.

Pancha Tulsi is a mixture of the liquid extract of five types of Tulsi (basil), namely Drudriha Tulsi, Rama Tulsi, Shama Tulsi, Babi Tulsi, and Tukshmiya Tulsi which are mixed in right proportions to attain the desired benefits.

Phytochemical analysis of Pancha Tulsi showed antimicrobial compounds like glycosides, tannins, alkaloids, anthraquinones, saponins, resins, polysaccharides, steroidal terpens, cardiac glycosides, steroidal ring and flavonoids[21].

The medicinal properties of Tulsi have been studied in hundreds of scientific studies inclusive of in-vitro, animal and human experiments. These studies reveal that Tulsi has a unique combination of actions that include: Antimicrobial (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, anthelmintic), chemo-preventive, radio-protective, anti-pyretic, anti-allergic, immunomodulatory, anti-coagulant activities[22]. Tulsi leaves have antibacterial agents mainly in the form of essential oils. The essential oils and biologically active compounds have antibacterial properties and are effective against gram-positive and gram-negative bacteria[23].

The therapeutic potential of the essential oils extracted from the fresh leaves of Tulsi has been found to be largely due to eugenol (major constituents of the essential oils), which is a phenolic compound (1-hydroxyl 2-methoxy-4-allylbenzene)[24]. Shenoi PR et al., evaluated the efficacy of three herbal gels in disinfecting gutta-percha cones. They concluded that all the gels showed inhibition zones nearly equal to 5.25% sodium hypochlorite[4]. In other study, Pancha Tulsi exhibited good disinfection action against *S. aureus* and *E. faecalis*, followed by Amla juice[25]. In the present study, Pancha Tulsi exhibited least disinfection when compared to the other test groups.

McFarland Standards are used to standardize the estimated number of bacteria in a liquid suspension by comparing the turbidity of the test suspension with that of the McFarland Standard. A McFarland Standard is a chemical solution of barium chloride and sulphuric acid; the reaction between these two chemicals results in the production of a fine precipitate, barium sulphate. In this study, McFarland Standard was used to compare the turbidity within the test samples.

Topographic alterations on the surface of gutta-percha cones showed the presence of granular deposits, crystal deposits and surface irregularities. In the previous studies effect of various disinfectants on the surface of the gutta-percha cones and the methods to eliminate them have been evaluated[2,8,26,27]. However, the literature lacks sufficient data regarding the action of Pancha Tulsi on the surface topography of the gutta-percha cones. According to the inference of the present study, no alterations on the surface of gutta-percha cones were observed after disinfection with Pancha Tulsi.

CONCLUSION

Within the limitations of the present study, all the tested solutions including chemical and herbal disinfectants showed disinfection action against *E faecalis*.

Among the tested groups, 1% Peracetic acid exhibited rapid disinfection of the gutta-percha cones, followed by 2% Chlorhexidine Gluconate and Pancha Tulsi showed least disinfection potential when compared with the above test groups at 1 minute immersion time. However, at 5 minutes' immersion time, all the tested solutions showed rapid disinfection potential.

Further studies should evaluate the effect of these disinfectants on changes in the physical and mechanical properties of the gutta-percha for clinical relevance.

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