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CHAIR SIDE DISINFECTION OF GUTTA PERCHA POINTS - AN *IN VITRO* COMPARATIVE STUDY BETWEEN A HERBAL ALTERNATIVE PROPOLIS EXTRACT WITH 3% SODIUM HYPOCHLORITE, 2% CHLORHEXIDINE AND 10% POVIDONE IODINE

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Abstract: The aim of this study was to compare the effectiveness of 30% propolis extract, 3% Sodium hypochlorite (NaOCl), 2% chlorhexidine gluconate (CHX), 10% povidone iodine and 0.9% saline solution to disinfect Guttapercha (GP) cones contaminated by *Enterococcus faecalis (E. faecalis)*. Fifty four size 80 GP cones were used. The cones were contaminated by *E faecalis*. GP cones were immersed in the disinfecting solutions for periods of 1 and 10 min. After the disinfection procedure, the cones were incubated in brain heart infusion and the presence of bacterial growth was analysed by turbidity of the medium. Result showed that that the propolis and the saline solution did not produce any bactericidal action, resulting in intense turbidity in all samples and in both time periods. In all samples for both time periods 3% NaOCl and 2% CHX demonstrated absence of the turbidity in the test tubes; indicating no bacterial growth. In Group4, all cones contaminated by *E. faecalis* showed bacterial growth after 1 min in povidone iodine and in 20% of the cones after the immersion for 10 min. According to the results, it can be concluded that the immersion of GP cones in a solution of 2% CHX and 3% NaOCl for 1 min is an efficient method to promote their disinfection.Propolis was not effective against clinical strain of *E. faecalis* when used as a GP disinfectant.

Key words: Propolis extract, Sodium hypochlorite, Chlorhexidine gluconate, *Enterococcus faecalis,* Guttapercha.

INTRODUCTION

One of the most important reasons of endodontic treatment failure is the persistence or survival of microorganisms in the complex root canal system or periapical area (1). Therefore, the maintenance of the disinfection obtained during the treatment is critical (2). Obturation is the final stage of endodontic treatments which promotes healing and prevents percolation or ingress of microorganisms into the periapical area (3). Guttapercha (GP) points are the most commonly used material for the obturation of the root canal system. Even though gutta-percha cones are produced under aseptic conditions, once exposed to the dental office environment or even by handling, they can be contaminated by variety of microorganisms (4). Gutta-percha cones cannot be sterilized by the conventional process in which moist or dry heat is used because this may cause alteration to the gutta-percha structure due to their thermoplastic characteristics (5). Therefore, a rapid chair side chemical disinfection is mandatory. Various chemical agents have been proposed as GP disinfectants, including sodium hypochlorite (NaOCl), Chlorhexidine (CHX), glutaraldehyde, alcohol, iodine compounds and hydrogen peroxide (6,7). Sodium hypochlorite (NaOCl) is one of the most widely used endodontic solution for GP disinfection.

The recommended method consists of treating the cones using a 1% Sodium hypochlorite for 1 minute (Milton's solution), or 0.5% Sodium hypochlorite for 5 minutes (Dakin's solution) (8). But Sodium hypochlorite produces crystal deposition within the canals and might causes the deterioration of GP points, including increased depth of surface irregularities and loss of elasticity which can impede the obturation4. Therefore the ideal disinfectant should be the one that can be used routinely in dental clinics, delivering a fast disinfection without modifying the structure of the cone6. Propolis (bee glue) is a by-product of honeybees having antibacterial, antiviral, and antifungal properties (9). Several studies reported the antimicrobial activity of propolis against *E. faecalis* (10, 11). It is well documented that propolis can be used for pulp capping, intracanal dressing, storage media, anti-inflammatory agent, periodontal applications and dentinal hypersensitivity (12-17). The medicinal values of propolis lie in their component phytochemicals such as flavonoids, aromatic acids, diterpenic acids and phenolic compounds which prevents bacterial cell division and breakdown the bacterial cell wall and cytoplasm, suggesting its potential to be used as a GP disinfectant (18). Hence the aim of this study is to compare the effectiveness of 30% propolis extract, 3% NaOCl, 2% chlorhexidine gluconate (CHX), 10% polyvinylpyrrolidone-iodine (povidone iodine, PVPI) and 0.9% saline solution to disinfect GP cones contaminated by Enterococcus faecalis (*E. faecalis*).

MATERIALS AND METHODS

In this study, 54 size 80 standardised GP cones (Dentsply, Petrópolis, RJ, Brazil) were used. Prior to the experiment, the cones were sterilized by ethylene oxide. Samples of *E. faecalis* were obtained clinically from an infected root canal and cultured in bile esculin agar medium. After that it was observed under the light microscope and type of bacteria was confirmed. GP cones were contaminated by immersion in 20 mL of a pure culture of *E. faecalis* that was inoculated in a brain heart infusion (BHI) broth. All samples were incubated at 37°C for 72 h. After the incubation period, the cones were dried using sterilise gauze and divided into four groups of 10 samples according to the chemical agent used.

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Study groups

Group A – 30% Propollis
Group B – 3% NaOCl
Group C – 2% Chlorhexidine
Group D – 10% Povidone Iodine
Group E – 0.9% Saline Solution

Preparation of Propolis extract

Seven gram of 96% ethanol was combined with 3 gram of Propolis and the mixture was filtered using Chromafil CA-20/25 filter paper for elimination of the impurities to obtain 30% ethanolic extract of propolis. Five GP cones were immersed for 1 min in one of the agents and other five were immersed for 10 min. The same procedure was repeated for all the groups. The positive control group comprised two cones contaminated by *E. faecalis*and the negative control was two cones that were kept sterilise after the initial sterilisation by ethylene oxide. The cones were once again dried and inserted individually into test tubes containing 20 mL of sterile BHI broth and incubated at 37°C for 72 h. Bacterial growth was evaluated by the presence of turbidity in the broth. The results were statistically analysed by Kruskal–Wallis test. Statistical significance level was established at $P < 0.05$.

Antimicrobial activity assay of disinfectants

The antibacterial activity of the disinfecting agents was tested using Agar well diffusion technique. The *E. faecalis* strains were cultured overnight in thioglycolate broth, and the culture was streaked on a plate of blood agar. Five wells of 5 mm × 5 mm measure were made with the help of a template on the surface of the agar plate. About 0.1 ml of each disinfecting agent was delivered into the corresponding well using a micropipette. They were then incubated at 37°C for 24 hours, and closely monitored for the development of clear zones around the extracts. The antibacterial activity was assessed by the diameter of the inhibition zone.

RESULTS

The comparison between the bactericidal activities of the chemical agents in disinfecting GP cones in this study is shown in Table 1. It was demonstrated that the propolis and the saline solution did not produce any bactericidal action, resulting in intense turbidity in all samples and in both time periods. In all samples for both time periods 3% NaOCl and 2% CHX demonstrated absence of the turbidity in the test tubes; indicating no bacterial growth. In Group4, all cones contaminated by *E. faecalis* showed bacterial growth after 1 min in povidone iodine and in 20% of the cones after the immersion for 10 min. The antimicrobial efficacy was assessed by the presence of zones of inhibition. The NaOCl, CHX, and Povidone Iodine showed 12 mm, 21 mm and 6 mm inhibition zones respectively. The propolis extract and saline did not produce any zones of inhibition.

Table 1: Bacterial growth (turbidity) between samples

Time (min)	Propolis (30%)	povidone iodine (10%)	3% NaOCl	2% CHX	Saline
1	+++++	+++++	-----	-----	+++++
10	+++++	---+-	-----	-----	+++++

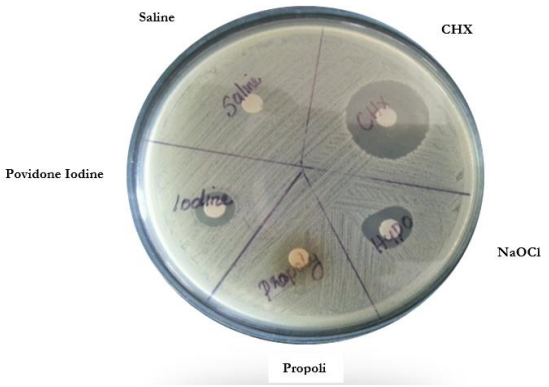

Figure 1: Antimicrobial activity assay of disinfectants (Disk diffusion test)

DISCUSSION

The important step during the endodontic treatment is sterilization of endodontic instruments and Materials. GP cones have been selected as the material of choice for root canal obturation because of properties such as biocompatibility, radio opacity, dimensionally stability, and antibacterial activity and are also easily removed from root canal (19). In endodontc therapy the natural contamination of the GP cones consists mainly of vegetative bacterial cells rather than resistant bacteria spores (20). Therefore the decontamination of gutta-percha cones can be accomplished with effective chemical agents. The result of the study showed that Chlorhexidine and NaOCl are equally effective in disinfection of gutta-percha cones for both 1 minute and 10 minute immersion. At the same time 2% Chlorhexidine has more antimicrobial efficacy than 3% NaOCl and 10% povidone iodine against clinical strain of E faecalis. The result of the present study is consistent with the result obtained by Gomes et al, who stated that 2% Chlorhexidine liquid took less than 30 seconds to completely eliminate *E. faecalis* from contaminated GP cones. CHX is a cationic bisbiguanide with broad antibacterial activity. The CHX molecule reacts with negatively charged groups on the bacterial cell surface, causing an irreversible loss of cytoplasmic constituents, membrane damage, and enzyme inhibition. In the study CHX at a concentration of 2% has been taken as a GP disinfectant as it has been demonstrated that the antibacterial efficacy of CHX depends on its concentration level and 2% CHX has a better antibacterial efficacy than 0.12% CHX in vitro. The disinfecting efficiency of NaOCl depends on the concentration of undissociated hypochlorous acid (HClO) in solution. HClO exerts its germicidal effect by an oxidative action on sulphydryl groups of bacterial enzymes (21-23).

Therefore NaOCl can be used effectively for the disinfection of gutta-percha cones. Several studies recommend the use of NaOCl for disinfecting GP cones. (24, 25) However, at very high concentrations (5.25%), NaOCl produces a large quantity of chloride crystals on the GP cone surface and might causes the deterioration and loss of elasticity of GP points, which could impede the obturation and impair the hermetic seal.(26) But lower concentrations will take more time to inhibit bacterial

growth than higher concentrations (11). Hence to obtain an optimal effect with minimum disadvantages, NaOCl has been taken at a concentration of 3% as a GP disinfectant in the present study. Iodine compounds are fast-acting and efficient bactericidal, fungicidal and sporicidal agents, where the molecular iodine is responsible for the antimicrobial activity. (27) The results of this study showed that the use of povidone iodine demonstrated an adequate disinfection of the GP cones contaminated by *E. faecalis* after 10 min of immersion. The result clearly indicated that Propolis was not effective against the clinical strain of *E. faecalis* when used as a GP disinfectant. The factor responsible for the ineffectiveness of propolis solution may be their low ph values. Mc Hugh et al reported that growth of *E. faecalis* is retarded only at a ph of 10-11 and it is destroyed above ph 11.5. (28) Another possible reason may be Propolis of different origins have different compositions and antimicrobial activities, requires standardization. Location, season, and vegetation of the area from which Propolis is collected influence its composition and biological activity. (29) In the current study clinical strain of *E. faecalis* from an infected root canal is used against propolis. Clinical strains are normally more virulent than standard strains. This might be another reason.

CONCLUSION

According to the results, it can be concluded that the immersion of GP cones in a solution of 2% CHX and 3% NaOCl for 1 min is an efficient method to promote their disinfection. The use of 10% povidone iodine required 10 min to provide an effective action and the use of Propolis and 0.9% saline solution produced no action to disinfect GP cones.

REFERENCES

1. Haapasalo M, Endal U, Zandi H, Coil JM. "Eradication of endodontic infection by instrumentation and irrigation solutions." *Endod Top* 10 (2005): 77–102.

2. Schirrmeister JF, Liebenow AL, Braun G, Wittmer A, Hellwig E, Al-Ahmad A. "Detection and eradication of microorganisms in root-filled teeth associated with periradicular lesions: an in vivo study. " *J Endod* 33 (2007) 33: 536–40.

3. Siqueira JF Jr., da Silva CH, Cerqueira M das D, Lopes HP,de Uzeda M. "Effectiveness of four chemical solutions in eliminating Bacillus subtilis spores on gutta-percha cones." *Endod Dent Traumatol* 14 (1998): 124–6.

4. Short RD, Dorn SO, Kuttler S. "The crystallization of sodium hypochlorite on gutta-percha cones after the rapid-sterilization technique: an SEM study." *J Endod* 29 (2003): 670–4.

5. Senia ES, Macarro RV, Mitchell JL, Lewis AG, Thomas L. "Rapid sterilization of gutta-percha cones with 5.25% sodium hypochlorite." *J Endod* 1 (1975): 136-40.

6. Cleber K. Nabeshima, DDS; Manoel Eduardo de Lima Machado; Maria Leticia Borges Britto and Raul Capp Pallotta. "Effectiveness of different chemical agents for disinfection of gutta-percha conesaej_25Aust" *Endod J* 37 (2011): 118–1216

7. Özalp N, Ökte Z, Özcelik B. "The rapid sterilization of gutta-percha cones with sodium hypochlorite and glutaraldehyde." *J Endod* 32 (2006): 1202–4.

8. Cardoso CL, Kotaka CR, Redmerski R, Guilhermetti M, Queiroz AF. "Rapid decontamination of gutta percha cones with sodium hypochlorite." *J Endod 25 (*2009): 498–501

9. Kujumgiev A, Tsvetkova I, Serkedjieva Y, Bankova V, Christov R, Popov S. "Antibacterial, antifungal and antiviral activity of propolis of different geographic origin." *J Ethnopharmacol.* 64.3 (1999): 235-40.

10. Oncag O, Cogulu D, Uzel A, Sorkun K. "Evaluation of in vitro antimicrobial activity of various propolis samples against Enterococcus faecalis." *Balkan Journal of Stomatology* 10.2 (2006): 122-5.

11. Stepanovic S, Antic N, Dakic I, Svabic-Vlahovic M. "In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs." *Microbiological Research 158.4* (2003): 353-7.

12. Bretz WA, Chiego DJ, Marcucci MC, Cunha I, Custódio A, Schneider LG. "Preliminary report on the effects of propolis on wound healing in the dental pulp." *Z Naturforsch* 53 (1998): 1045-8.

13. Gafar M, Sacalus A, David E, David N. "Tratamentul gangrenei pulpare simple cu produsul apiterapic "propolis." Stomatologia 33 (1986): 115-7.

14. Martin MP, Pileggi R. "A quantitative analysis of propolis: a promising new storage media following avulsion." *Dent Traumatol* 20 (2004): 85-9.

15. Paulino N, Teixeira C, Martins R, Scremin A, Dirsch VM,Vollmar AM, et al. "Evaluation of the analgesic and anti-inflammatory effects of a Brazilian green propolis." *Planta Med* 72 (2006): 899-906.

16. Santos FA, Bastos EM, Rodrigues PH, de Uzeda M, de Carvalho MA, de Macedo Farias L, Andrade Moreira ES. "Susceptibility of Prevotella intermedia/Prevotella nigrescens (and Porphyromonas gingivalis) to propolis (bee glue) and other antimicrobial agents." *Anaerobe* 8 (2002): 9-15.

17. Almas K, Mahmoud A, Dahlan A. "A comparative study of propolis and saline application on human dentin A SEM study." *Indian J Dent Res* 12 (2001): 21-7.

18. Sibel Silici, Kutluca semiramis. "Chemical composition and antibacterial activity of propolis collected by three different races of honey bees in the same region." *J Ethnopharmacol* 99 (2005): 69-73.

19. Siqueira JF Jr., da Silva CH, Cerqueira M das D, Lopes HP, de Uzeda M. "Effectiveness of four chemical solutions in eliminating Bacillus subtilis spores on gutta-percha cones." *Endod Dent Traumatol* 14 (1998): 124–6.

20. Frank RJ, Pelleu GB. "Gluraraldehyde decontamination of gutta percha cones." *J Endod* 9 (1983): 368-70.

21. Gomes BPFA, Vianna ME, Matsumoto CU et al. "Disinfections of gutta-percha cones with chlorhexidine and sodium hypoclorite." *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 100 (2005): 512–7.

22. Basrani B, Tjaderhane L, Santos JM, Pascon E, Grad H, Lawrence HP, Friedman S. "Efficacy of chlorhexidine- and calcium hydroxide-containing medicaments against Enterococcus faecalis in vitro." *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 96 (2003): 618–624.

23. Dychdala GR. "Chlorine and chlorine compounds. In: Block SS,ed." *Disinfection, Sterilization, and Preservation* 4 (1991): 133–5.

24. Cardoso CL, Kotaka CR, Redmerski R, Guilhermetti M, Queiroz AF. "Rapid descontamination of gutta-percha cones with sodium hypochlorite." *J Endod* 25 (1999): 498–501.

25. Motta PG, Figueiredo CBO, Maltos SMM *et al.,* "Efficacy of chemical sterilization and storage conditions of gutta percha cones." *Int Endod J* 34 (2001): 435–9.

26. Valois CRA, Silva LP, Azevedo RB. "Effects of 2% Chlorhexidine and 5.25% sodium hypochlorite on gutta-percha cones studied by atomic force microscopy." *Int Endod J* 38 (2005): 425–9.

27. Haapasalo M, Endal U, Zandi H, Coil JM. "Eradication of endodontic infection by instrumentation and irrigation solutions." *Endod Top* 10 (2005): 77–102.

28. MCHugh C P,Zhang P,Michaelek S,Elaezer P "D.PH required to kill *E. faecalis*in vitro." *J Endond* 30 (2004): 218.

29. Bankova V. "Chemical diversity of propolis and the problem of standardization." *J Ethnopharmacol* 100.2 (2005): 114-7.

30. Marcucci MC. "Propolis: chemical composition, biological properties and therapeutic activity." Apidologie. 26.2 (1995): 83-99.

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