



## RESEARCH ARTICLE

### EVALUATION OF EFFICACY OF DISINFECTING AGENTS FOR GUTTA PERCHA CONES: HERBAL VERSES CHEMICAL –AN IN VITRO STUDY

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#### ABSTRACT

The major goals of root canal therapy are the complete elimination of microorganisms from the root canal system and the prevention of subsequent reinfection. In clinical practice the dentist is occasionally come across with a problem of infection that occurs after obturation of root canal space. Guttapercha is the most common obturating material. Unlike most instruments used in endodontic treatment, guttapercha cones cannot be sterilized by conventional autoclaving or in a hot-air oven, therefore, they require rapid decontamination before use to maintain the aseptic condition required for successful endodontic therapy. Various chemical agents have been proposed as GP cones disinfectant, including sodium hypochlorite, glutaraldehyde, alcohol, iodine compounds and hydrogen peroxide. The objective of this study is to evaluate the efficacy of a herbal alternative, *Aloe vera* and Amla for rapid decontamination of guttapercha cones.

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## INTRODUCTION

A successful root canal therapy needs a considerable effort for removal of microorganisms from root canal system and a proper aseptic condition while performing the treatment to prevent any kind of external cross contamination from the instruments or filling material. Root canal obturation is the final and one of the most critical step in root canal therapy which determines the future success of the treatment. Gutta percha cones are the most widely used obturating material since many a years. Gutta-percha points are usually sterilized during the manufacturing process but once their packages are open they can be contaminated while handling them with gloves in hand or coming in contact with microorganisms of oral mucosa (Linke and Chohayeb, 1983; Peciulienė et al., 2001). They can also be contaminated by aerosols and different physical sources during the storage process. Gutta-percha cones cannot be sterilized by the conventional process because of their thermoplastic properties in which moist or dry heat is used because this may cause alteration to the gutta-percha structure. Therefore, rapid chairside disinfection is needed (Frank and Pelleu, 1983; Senia et al., 1975). However, it is suggested that as gutta percha is heat labile, hence chemical disinfectants like Ethyl alcohol, Paraformaldehyde, Formocresol Glutaraldehyde, Chlorhexidine and Sodium Hypochlorite are routinely used, which usually take 3-25 minutes for disinfection. Amongst all the various methods of rapid chair side disinfection, Sodium

Hypochlorite 5.25% is most effective in 1 minute (Retamozo et al., 2010; Senia et al., 1975). The appropriate disinfecting agent should be effective in killing different bacterial species. The objective of this study is to evaluate the effectiveness of a herbal alternative, *Aloe vera*, Neem and Amla for rapid decontamination of guttapercha cones. *Aloe barbadensis* Mill has strong Antibacterial, Antifungal and Antiviral properties (Ramasubramanian et al., 2010; Arunkumar and Muthuselvan, 2009). The antimicrobial effects have been attributed to its natural anthraquinones component (Prakash P Athiban et al., 2012). Neem has an established antibacterial, antifungal and antiviral effect (Fariha Natasha et al., 2015). Neem have proven anti-inflammatory, antiseptic, astringent and analgesic properties (Sheila et al.,). So, it can be very effective in decontaminating gutta percha cones. Amla has antibacterial and astringent properties that help to prevent infection which makes it a desired disinfecting agent for gutta-percha cones disinfection (Singh et al., 2011). Therefore the objective of the present study was to evaluate the antimicrobial properties of three commonly used chemical agents namely 5% sodium hypochlorite, 2% chlorhexidine and 2% glutaraldehyde and three herbal agents namely Amla, *Aloe vera* and Neem, against the most commonly isolated micro-organism from the root canal i.e. *E. faecalis*.

## MATERIALS AND METHODS

In the present study, sixty four new Gutta-percha (Dentsply Maillefer, USA) cones were randomly chosen. These were broadly divided into 2 major groups of 32 gutta percha cones.

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The Group I having 32 gutta percha were not contaminated or infected by *E. Faecalis* whereas the Group II of 32 gutta percha cones were contaminated by *E.faecalis* for 1 minute.

GROUP I (32 Gutta Percha cones which were not contaminated by *E.Faecalis*)

- GROUP I(A)- Amla
- GROUP I(B)- *Aloe vera*
- GROUP I(C)- Neem
- GROUP I(D)- Sodium Hypochlorite
- GROUP I(E)- Chlorhexidine
- GROUP I(F)- Gluteraldehyde
- GROUP I(G)- Control
- GROUP I(H)- Saline

GROUP II (Gutta Percha cones which were contaminated by *E.Faecalis*)

- GROUP II(A)- Amla
- GROUP II(B)- *Aloe vera*
- GROUPII(C)- Neem
- GROUP II(D)- Sodium Hypochlorite
- GROUP II(E)- Chlorhexidine
- GROUP II(F)- Gluteraldehyde
- GROUP II(G)- Control
- GROUP II(H)- Saline

These 32 gutta percha were further subdivided into 8 subgroups comprising of 4 gutta percha each. In Group I, 8 test tubes were taken in which brain heart infusion broth was kept. For each test tube, 4 gutta percha were taken out from the sterile closed pack followed by decontamination by amla, aleovera, neem, 3% sodium hypochlorite, 2% chlorhexidine, 2% gluteraldehyde and saline respectively for 1 minute and then placed into the test tube containing brain heart infusion broth. Whereas in the control group, 4 gutta percha were directly taken out of pack and placed inside one of the test tube containing broth and the last one is not decontaminated by any of the agents. In Group II, remaining 32 gutta percha cones were further subdivided into 8 subgroups. For each test tube, 4 gutta percha were taken out from the sterile pack and firstly contaminated with *E.faecalis* for 1 minute, are taken and decontaminated by amla, aleovera, neem, 3% sodium hypochlorite, 2% chlorhexidine, 2% gluteraldehyde and saline respectively for 1 minute and then placed into the test tube containing brain heart infusion broth. In the control group, 4 contaminated guttapercha were placed inside one of the test tube containing broth without decontaminating. All these guttapercha cones placed inside the test tube are then incubated at 37°C for 24 hours. After 24 hrs, these test tubes are taken out and the solution is checked by presence or absence of turbidity indicates proper disinfection.

## RESULTS

The decontaminating efficacy was assessed by the occurrence of turbidity in the brain heart infusion broth. The GP cones which were not decontaminated and directly placed in the broth developed turbidity. The cones which were not contaminated by *E. Faecalis* and placed in the broth, in that only Control group and saline developed turbidity (Fig.1) whereas those disinfected with the chemical agents and herbal agents remained free of turbidity (Fig.2). When the guttapercha

cones were contaminated with *E.Faecalis*, in those; control group, saline, neem, *Aloe vera* developed turbidity whereas



Fig.1. After 24 hours of incubation turbidity is checked in the samples not contaminated with *E. faecalis*

Table 1. After 24 hours of incubation turbidity is checked in the samples not contaminated with *E faecalis*

Groups	Presence or absence of turbidity
Group1a - amla	-
Group1b - <i>Aloe vera</i>	-
Group1c - neem	-
Group1d - sodium hypochlorite	-
Group1e - chlorhexidine	-
Group1f - glutaraldehyde	-
Group1g - saline	+
Group1h - control	+

+ Indicates presence of turbidity

- Indicates absence of turbidity



Fig.2. After 24 hours of incubation turbidity is checked in the samples contaminated with *E.faecalis*

Table 2. After 24 hours of incubation turbidity is checked in the samples contaminated with *E.faecalis*

Groups	Presence or absence of turbidity
Group2a - amla	-
Group2b - <i>Aloe vera</i>	+
Group2c - neem	+
Group2d - sodium hypochlorite	-
Group2e - chlorhexidine	-
Group2f - glutaraldehyde	-
Group2g - saline	+
Group2h - control	+

+ Indicates presence of turbidity

- indicates absence of turbidity

amla, chlorhexidine, glutaraldehyde and sodium hypochlorite were free from turbidity. The result of the study showed that Chlorhexidine was effective in disinfection of gutta-percha cones for 1 minute followed by glutaraldehyde and sodium hypochlorite. Whereas Amla was the most effective herbal extract in disinfecting gutta-percha followed by *Aloe vera* and neem.

## DISCUSSION

The disinfection of gutta percha cones prior to its use as obturating material is important for a successful endodontic treatment. Although gutta-percha cones are produced under aseptic conditions, but they may get contaminated by aerosols, improper storage and physical handling. Gutta-percha cones can be easily contaminated by the different microorganisms by physical contact or aerosol and needs disinfection, despite the fact that some authors have said that there is no need to use disinfectant solutions on cones. Cones contaminated with well-defined microbial populations were sterilized in different antimicrobial solutions for comparison. The thermoplastic characteristic of gutta-percha cones prohibits sterilization by standard autoclave, which may cause deformation of cones. Therefore, the cones were sterilized with ethylene oxide. Several other chemical solutions have been proposed for a rapid decontamination of gutta-percha cones. The time window to apply these solutions for disinfection ranges from a few seconds to several minutes. Sodium hypochlorite has antibacterial and sporicidal activities related to the liberation of active chlorine (Bloomfield and Miler, 1979; Dychdala, 1991). Sodium hypochlorite is being used in several different concentrations for gutta percha disinfection. Gomes *et al.* (2005) found that the time required for sodium hypochlorite to eliminate the microorganisms was inversely proportional to its concentration. The sodium hypochlorite solution at 5.25%, for example, eliminated *C. albicans* and *E. faecalis* after 45 seconds of contact, while at 2.5%, ten minutes of contact was needed to eliminate these microorganisms. Therefore NaOCl can be used effectively for the disinfection of gutta-percha cones. Several studies recommend the use of NaOCl for disinfecting GP cones (Cardoso *et al.*, 1999; Motta *et al.*, 2001). 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 (Valois *et al.*, 2005). But lower concentrations will take more time to inhibit bacterial growth than higher concentrations (Stepanovic *et al.*, 2003). Chlorhexidine 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. Gomes *et al.*, who stated that 2% Chlorhexidine liquid took less than 30 seconds to completely eliminate *E. faecalis* from contaminated GP cones. A 2% CHX solution was found effective at 15 seconds to 2 hours in direct contact with infected gutta-percha cones (Murray *et al.*, 1994). Glutaraldehyde has been effectively used as chemosterilizer or high-level disinfectant (Borick, 1968). Aqueous 2% glutaraldehyde solutions display a broad spectrum of activity and rapid destruction rate against the majority of microorganisms. They are capable of killing vegetative bacteria in less than 1 min of exposure. Spores may be destroyed in 3 h or less (Lawrence *et al.*, 2009).

Recently herbal alternatives are also being explored as disinfectants for gutta percha cones. In this study, Amla, *Aloe vera* and Neem have been used as a herbal alternative for gutta-percha cones disinfection. *Aloe vera* has been used from past many years for the treatment of a multiple ailments ranging from peptic ulcers to its use in cosmetics. It has a well-established antimicrobial activity ascribed to compounds that are now specifically identified as p-coumaric acid, ascorbic acid, pyrocatechol and cinnamic acid (Athiban *et al.*, 2012). Another major advantage is that *Aloe vera* gel has been found to be effective in decontaminating GP cones within one minute. Rajshekharan *et al.* concluded that the leaf extracts of Neem exhibited significant antibacterial activity against all the test microorganisms. However, the inhibitory activities of the leaf extracts were both organism- and solvent-dependent. The leaf extracts limited the growth of both Gram-positive and Gram-negative bacterial species (Rajasekaran *et al.*, 2008). It has anti-adherence activity by altering bacterial adhesion and has been helpful in the prevention of colonization by microorganisms inside the root canals (Botelho *et al.*, 2008). For the evaluation of the efficacy of these disinfecting solutions, 32 Gutta Percha cones were taken (25 No. ISO standardized) 4 Gutta Percha cones were placed inside the freshly prepared brain heart infusion broth and incubated for 24 hours. Then four new gutta percha cones were decontaminated for 1 minute with the different disinfecting solutions. These cones were incubated for 24 hours in brain heart infusion broth. 32 gutta Percha cones were submerged in test organism (*E. Faecalis*) for ten minute these cones were then kept in different herbal and chemical agents for 1 minute and further placed inside the brain heart infusion broth and incubated at 37°C for 24 hours. As a control, Gutta Percha cones immersed in test organism were directly incubated in brain heart infusion broth. The broth were then checked for turbidity in both the cases. The result of the study showed that Chemical agents were effective in disinfection of gutta-percha cones for 1 minute as the samples were free from turbidity for 1 minute. Whereas Amla was the most effective herbal extract in disinfecting gutta percha cones.

## Conclusion

Within the limits of this study, it can be concluded that although Chemical Agents (Chlorhexidine and Glutaraldehyde, sodium hypochlorite) are efficient chemical disinfectants but Herbal agents also hold a promising future as a medium for disinfection, in which amla gave the best results within a short duration of time.

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