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To Evaluate the Antimicrobial Activity of Herbal Extracts and their Efficacy in Disinfecting Gutta Percha Cones Before Obturation- an in Vitro Study

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Abstract

Context: *Gutta percha are known to be contaminated during storage and intra operative manipulation. Chair side disinfection of these points is important and critical for success of endodontic therapy. Gutta percha being heat labile, chemical disinfection is the only viable alternative which takes longer time and may leave a residue.*

Herbal gels are known to have antimicrobial , antiseptic properties and were not explored till date as an alternative pre operative disinfective medicament for gutta percha points. This paper focuses on use of Herbal gels for disinfection of Gutta percha points.

Aim: *To evaluate Herbal extracts for their antimicrobial activity and their efficacy in disinfecting Gutta percha cones before obturation.*

Materials and Methods: *Concentrated extracts of Aloe vera, Neem bark and Turmeric were used to check for their antimicrobial efficacy using agar well diffusion method. Presence of inhibition zones were identified against three common gutta percha contaminants namely E-Coli, E-faecalis and Stap Aureus. Sodium hypochlorite(5.25%) and Saline (0.9%) were used as controls. Forty-five Gutta percha cones were*

contaminated by glove handling during root canal treatment and were cut into two equal halves by sterilized Gutta percha cutter. First half of all the samples were placed in the broth and incubated to ascertain and confirm that the gutta percha points are contaminated. The second part of each contaminated cone was then treated with the herbal gels for 3 minutes and then incubated in the broth. This was done to observe any inhibition in bacterial growth due to treatment with herbal gels.

Results:

1) Herbal gels are effective against E-coli, E-faecalis and Staph-aureus.

2) Gutta percha cones do get contaminated during storage and Root canal treatment due to glove handling.

3) Placing gutta percha points in herbal gels for 3 minutes effectively decontaminate them,with neem gel showing decontamination equivalent to sodium hypochlorite

Conclusion: *Herbal extracts which are known to be antiseptics in Naturopathy are indded effective as Gutta percha decontaminants. The Neem bark extract is as effective as 5.25% sodium hypochlorite within a minute and holds a promising future.*

Key words: *Aloe vera, disinfection, gutta percha cones, Neem bark, Turmeric.*

INTRODUCTION

For successful endodontic therapy considerable efforts should be done in removal of micro-organisms from the canal and prevention of their introduction in root canal.[1] **Gutta percha (Edwin Truman, 1847) is most frequently used obturating material as it is** is biocompatible, dimensionally stable, radio opaque thermoplastic and also possesses antimicrobial properties. Moorer and Genet et al[2] suggested disinfection of gutta percha cones before obturation as unnecessary due to presence of zinc owing to its anti-microbial properties. Higgins et al [3] reported that mostly gutta percha cones are directly used from their packages with no concern for their sterility.The risk of gutta percha cones contamination by glove handling and from other physical sources during storage also cannot be ruled out.[4,5]

Rapid chair side disinfection of GP cones is of utmost importance to maintain aseptic chain during root canal treatment. However, it is hampered as gutta percha is heat labile, hence chemical disinfectants like ethyl alcohol, paraformaldehyde, formocresol 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. Milton's solution (1%) and Dakin liquid (0.5%) are also used.[6] However in all the concentrations crystal deposition within the canals is reported hampering the bond of sealers with canal walls and leading to microlekage.[7]

Indiscriminate use of antimicrobial agents has led to development of many resistant microbial strains which forced scientist to search for new antimicrobial substances from herbs. Neem, Aloe vera and Turmeric are household names in this

part of the world and are routinely used as home remedies for common illness. Extensive scientific literature is also available supporting their antimicrobial, antiviral, antifungal, anti-diabetic and anti cancer, immune-modulatory and soothing properties, food industry, pharmaceutical industry, cosmetics and toiletries, is cost effective with no or little side effects. [8, 25, 26]

Since, we seem to be at crossroads in achieving disinfection of gutta percha cones (GP) before obturation with use of chemicals and heat. It was hypothesized that herbal gels of Neem, turmeric and Aloe vera which have known and proven antimicrobial properties may have a positive role to play in achieving disinfection of GP cones prior to obturation.

Therefore the objective of the present study was to evaluate the antimicrobial properties of Neem, turmeric and Aloe vera herbal gels against the commonly isolated root canal microorganisms like E. faecalis, E. coli and S. aureus. [9] Second part of the study was to verify whether the GP cones are indeed contaminated during storage and glove handling and lastly to verify if the above mentioned herbal gels effectively disinfect the GP cones within specific time.

MATERIALS AND METHODS

The study was carried out in two stages and described as such for convenience in presentation and understanding.

Stage I: Evaluation of antimicrobial properties of herbal gels.

In this stage, herbal extracts of aloevera, neem and turmeric were prepared by soxhlation method[9-12,23,24.] Later the effectiveness of these 3 herbal gels was ascertained by agar well diffusion method against 3 microorganisms S. Aureus, E. Coli and E.faecalis which are commonly encountered in oral cavity and it's contamination. The inoculums of these test organisms were incubated overnight on nutrient agar slope in a test tube to collect sufficient number of microbial colonies for the experiment.

To verify the antimicrobial properties of herbal gels against test organisms, six petri dishes were prepared by pouring 10%blood agar at 500^0 C with minimum thickness of 4mm in each plate. They were then divided into 3 groups (A,B,C) in which microbes of E faecalis, E coli and S aureus were lawn cultured respectively. Each group of microbes were grown in two petri dishes namely A1 and A2, B1 and B2, C1 and C2.

In first three petri dish (A1,B1,C1), 3 wells of 5mmx5mm were punched with the template and the wells were designated as A,N,T for Aloevera, Neem and Turmeric extracts respectively. A 0.5 ml of the extracts were placed in their respective wells. In the remaining 3 petri dishes (A2,B2,C2), 2 wells each were punched with the same template. Each well was then filled with 0.5ml of NaOCl (Na) positive control and Saline (Sa) negative control respectively. Similar procedure was carried out for 10 times so as to calculate the mean inhibition zones. All the plates were then incubated at 37^oC for 24hrs and antimicrobial activity assessed by noting inhibition zones around each well.

Stage II: To verify contamination of gutta percha cones.

45 GP cones (Dentsply) were selected from freshly opened pack and glove handled during ongoing endodontic procedure in PG Clinic to mimic the clinical situation. These cones were then collected in a sterile container and carried to Department of Microbiology to verify the contamination of gutta percha cone. The cones were then cut into 2 equal halves with a sterile GP cutter. First half of each cone was then placed in individual test tube containing Brain Heart infusion broth (High media) and incubated for 24 hrs. [9] Thus, 45 test tubes were monitored for the presence or absence of turbidity, to confirm the presence of bacterial colonies. In this study turbidity was observed in all the test tubes confirming that glove handling and storage may contaminate the GP cones.

Stage III: Verification of antimicrobial activity of herbal gels in decontamination of GP points

The second half of the contaminated GP cones were randomly divided into 3 groups containing 15 each as Group I,II and III.

The GP points were treated with herbal gels according to the herbs prescribed to individual group and kept immersed in it 3 min. Excess herbal gel from GP points was removed by wiping. Each cone was then placed in the tube and incubated for 24 hours and presence or absence of turbidity was noted.

RESULTS

1. **Evaluation of antimicrobial properties of herbal gels:**

The antimicrobial efficacy of herbal extracts were assessed by measuring inhibition zones in mm and their mean was compared with positive control using unpaired t-test STATA version 10.0 (Table 1).The p values obtained for aloevera, neem and turmeric were 0.0241(significant),1.00(non-significant) and 0.0075(highly significant) respectively.

2. **Asserting the contamination of GP cones:**

In current study, presence of turbidity indicated presence of microbes after placing contaminated gutta percha cones in BHI broth.

3. **Asserting the effectiveness of herbal gels to decontaminate GP cones:**

In our study, the efficacy of 3 herbal gels were measured by absence of turbidity and a categorical data obtained was compared (Table 1.2) within the stipulated time period of 3 minutes and analyzed by Chi Square test. Neem bark showed (100%) efficacy for decontamination of GP in 3 minutes, followed by Aloe vera (86.7%) and Turmeric (66.7%) with significant p value- 0.040. Thus Neem bark extract showed maximum efficacy in chair side decontamination of GP cones compared to other 2 herbal gels.

DISCUSSION

GP cones are consistently used for obturation for successful root canal treatment for several years, the only drawback being inability to disinfect them effectively before obturation.Though various chemicals and disinfecting agents are available, they are known to take considerable time to achieve disinfection ranging from 3-15 minutes. Glutaraldehyde has been effectively used as chemosterlizer for 6-12hrs. Sporicidin is effective

in decontaminating gutta percha cones after 5 minutes. Cidex 7 reduced the contamination level by 99.90% after 15 minutes whereas, aqueous 5.25% NaOCl sterilize cones in 1 minute.[14] However, studies stated that concentrated ethanol provides an intermediate level of disinfection and the surface requires to be submerged at least for 10 minutes[15]. 0.2%, 1% and 2% Chlorhexidine were not effective at the high disinfection level of GP cones contaminated with spores of Bacillus Subtilis even after 72 hrs of contact.[13]

Bloomfield et al [16-17] noticed that NaOCL has anti-bacterial and sporicidal activities related to the liberation of active chlorine. Chlorine may inhibit the germination and outgrowth of bacterial spores[18]. 5.25% NaOCL was effective after 10 minutes of contact with vegetative forms of Bacillus Subtilis. But Senia et al [6] observed that 5.25% Sodium Hypochlorite disinfects gutta percha cones contaminated with Bacillus Subtilis spore after 1 minute exposure. The anti-microbial activity of NaOCL was related to its concentration, that is higher concentration took less time to inhibit bacterial growth than lower concentrations.[19] 5.25% NaOCL took 15 sec to 1 minute to kill all micro-organisms whereas 0.5% took 30 minutes. Rico D et al [7] in their SEM study showed that GP cones immersed in 5.25% and 2.5% NaOCl revealed chloride crystal formation may interfere with bonding thus affecting the monobloc concept.

GP cones immersed for 1 minute in both 5.25% and 2.5% NaOCl and then rinsed with 96% ethyl alcohol or 70% isopropyl alcohol did not display any chloride crystal activity. These cuboidal crystals that are likely to be partial lysis products of some component of GP cone, may affect the apical sealing at the time of obturation.[7] Also, such GP cones has a tendency to converge and cluster in the centre of the petri dish probably due to the tensoactive properties in the solution. Therefore care has to be taken to submerge each cone in the solution during disinfection.[5] Nan-Shim Pang et al[20] studied the surface changes and physical properties of GP cones checked after chemical disinfection with 2 % Chlorhexidine,5.25% NaOCl and Chloraprep. These disinfectants significantly increased their elongation rate compared to fresh GP cones, especially in the Chloraprep.[20] Also1 minute treatment with 5.25% NaOCl increased the elasticity of GP cones.[21]

Herbal gels are commonly available in Indian market have shown dramatic results as antimicrobials, anti cancer, anti diabetic, immune-modulatory, respiratory diseases, liver disorders and cosmetics agents.[8,10,22] The popularity of Herbal medications has increased due to the search for cheaper, more accessible and natural form of alternatives. However, data is still lacking for their use in endodontic practice and sterilization of GP cones. Therefore, this study was taken up to analyze the anti microbial property of herbal gels, contamination of GP points by glove handling and lastly to verify the ability of the herbal gels to disinfect GP in a stipulated time period of 1, 3 and 5minutes.

In our study, freshly prepared extracts from Aloe vera, Neem and Turmeric which were t analyzed for their anti microbial property against E. coli, E.

faecalis and S. aureus.[9] All 3 herbal gels showed the antimicrobial efficacy against the 3 microbes by forming an effective inhibition zones which was almost equivalent to NaOCl(positive control). P Athiban et al[9] has also proved the antimicrobial efficacy of Aloe vera gel against the 3 microbes which also stated inhibition zones nearly equal to NaOCl.

The next part of the study was to confirm the contamination of GP. After intentional manipulation of cones by glove handling, it was found that all the cones were contaminated with Staphylococcus (100%), followed by Micrococcus (33.3%), Streptococcus (26.7%), Bacillus (20%) and Lactobacillus (13.3%). Thus even though GP cones are usually sterile during storage but can be easily contaminated when handled with gloves[13].Our study confirmed the findings of Brenda Paula et al[13] that the GP cones are indeed contaminated by glove handling during obturation as all of them showed presence of turbidity in BHI broth.

The last part of the study was to analyze the capacity of herbal gels to disinfect the GP cones and the time period needed for their effective rapid chair-side disinfection. All the 3 herbal gels were able to disinfect GP cones within the acceptable time needed for chair-side decontamination. Our microbial assays showed that Neem bark extract was 100% effective in 3 minutes. Aloe vera extract was 86.7% % effective in 3 minutes and Turmeric extract was 66.7% effective in decontamination of GP cones.(Graph 2.1)

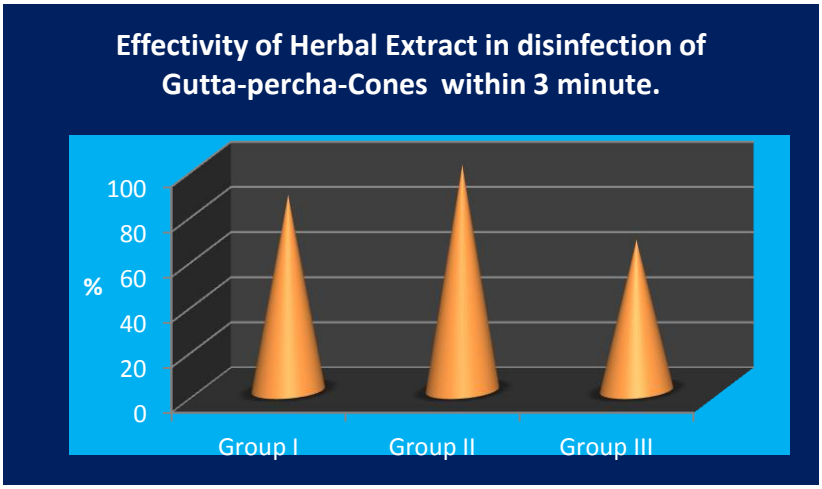
Within the limits of this study, it can be concluded that Herbal extracts are effective substitutes to the chemical disinfectants. Neem bark extract is as effective as 5.25% NaOCl for disinfection of GP cones in within 3 minutes. Thus Herbal extracts can hold a promising future in rapid decontamination of GP cones. The study is still in progress and the findings will be reported soon. The gel extract has been applied for patent.

(Table 1.1) Inhibition Zones on blood agar in mm

Test organism	Zones of inhibition on blood agar in mm				
	Aloe vera (n=10)	Neem bark (n=10)	Turmeric (n=10)	5.25%NaOCl (n=10)	Saline (0.9%) (n=10)
E. faecalis	21mm	23mm	19mm	22mm	0mm
E. coli	20mm	21mm	18mm	22mm	0mm
S. aureus	21mm	23mm	20mm	23mm	0mm
Mean	20.66	22.23	19.0	22.33	0
SD	0.57	1.15	1.0	0.58	0

(Table 1.2) Efficacy of herbal extracts in decontamination of gutta percha cones within specific time of 3 minutes (chi square test)

	Group I (n=15)	Group II(n=15)	Group II(n=15)	Chi2=6.428 p=0.040, Significant.
Absence of turbidity	13	15	10	
Effectivity in % percentage	86.7	100	66.7	



Graph 2.1 Effectivity of herbal extracts in disinfection of gutta percha cones within 3 minutes in percentage

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