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Original Article

Microbiology Section

Role of Herbal Agents - Tea Tree Oil and *Aloe vera* as Cavity Disinfectant Adjuncts in Minimally Invasive Dentistry- An In vivo Comparative Study

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ABSTRACT

Introduction: The prevention and control of caries necessitates the elimination of cariogenic bacteria and cavity disinfectants have proved to play a major role in achieving the goal. The use of phytotherapy is trending and many natural products have shown anti-microbial properties which can be used as cavity disinfectant in the field of dentistry.

Aim: The aim of this study was to compare the efficacy of herbal antibacterial agents (Tea Tree Oil (TTO) and *Aloe vera*) with commercially available 2% chlorhexidine (CHX) as cavity disinfectant for use in minimally invasive dentistry.

Materials and Methods: The study included three test groups, Group I (2% chlorhexidine), Group II (tea tree oil), Group III (*Aloe vera* gel) with a control group (distilled water). Ten patients with atleast one tooth with an occlusal or occluso-proximal lesion suitable for Atraumatic Restorative Treatment (ART) were selected for each group, dentinal samples were collected using sterile spoon excavators at three stages from each tooth viz., pre-excavation, post-excavation and post-disinfection of the cavities. These dentinal samples were subjected to microbiological analysis for Total Viable Count (TVC). The data collected were statistically analysed using ANOVA and Bonferroni post-hoc test.

Results: The results of present study showed that there was a statistically significant reduction in TVC when compared between pre and post excavation in all the groups ($p<0.05$) and post- excavation and post-disinfection in all the test groups ($p<0.05$) (except control group). Post-disinfection, 2% chlorhexidine showed highest reduction in TVC followed by 1% tea tree oil and aloe vera gel.

Conclusion: Natural antibacterial agents like tea tree oil and aloe vera could be effectively used as cavity disinfectants which will help in minimizing secondary caries and rendering a long term restorative success.

Keywords: Atraumatic restorative treatment, Dental caries, Phytotherapy, Phytotherapeutics

INTRODUCTION

Dental caries remains to be a major oral health problem afflicting people, young and old, especially in developing and underdeveloped countries [1]. The prevention and control of caries necessitates the elimination of cariogenic bacteria that produce acids responsible for the decrease of pH and starting the process of demineralization [2].

Modern dentistry shuns the principle of "extension for prevention" and professes minimum tooth tissue loss, minimum patient discomfort and maximizing micro-organism removal from the carious defect. ART, a minimally invasive treatment modality has gained tremendous popularity as only hand instruments are used to excavate soft, demineralized dental tissue followed by placement of adhesive restorative material that creates an environment for arresting caries progression with minimum invasive intervention [3]. However, limitations such as difficulty in accessibility and operator's fatigue can lead to incomplete excavation [4-6] and thus may cause secondary caries and failure of the restoration. Thus, the concept of cavity disinfection using antibacterial agents came into play. Chlorhexidine is one such agent which has been studied extensively and was found to reduce residual bacteria after cavity excavation. It is the most efficient chemotherapeutic agents against *S. mutans* and dental caries.

Due to indiscriminate use of antimicrobials more and more pathogens are becoming resistant and posing a serious threat in rendering successful treatment of the diseases [7,8]. With the rise in bacterial resistance to various synthetic antimicrobial agents, there is a considerable interest and a growing trend in the field of phytotherapeutics. Phytotherapy is the medicinal use of plant extracts. In this quest many natural products such as garlic extract, cinnamon oil, thyme oil, tea tree oil, *Aloe vera* etc., have shown anti microbial properties [9-13].

Literature search revealed limited studies evaluating the efficacy of these antimicrobial natural products as cavity disinfectants. Thus, the aim of our study was to evaluate and compare the efficacy of herbal antibacterial agents, TTO and *Aloe vera* gel as cavity disinfectant with 2% CHX.

MATERIALS AND METHODS

The in vivo study was conducted in the Department of Conservative Dentistry and Endodontics in Kalinga Institute of Dental Sciences, Bhubaneswar, Odisha, India, during August 2016 and December 2016. Patients reporting to the department suitable for ART, irrespective of sex, age, race and socio-economic status were included in this study.

Inclusion criteria consisted of occlusal or occluso-proximal lesion suitable for ART such as deep dentinal lesions without involving pulp as diagnosed on radiograph, without pain, abscess, swelling or adjacent soft tissue lesions.

Forty patients were divided into four groups consisting of ten patients each. The groups were as described in the [Table/Fig-1].

Chlorhexidine Gel

Commercially available 2% chlorhexidine gel (Consepsis, Ultradent) in syringe form was used in this study.

Groups	Test agents
I	2% Chlorhexidiene
II	1% Tea tree oil
III	*Aloe vera* gel
IV	Distilled water (control)

[Table/Fig-1]: Groups and test agents used.

Preparation of 1% Teatree Oil

For preparing 1% TTO, 1 ml of commercially available pure TTO (Mother Herbs Private Limited, New Delhi) was mixed with 0.5% polyoxyethylene sorbitan monolaurate-Tween 80, an emulsifying agent (with no antimicrobial activity) and 1 ml of distilled water. After mixing it well, distilled water was again added to make it a solution of 100 ml. As TTO is sparingly soluble in water, an emulsifying agent was added to enhance its solubility [1]. This preparation was stored in sterile syringes for easy application.

Preparation of *Aloe vera* Gel

The leaves of *Aloe vera* plant were washed with distilled water, cut opened and fresh pulp was collected in a sterile container. Slurry was formed with the help of mortar and pestle and stored in sterile syringes for easy application [14].

Distilled Water

Distilled water was put in syringes for ease in application and for maintaining the uniformity in test agents application.

Dentin Sampling Procedure

A baseline sample of the carious lesion was obtained using a sterile spoon excavator from the center of the lesion, after isolation with a rubber dam prior to excavation of caries in each sample.

After complete excavation of caries, a second dentin sample for microbial evaluation was collected from the hard dentin using another sterile spoon excavator. The teeth in each group were then disinfected with the test agent according to the group. Approximately 1 ml of the disinfectant test agent was syringed out into the cavity for one minute and then the cavity was washed with distilled water and air dried. Another dentin sample after disinfection was then collected for microbial analysis using a spoon excavator from the same place. After collection of the third sample, the tooth was restored with glass ionomer cement (GC Fuji II®).

Thus, dentinal samples were collected three times from each carious tooth, viz., baseline- before excavation of caries, after hand excavation of caries and after disinfection of the cavity. These samples were subjected to microbiological evaluation for TVC [15,16].

Microbiological Procedure

The samples collected were immediately transferred to brain heart infusion broth and was incubated overnight for microbial growth in it. The samples were homogenized in a tube shaker for three minutes, and 25 µl aliquots of this solution were placed onto the plate surfaces containing blood agar, with a micropipette. Each culture plate was inoculated with samples collected in three phases from a single tooth. Subsequently, the cultures were incubated at 37°C for 24 hrs, after which a visual assessment of the total number of viable bacterial colonies were counted and subjected to statistical analysis [15,17].

STATISTICAL ANALYSIS

The data obtained were analysed using analysis of variance (ANOVA) to test the significance. Bonferroni post-hoc test was used for pair-wise comparison between the means when ANOVA test was significant. The level of significance was set at $p \leq 0.05$. Statistical analysis was carried out using the SPSS 14.0 software (Statistical Package for Scientific Studies Inc. Chicago, IL, USA.).

Group	Procedure	Mean	SD	F-value	p-value
2% CHX*	Pre-excavation	131.5	9.107	1031.67	<0.001
	Post-excavation	69.2	5.959		
	Post-disinfection	3.1	1.197		
	Total	67.93	53.671		
1% TTO*	Pre-excavation	135.7	6.8	1197.11	<0.001
	Post-excavation	69.4	6.72		
	Post-disinfection	13.1	1.729		
	Total	72.73	51.25		
Aloe vera gel	Pre-excavation	130.8	9.151	777.47	<0.001
	Post-excavation	67.5	6.399		
	Post-disinfection	16.9	0.994		
	Total	71.73	47.802		
Distilled water	Pre-excavation	130.9	6.136	336.37	<0.001
	Post-excavation	69.9	7.81		
	Post-disinfection	66.8	8.626		
	Total	89.2	30.9		

[Table/Fig-2]: Comparison of mean of total viable count (TVC) in control and test groups (ANOVA).
*CHX-chlorhexidine; TTO-tea tree oil

Group	Intragroup Comparision		Mean difference	SE	Percentage reduction	95% CI		p-value
						Lower bound	Upper bound	
2% CHX	Pre-excavation	Post-excavation	62.3	2.827	47.4	55.08	69.52	<0.001
	Pre-excavation	Post-disinfection	128.4	2.827	97.6	121.18	135.62	<0.001
	Post-excavation-	Post-disinfection	66.1	2.827	95.5	58.88	73.32	<0.001
1% TTO	Pre-excavation	Post-excavation	66.3	2.508	48.9	59.9	72.7	<0.001
	Pre-excavation	Post-disinfection	122.6	2.508	90.3	116.2	129	<0.001
	Post-excavation	Post-disinfection	56.3	2.508	81.1	49.9	62.7	<0.001
Aloe vera gel	Pre-excavation	Post-excavation	63.3	2.894	48.4	55.91	70.69	<0.001
	Pre-excavation	Post-disinfection	113.9	2.894	87.1	106.51	121.29	<0.001
	Post-excavation	Post-disinfection	50.6	2.894	75.0	43.21	57.99	<0.001
Distilled water	Pre-excavation	Post-excavation	61	3.397	46.6	52.33	69.67	<0.001
	Pre-excavation	Post-disinfection	64.1	3.397	49.0	55.43	72.77	<0.001
	Post-excavation	Post-disinfection	3.1	3.397	4.4	-5.57	11.77	1.000

[Table/Fig-3]: Pair-wise comparison of mean bacterial counts in different groups (Bonferroni post-hoc test).

RESULTS

The first test group (2% CHX) showed a mean TVC pre-excavation, post-excavation and post-disinfection of 131.5±9.107, 69.2±5.959, 3.1±1.197 respectively. Similarly second test group (1% TTO)

Group	Mean	SD	F-value	p-value
2% CHX	3.1	1.197	406.638	<0.001
1% TTO	13.1	1.729		
Aloe vera gel	16.9	0.994		
Distilled water	66.8	8.626		
Total	24.98	25.348		

[Table/Fig-4]: Comparison of mean bacterial counts among different groups in post-disinfection procedure (ANOVA).

Intergroup Comparision		Mean difference	SE	95% CI		p-value
				Lower bound	Upper bound	
2% CHX	1% TTO	-10	1.998	-15.58	-4.42	<0.001
2% CHX	*Aloe vera*	-13.8	1.998	-19.38	-8.22	<0.001
2% CHX	Distilled water	-63.7	1.998	-69.28	-58.12	<0.001
1% TTO	*Aloe vera*	-3.8	1.998	-9.38	1.78	0.391
1% TTO	Distilled water	-53.7	1.998	-59.28	-48.12	<0.001
Aloe vera	Distilled water	-49.9	1.998	-55.48	-44.32	<0.001

[Table/Fig-5]: Pair-wise comparison of mean bacterial counts in post disinfection procedure (Bonferroni post-hoc test).

showed values of 135.7±6.8, 69.4±6.72, 13.1±1.729 and third test group (*Aloe vera*) values of 130.8±9.151, 67.5±6.399, 16.9±0.994 respectively as shown in [Table/Fig-2].

The results of present study showed that there was a statistically significant reduction in TVC when compared between pre and postexcavation in all the groups ($p<0.05$) and postexcavation and post-disinfection in all the test groups ($p<0.05$) (except control group) using Analysis of Variance (ANOVA)

Pair-wise comparison of viable bacterial colony using Bonferroni post-hoc test was done for each study group at different phases viz., pre-excavation, post-excavation and post-disinfection which showed statistically significant difference in the number of bacterial colonies between each phase in all the three study groups ($p<0.05$) [Table/Fig-3].

An important and clinically relevant finding of the present study is that there was a statistically significant reduction in the bacterial counts after cavity disinfection in all the three groups as compared to the control group (distilled water). ($p<0.05$) as shown in [Table/Fig-4].

A pair-wise comparison of mean bacterial colony counts in post disinfection procedure showed maximum reduction of TVC with 2% CHX followed by 1% TTO and *Aloe vera* in which the difference in results were statistically significant.

Both TTO and *Aloe vera* showed reduced bacterial count post disinfection, but 1% TTO was found to be better than *Aloe vera* although the difference was not statistically significant.($p\geq0.05$) [Table/Fig-5].

DISCUSSION

Traditional restorative dentistry propagated early operative intervention to remove diseased tissue and bacteria. Modern dentistry, however, emphasises on arresting the caries progression and restoring the tooth with minimum tissue destruction. Minimal invasive dentistry is based on this axiom.

A drawback in restorative dentistry is the occurrence of secondary caries [18,19] that has compelled us to practice a more extensive form in the past. Minimally Invasive Dentistry (MID) also displays the same drawback. Our study, in accordance with other studies has also shown incomplete bacterial elimination with excavation only [20]. Several antimicrobial agents have been tried and tested to eliminate bacteria underneath the restorations, and are now being used as cavity disinfectants [21]. With the advent of cavity disinfectants, there is an upsurge in the use of MID in the recent years.

Varied concentrations of CHX; 0.2% [22], 0.12% [23-25], 0.06% [23] have been tested and shown to act effectively against *S. mutans*. A major disadvantage of this agent is that it adversely affects the micro-tensile [26] and shears bond strength [27] of composite resins as well as increases the microleakage [26,28]. Thus, there is the need for an alternate agent which could overcome these difficulties as well as serve the purpose of a cavity disinfectant.

Two antimicrobial herbal agents, TTO and *Aloe vera*, were used in our study to compare and illustrate their efficacy as cavity disinfectants.

Mechanism of Action of TTO

Tea tree oil is the volatile essential oil obtained primarily from the Australian native plant, Melaleuca alternifolia. It consists of terpene hydrocarbons, mainly monoterpenes, sesquiterpenes and their associated alcohols which are volatile, aromatic hydrocarbons [29-33].

The terpinen-4-ol, α-terpineol and 1, 8-cineole, are the antimicrobial components in TTO which cause leakage of 260 nm-light absorbing material and render cells susceptible to sodium chloride [34]. Thus, tea tree oil shows antimicrobial effect through cell lysis and the loss of membrane integrity which leads to leakage of ions and the inhibition of respiration [29].

Mechanism of Action of *Aloe vera*

Aloe barbadensis Mill (*A. vera)* is a short succulent herb filled with a clear viscous gel which has potent antibacterial, antifungal, and antiviral properties [35,36]. The therapeutic use of *Aloe vera* has been known to us since inception.

The antimicrobial activity of *Aloe vera* is attributed to its constituent compounds, the most important being the anthraquinones [37]. *Aloe vera* gel has a number of components such as aloin, aloe emodin, aloetic acid, anthracene, aloe mannan, aloeride, antranol, chrysophanic acid, resistanol, and saponin [38]. Aloin and aloe emodin possess strong antibacterial and antiviral activities. They inhibit protein synthesis from bacterial cells, thus explaining their antimicrobial activity [39]. It is noteworthy that some compounds like anthraquinones and saponin present in *Aloe vera* gel have direct antibacterial activities while some other components, such as acemannan, have been considered to exert indirect bactericidal activity through stimulation of phagocytosis [40].

Many studies on TTO have advocated its antibacterial property. Studies conducted by Takarada K et al., Groppo FC et al., showed antibacterial activity of TTO on *S. mutans* [41,42]. Filoche SK et al., have shown that combination of TTO and CHX mouthwash as a better antibacterial agent for oral pathogens [43]. Our study also, in accordance with other studies has shown TTO to be an effective antibacterial when used as a cavity disinfectant. Another in vitro study showed significant reduction in *S. mutans* count when tooth brushes were soaked for 12 hrs in 0.2% TTO and also showed more reduction in salivary *S. mutans* counts when compared with 0.12% CHX mouthwash [1,42]. However, in the present study 2% CHX was found to be more effective than 1% TTO. This could be due to the difference in the concentration of CHX and TTO used and the time duration it was applied for. Most bacteria are susceptible to TTO at concentrations of 1.0% or less, however, for organisms like *Enterococcus faecalis*, and *Pseudomonas aeruginosa* the MICs was found to be 2% [29,44,45]. Although TTO has shown its antibacterial efficacy against various oral microfloras, there is no literature available on its use as a cavity disinfectant.

It was also shown in various studies that mouth rinses and dentifrices containing *Aloe vera* have shown a prodigious reduction in gingivitis and plaque accumulation after its use. Gupta RK et al., in his study

observed *Aloe vera* mouth rinse to be equally effective as 0.2% CHX [46], but in our study 2% CHX was found to have better antibacterial efficacy than pure *Aloe vera* gel. This could again be attributed to the difference in the concentration and type of *Aloe vera* preparation used. Mohammadmehdi Fani M et al., have reported mean MIC values for *Aloe vera* gel against clinical isolates of *S. mutans* to be 12.5 µg/ml [47]. A study by Prabhakar R et al., has shown *Aloe vera* to be a more effective cavity disinfectant when compared to propolis [15].

In our study, although TTO showed more reduction of TVC than *Aloe vera*, the difference was statistically insignificant.

LIMITATION

Limitations of this study were that neither specific bacteria were isolated from the pre excavated dentin samples nor the test agents were tested against any specific strain which could provide a better understanding of the pathogens and the effect of the test agents on them in isolation.

CONCLUSION

The present study concluded that only excavation cannot eliminate all the cariogenic bacteria. Natural antibacterial agents like tea tree oil and *Aloe vera* could be effectively used as cavity disinfectants which will help in minimizing secondary caries and rendering a long term restorative success. However, further scientifically sound clinical research and studies should be carried out to broaden our understanding of various antimicrobial agents, particularly natural agents, in the prevention of dental caries.

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