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Original Article

Herbal combat against *E. faecalis* – An in vitro study

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ABSTRACT

Background: Herbs have renewed importance in this modern era for their antimicrobial properties and fewer side effects. So a novel idea to use these herbs for pulpectomy; was implemented. There are chances of failure of pulpectomy due to inefficient removal of microbial load. Thus, for a successful endodontic treatment, obturating material with optimum antimicrobial properties is advocated in the present study.

Aim: To compare and evaluate the antibacterial efficacy of zinc oxide eugenol, zinc oxide with tulsi extract and zinc oxide with aloe vera as obturating materials against *E. faecalis* bacteria.

Materials and methods: Antimicrobial efficacy of zinc oxide eugenol as control, zinc oxide with tulsi extract and zinc oxide with aloe vera as experimental groups were assessed by using agar diffusion method. The statistical analysis was done.

Results: Intergroup comparison revealed significant difference amongst all the groups except between zinc oxide eugenol and zinc oxide with tulsi extract. Zinc oxide eugenol had significantly higher zone of inhibition among all the groups.

Conclusion: According to results obtained from the present study can be summarized as follows: Zinc oxide eugenol > Zinc oxide with tulsi extract > Zinc oxide with aloe vera.

1. Introduction

The once obsolete archaic medicine has commenced an *"Herbal Revolution"*. The herbal products today symbolize safety in contrast to the synthetic antibiotics. They have gained renewed importance in this modern era for their antimicrobial properties, better patient tolerance, renewable in nature and economic.[1]

Herbal drugs used in dentistry include aloe vera, bloodroot, calendula, Echinacea, goldenseal, and grapefruit seed extract which are useful for treating periodontal problems, while clove oil, garlic, lemon balm, licorice and propolis are helpful for fighting toothaches.[1] Among these the most extensively researched herbs are tulsi and aloe vera.

The commonly used obturating materials for primary teeth are zinc oxide eugenol, calcium hydroxide and Iodoform based materials. Zinc oxide eugenol cement was introduced by Richert and Dixon in 1931, a gold standard material for obturating in primary teeth. It has a history of successful use over an extended period of time. An advantage to this cement group is its antimicrobial activity. But exhibit a slow setting time and shrinkage on setting and its eugenol content has been reported to be irritating to periapical tissues, causing necrosis of bone and cementum, as it is proven to be cytotoxic at higher concentrations.[2]

Thus if we could replace eugenol with some other less irritating and biocompatible material, we might formulate an ideal obturating material for deciduous dentition. Obturating the root canal with a material with efficient antimicrobial efficacy as well as less toxicity might lead to increase in treatment success.

Therefore, the present study has been undertaken to evaluate the antimicrobial efficacy of zinc oxide with tulsi and zinc oxide with aloe vera, as obturating material against the most prevalent bacteria in root canals.

2. Aim & objectives

To compare and evaluate the antibacterial efficacy of zinc oxide eugenol, zinc oxide with tulsi extract and zinc oxide with aloe vera as obturating materials against *E. faecalis* bacteria.

3. Materials and method

A total of 45 obturating material pellets were prepared for the study, which were divided in three groups having 15 pellets in each

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group: GROUP 1: Zinc oxide and eugenol, GROUP 2: Zinc oxide and tulsi liquid extract and GROUP 3: Zinc oxide and aloe vera gel mixture. The antimicrobial efficacy of obturating materials used in primary teeth was evaluated against *E. faecalis* (ATCC 29212) by agar diffusion method. The standard bacterial strain of *E. faecalis* was obtained from Himedia Labs.

E. faecalis was cultivated in Brain Heart Infusion (BHI) broth at 37 °C for 24 h in incubator as the primary broth culture. The turbidity of this culture suspension was adjusted until it was equivalent to no.1 McFarland Standard (Approximately 3×10^8 cells/mL). Several drops of this primary broth were inoculated on Trypticase soy agar with defibrinated sheep blood (Blood agar) using a micropipette. The inoculated blood agar plate was incubated in the incubator at 37 °C for 24 h. Rest of the primary broth was kept in the refrigerator at 2–8 °C.

Confirmation of the primary broth growth was done after 24 h by observing the colonies on blood agar which appeared circular, entire, glistening, smooth, and low convex according to the ATCC product sheet and on Gram staining gram positive cocci in short chains were observed in compound microscope also confirming the growth of the microbial indicator as *E. faecalis*. Hence, confirming the growth of the *E. faecalis* (ATCC 29212).

To assess antimicrobial efficacy by agar diffusion test prepared 15 mueller hinton agar plates were used as culture medium. The back side of each mueller hinton agar plate was labelled using a permanent marking pen of respective group. The mueller hinton agar plates were inoculated with *E. faecalis* bacteria; 0.1 mL from the primary broth vial using sterile swab in one swiping motion all over the mueller hinton agar plate. Disposable droppers were cut at 1.5 ml marking using a sterile pair of scissors. Using the dropper, agar was punched out from the agar plates to create an agar well for the placement of obturating material pellet. The 3 wells (4 mm of depth X 6 mm of diameter) were made in each of the agar plates i.e. total 45 wells were prepared in 15 agar plates.

Preparation of all obturating material pellets was done simultaneously in microbiological laboratory and placed in the agar wells for agar diffusion test.

Zinc oxide powder and eugenol (4:1 (0.28:0.07 mg by weight) powder liquid proportion) were dispensed on a cool glass slab and mixed to achieve putty consistency using a stainless steel spatula. Once a pellet was made it was directly placed in the labeled agar well with a gloved finger. Likewise zinc oxide and tulsi liquid extract (preweighed) (TULSI 51–JOLLY PHARMA) were mixed in same manner in 4:1 ratio. Aloe vera gel (preweighed) was directly obtained from the plant, scooping the gel after the removal of the covering. Zinc oxide and aloe vera gel were dispensed in 4:1 powder gel proportion and mixed in a similar manner. The agar plates were kept for 2 h at room temperature for diffusion of the materials, and then incubated for 24 h at 37 °C. After 24 h the agar plates were evaluated. Most uniform outer diameter of the inhibitory zone was recorded in millimetres using a vernier calliper. (Fig. 1) Data was collected, tabulated and sent for statistical analysis.

Results and observations

The data was statistically analyzed using SPSS Version 15.0. The Analysis of Variance (ANOVA) and Post-Hoc Test (Tukey-HSD) were performed to know the effect of each variable and to reveal the statistical significance. The values were represented in number and mean ± SD. For the purpose of statistical interpretation p value of 0.05 was considered statistically significant.

The mean value of the inhibition zone for Group 1 (Zinc oxide Eugenol), Group 2 (Zinc oxide with Tulsi) and Group 3 (Zinc oxide with Aloe Vera) were 10.7867, 8.312 and 0 respectively. It was found that Group 1 (ZoE) had highest mean value followed by Group 2 (Zinc oxide with Tulsi) and lowest in Group 3 (Zinc oxide with Aloe Vera). (Table 1, Fig. 2)

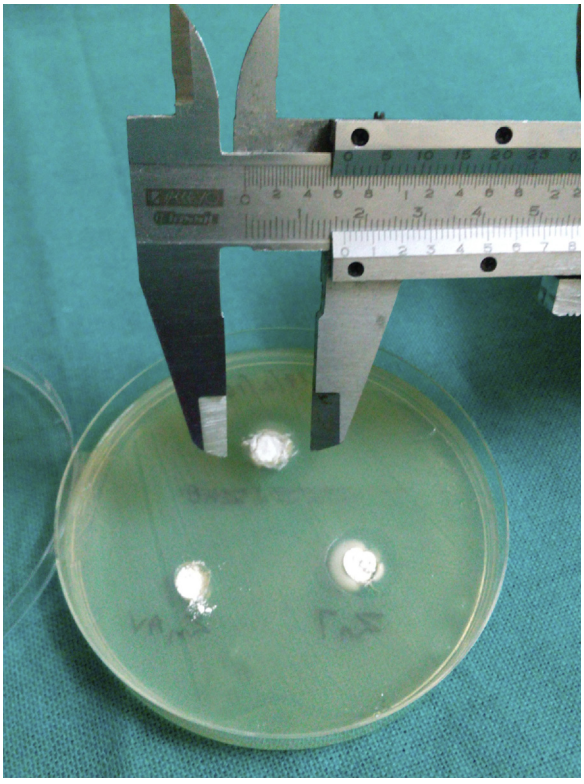

Fig. 1. Vernier caliper measuring inhibition zone.

On applying one way ANOVA, the inhibition zone in all the groups had a significant difference as p value was 0.000 at 0.05 at 95% confidence interval (Table 2).

When intercomparison of various groups were done using Tukey's (2-sided) test (Post Hoc tests). There was statistical non-significant difference between Group 1 (Zinc Oxide Eugenol) and Group 2 (Zinc Oxide with tulsi extract). There was statistical significant difference between Group 1 (Zinc Oxide Eugenol) and Group 3 (Zinc Oxide with aloe vera) and between Group 2 (Zinc Oxide with tulsi extract) and Group 3 (Zinc Oxide with aloe vera) (Table 3).

4. Discussion

The objective of pulpectomy is the removal of pathologic pulpal tissue, sterilization of the canals and restoration with appropriate obturating material. Obturating material should be resorbable, antiseptic, non-inflammatory and non-irritating to the underlying permanent tooth germ, radiopaque for visualization on radiographs, easily inserted and easily removed.[3]

Rôças IN et al (2004) stated that the prevalence of *E. faecalis* is 4 to 40% of primary endodontic infections and consistently higher percentages (67–77%) in secondary infections.[4] *E. faecalis* is able to form a biofilm that helps it resist destruction by enabling the

Table 1
Mean values of inhibition zone in different groups.

Groups	N (Sample Size)	Mean	Standard deviation	Minimum	Maximum
Group I	15	10.7867	0.806	8.9	12
Group II	15	8.312	0.787	4.2	10
Group III	15	0	0	0	0

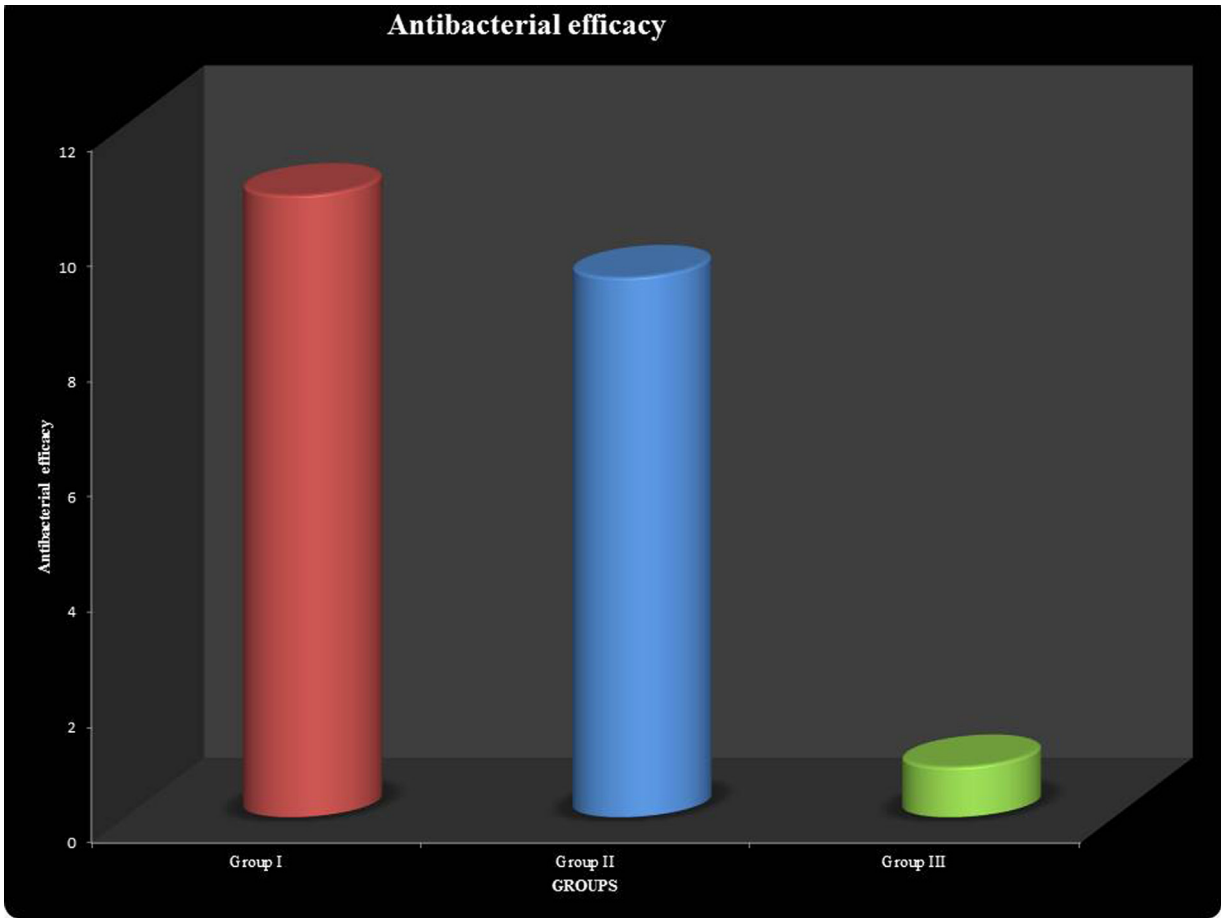

Fig. 2. Graphical representation of mean of antibacterial efficacy of all the groups.

Table 2
Comparison of Means of inhibition zone in all groups.

ANOVA					
	Sum of Squares	df	Mean Square	F	p value
Between Groups	876.0053	2	438	817.36	0.000*
Within Groups	22.5067	42	0.54		
Total	898.512	44			

* p value < 0.05 Significant.

Table 3
Intergroup Comparison of means of inhibition zone by Tukey's (2sided) Post-hoc Test.

Group	Mean difference	p value
1vs 2	2.474	0.8739**
1 vs 3	10.786	0.0216*
2 vs 3	8.312	0.0000*

* p value < 0.05 Significant.
** p value > 0.05 Not Significant.

bacteria to become 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms due to its ability to suppress lymphocytic action.[4] Thus *E. faecalis* was chosen as the microbial indicator for the present study.

The most widely and the oldest used obturating material for root canal filling of primary teeth is zinc oxide eugenol. Zinc oxide eugenol paste was the first root canal filling material to be recommended for primary teeth, as described by Sweet (1930). Inspite of having a high success rate, there are certain disadvantages of eugenol like slow resorption, irritation to the periapical tissues, necrosis of bone and cementum and alters the path of eruption of succedaneous tooth. Thus, still the onus is on the dentist to come up with robust, innovative, effective, feasible and new strategies to manage these drawbacks of zinc oxide eugenol.

Tulsi (*Ocimum sanctum*) and aloe vera (*Aloe barbadensis*) are one of the oldest known used herbs for its medicinal properties. Keeping this in mind the following study was aimed to evaluate the antibacterial efficacy of the obturating materials.

In the present study the highest zone of inhibition was seen with zinc oxide eugenol against *E. faecalis* among all the groups. This antimicrobial efficacy might be because of eugenol content of zinc oxide eugenol. Eugenol is bactericidal due to its ability to cause hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death.[5] Oyedemi et al (2009) reviewed the mechanism of bactericidal action of eugenol against gram positive and gram negative bacteria.[14] It was concluded that eugenol causes cell wall damage lading to protein leakage from the bacterial cell eventually causing bacterial cell death. Because of its potent antibacterial effects it is cytotoxic at higher concentrations. Similar results were observed by Markowitz et al (1992)[6] and Navit et al (2016)[7] where eugenol containing obturating materials showed highest antimicrobial activity as compared to non eugenol containing obturating materials.

The antimicrobial efficacy of zinc oxide with tulsi extract was found to be comparable with zinc oxide with eugenol but was

statistically better than zinc oxide with aloe vera. This antibacterial efficacy might be because of tulsi's most bioactive component being eugenol (70%) and methyl eugenol (20%). Other bioactive components that enhanced the antibacterial efficacy might be because of carracrol, Tetpene, Sesquiterpene B and caryophyllene and polyphenol rosmarinic acid –a powerful antioxidant. Thereby, justifying the bactericidal action against on *E. faecalis*. The concentration of eugenol in the tulsi extract was 70% which gave comparable antimicrobial efficacy to zinc oxide eugenol and thereby, demonstrate less toxic effects in comparison to higher eugenol content in case of zinc oxide eugenol. Similar antibacterial efficacy of tulsi extract against *E. faecalis* was seen in an in vitro study conducted by Chandrappa PM, Dupper A, Tripathi P, Arroju R, Sharma P, Sulochana K (2015)[8] to evaluate the antibacterial efficacy of herbal medicines.

The zinc oxide with aloe vera showed no antibacterial efficacy against *E. Faecalis* among all the groups because of the complex polysaccharide composition of fresh aloe vera gel which elicits less or none therapeutic actions as desired when used directly from the plant. On intergroup comparison the antibacterial efficacy of zinc oxide eugenol and zinc oxide with tulsi extract against *E. Faecalis* was found to be statistically more significant than zinc oxide with aloe vera. Similar in vivo study was conducted by Khairwa et al (2014)[9] using aloe vera gel was scooped from the freshly plucked aloe vera leaf and mixed with zinc oxide powder for 55 pulpectomies. Hamman et al (2008)[10] reported that there is difference in plant composition of aloe vera according to geographical location and seasonal changes. There are conflicting results according to geographical variation in their chemical composition. The antibacterial effect of aloe vera was studied by Sahebi et al (2014)[11] as root canal irrigant and they concluded by not recommending aloe vera as a root canal irrigant due to its inefficient antibacterial efficacy. Bhardwaj et al (2012)[12] conducted antimicrobial efficacy of plant extracts and concluded that aloe vera was the least effective against *E. Faecalis*. Karumari et al. (2014)[13] conducted antimicrobial efficacy of among herbal extract and concluded that tulsi extract showed better antibacterial activity in comparison to aloe vera.

Therefore, in the present study it can be concluded that zinc oxide with tulsi extract can be an alternate to zinc oxide eugenol as an obturating material for primary teeth. We recommend further studies to authenticate these results. Clinical trials should be made so as to check its biocompatibility and various other properties in the intraoral environment.

Conflict of interest

None.

References

1. Nandagopal B, Sankar S, Ramamurthy M, Sathish S, Sridharan G. Could the products of Indian medicinal plants be the next alternative for the treatment of infections? *Indian J Med Microbiol*. 2011;29(2):93–101.
2. Dogra S. Comparative evaluation of calcium hydroxide and zinc oxide eugenol as root canal filling materials for primary molars: a clinical and radiographic study. *World J Dent*. 2011;2(3):231–236.
3. Jha M, Patil SD, Jogani V, Shingare P. *Pediatr Obturating Mater Tech*. 2011;1 (2):27–33.
4. Fernanda Barja-Fidalgo, MicheleMoutinho-Ribeiro Maria Angelina AmorimOliveira, Maria Angelina Amorim Oliveira, Branca Helo'ısa de Oliveira. A systematic review of root canal filling materials for deciduous teeth: is there an alternative for zinc oxide-eugenol? *Int Sch Res Netw*. 2011;02 (2):1–7.
5. Bilal Ahmad Tantry, Avneesh Kumar, Shiak Rahiman, Mudasar Nabi Tantry. Antibacterial evaluation and phytochemical screening of methanolic extract of Ocimum sanctum against some common microbial pathogens. *Glob Adv Res J Microbiol*. 2016;5(1):1–6.
6. Markowitz K, Monihan M, Liu M, Kim S. Biologic properties of eugenol and zinc oxide eugenol. *Oral Surg Oral Med Oral Path*. 1992;73:729–737.
7. Navit S, Jaiswal N, Khan SA, et al. Antimicrobial efficacy of contemporary obturating materials used in primary teeth- an in-vitro Study. *J Clin Diagn Res*. 2016;10(9):ZC09–ZC12.
8. Pradeep Muttagadur Chandrappa, Akash Dupper, Pragya Tripathi, Ramakrishna Arroju, Preeti Sharma, Konthoujam Sulochana. Antimicrobial activity of herbal medicines (tulsi extract, neem extract) and chlorhexidine against Enterococcus faecalis in Endodontics: an in vitro study. *J Int Soc Prev Commun Dent*. 2015;5(December (Suppl. 2)):S89–S92.
9. Khairwa A, Bhat M, Sharma R, Satish V, Maganur P, Goyal AK. Clinical and radiographic evaluation of zinc oxide with aloe vera as an obturating material in pulpectomy: an in vivo study. *J Indian Soc Pedod Prev Dent*. 2014;32(1):33–38.
10. Hamman Josias H. Composition and applications of aloe vera leaf gel. *Molecules*. 2008;13:1599–1616.
11. Sahebi SA, Khosravifar NB, SedighShamsi MA, Motamedifar MC. Comparison of the antibacterial effect of sodium hypochlorite and aloe vera solutions as root canal irrigants in human extracted teeth contaminated with Enterococcus faecalis. *J Dent Shiraz Univ Med Sci*. 2014;15(March (1)):39–43.
12. Bhardwaj A, Ballal N, Velmurugan S. Comparative evaluation of the antimicrobial activity of natural extracts of Morinda citrifolia, papain and aloe vera (all in gel formulation), 2% chlorhexidine gel and calcium hydroxide, against Enterococcus faecalis: an in vitro study. *J Conserv Dent*. 2012;15 (3):293–298.
13. Jothi Karumari R, Vijayalakshmi K, Tamilarasi L, Ezhilarasi Balasubramanian. Antibacterial activity of leaf extracts of aloe vera, ocimum sanctum and sesbania grandiflora against the gram positive bacteria. *Asian J Biomed Pharm Sci*. 2014;4(35):60–63.
14. Oyedemi SO, Okoh AI, Mabinya LV, Pirochenva G, Afolayan AJ. The proposed mechanism of bactericidal action of eugenol, –terpineol and g-terpinene against Listeria monocytogenes, Streptococcus pyogenes, Proteus vulgaris and Escherichia coli. *Afr J Biotechnol*. 2009;8(7):1280–1286.