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Original Research Article

Comparative evaluation of antimicrobial efficacy of calcium hydroxide, curcumin and aloe vera as an intracanal medicament: An in-vivo study

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ABSTRACT

Background: For achieving predictable long-term success of nonsurgical endodontic treatment, complete debridement and effective disinfection of the root canal space are considered to be essential. The use of an intracanal medication remains an important adjunct in the total elimination of bacteria remaining even after chemo-mechanical instrumentation during endodontic treatment. Calcium hydroxide is most commonly used as intracanal medicament. Herbal alternatives like turmeric, neem, aloe vera etc. have the potential to be used as intracanal medicaments. The present study was undertaken for evaluating the antimicrobial efficacy of curcumin and aloe vera as an intracanal medicament for endodontic disinfection.

Materials and Methods: In this prospective randomised controlled study, 45 patients of age group of 18-55 years requiring endodontic therapy for single rooted, single canaled teeth with periapical lesions were selected. Patients were divided into 3 groups having 15 patients each, Group I (Curcumin), Group II (Aloe vera) and Group III (Calcium hydroxide). Under aseptic conditions preoperative and post-operative samples were collected & sent to the microbiology laboratory. Antimicrobial property was assessed using the microbial colony count.

Results: Distribution of median postoperative microbial colony counts among the cases studied did not differ significantly between Groups I and III (P-value>0.05) whereas it was significantly higher in Group II compared to Groups I and III (P-value<0.05 for both).

Conclusion: It can be concluded that antimicrobial efficacy of curcumin showed highest antibacterial efficacy followed by calcium hydroxide and aloe vera as an intracanal medicament.

1. Introduction

Micro-organisms and their by-products have a significant role in the etiology & perpetuation of pulp and periapical lesions.[1,2] Endodontic infections are polymicrobial with a synergistic relationship between bacteria. For achieving predictable long-term success of nonsurgical endodontic treatment, complete debridement and effective disinfection of the root canal space are considered to be essential. This might be accomplished by proper chemo-mechanical instrumentation of root canal.[3,4] Complete disinfection of root canal space cannot be achieved by chemo-mechanical instrumentation alone because of the anatomic complexity and diversity of root canals.[5,6] The use of an intracanal medication remains an important adjunct in the total elimination of bacteria remaining even after chemo-mechanical instrumentation during endodontic treatment.[7] Calcium hydroxide is used as intracanal medicament, it has many properties of an ideal root canal dressing material such as antibacterial action, tissue dissolving ability and induction of hard tissue.[8,9] In this modern era of dentistry we turn our attention towards herbal alternatives like turmeric, neem, aloe vera etc. These herbal alternatives have been used since many years in the field of Ayurvedic, Unani & Chinese medicine. Yet

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very few studies have been conducted involving their use in dentistry. Hence, the present study was undertaken for evaluating the antimicrobial efficacy of curcumin (Curcuma Longa) and aloe vera (Aloe Barbadenesis) as an intracanal medicament for endodontic disinfection. The aim of the present prospective randomized comparative study was to evaluate and compare the antimicrobial efficacy of curcumin, aloe vera and calcium hydroxide as an intracanal medicament for endodontic disinfection.

2. Materials and Methods

This prospective randomized comparative study was undertaken after gaining approval from the institutional ethics committee. The sample size was 45 i.e. 15 per group and was determined by using the effect sizes from the previously published study by Samta Khetarpal et al.

2.1. Selection of cases

A total of 45 patients were selected in the study. The inclusion criteria for patients were single rooted teeth with single canal, negative response to electric pulp vitality testing, sufficient crown structure for adequate isolation & age of patients between 18-55 years. The exclusion criteria were Teeth with external/internal resorption, calcified and curved canal; patients who did not consent to participate in study; teeth with open apices. Purpose of the study was explained to the selected patients and a written informed consent was obtained**.**

The patients were assessed for having one intact non-vital, single canal tooth. The vitality of all the experimental teeth was evaluated by electric pulp tester (C PULSE pulp tester, FOSHAN COXO Medical Instruments Co. Ltd.). Straight & angled views of the radiographs were taken to confirm the number of canals and to know the periapical status of the diseased tooth. Each tooth was randomly assigned according to the intracanal medicament used into three groups:

Group I – Curcumin (100% Pure turmeric curcumin, Perennial Life Sci Pvt Ltd. Ind)

Group II – Aloe vera (100% Natural aloe vera powder, Myra Ayurveda, India)

Group III - Calcium hydroxide (Prodent calcium hydroxide, Prodent Ratnagiri Pvt Ltd)

2.2. Clinical Procedure

After a thorough oral prophylaxis, tooth to be treated was isolated under rubber dam (hygienic, Coltene/Whaledent In., Mahwah. NJ). The field of operation was swabbed with 10 % povidone-iodine solution as an aseptic measure. Endodontic access cavity preparation was done on all teeth using a high speed rotary hand-piece (NSK dental Corp. Nakanishi, Japan) without water spray and with the use of round and safe end taper bur. Sterile No. 15 K file (MANI, Japan) was placed in the canal to establish the patency of the foramina and for determining working length of the root canal. The working length was determined by electronic apex locator Root ZX mini (J. Morita CO., Tustin, CA) and confirmed radiographically using RVG (Visiodent). If the canal was dry then a drop of sterile saline was introduced in root canal. The sterile paper points were left in the canal for a period of 1 min to absorb all the fluid from the canal which contains viable bacteria.[10] The paper point was inserted in the vials containing 5 ml brain heart infusion broth. The vials were tightly screw capped after collecting the samples. The preoperative sample was labelled as S1 and transported to the microbiology laboratory within 2 hours. Cleaning and shaping of the root canal was performed using step back technique. Excess normal saline was removed from the canal using sterile paper point. Intracanal medicament was introduced in the canal using lentulospiral and the coronal access was sealed with an intermediate restoration (Cavit G, 3M ESPE, Germany). The patients were recalled after 7 days. Removal of medicament was done by copious irrigation with sterile saline. The postoperative microbiological sample (S2) was taken in a manner similar to the preoperative sampling procedure.

Bacterial culture processing was done by a specialized technician who was blinded to the disinfection procedures. The paper point in the brain heart infusion broth was vortexed for 2 minutes and then inoculated onto blood agar plate, incubated upto 48 hours at 37^0C. Bacterial growth was observed using counter colony QUEBE spencer and the colony forming unit (CFU) count was obtained. The preoperative & postoperative colony counts for each sample were recorded using data recording sheet. The data was tabulated and statistically analyzed.

2.3. Statistical analysis

The data on categorical variables is shown as n (% of cases) and the data on continuous variables is presented as Mean and Standard deviation (SD) for normally distributed variables and for non-normally distributed variables median is used. The statistical comparison of distribution of categorical variables is tested using Chi-Square test. The inter-group statistical comparison of distribution of means of continuous variables is tested using analysis of variance (ANOVA). The inter-group statistical comparison of distribution of medians of continuous variables is tested using Kruskal-Wallis H test. The intra-group statistical comparison of distribution of medians of continuous variables is done using Wilcoxon's signed rank test. The entire data was statistically analyzed using Statistical Package for Social Sciences (SPSS ver 22.0, IBM Corporation, USA) for MS Windows.

3. Results

Distribution of mean age and sex of cases studied did not differ significantly across three study groups (Tables 1 and 2).

Distribution of average (median) postoperative microbial colony counts among the cases studied did not differ significantly between Groups I and III (P-value>0.05) whereas it was significantly higher in Group II compared to Groups I and III (P-value<0.05 for both). The percentage decrease in bacterial numbers was found to be 75.04%, 47.27% and 70.52% in group I, Group II and Group III respectively. (Table 3)

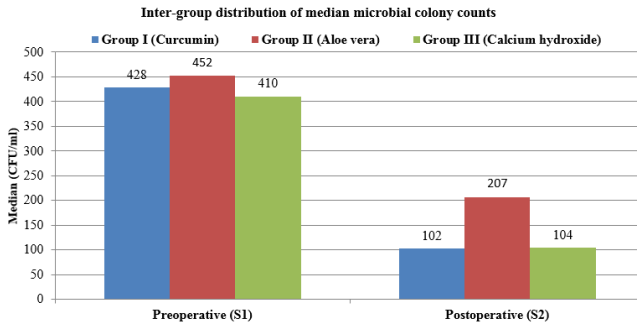

Fig. 1: Inter-group distribution of median microbial colony counts

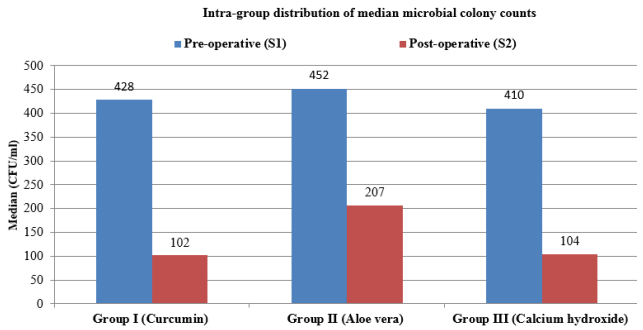

Fig. 2: Intra-group distribution of median microbial colony counts.

4. Discussion

The main goal of endodontic therapy is to eradicate the bacteria and their by-products from the root canal. Effective control of root canal infection determines the success of endodontic treatment of teeth with apical periodontitis.[11] In long-standing endodontic infection, bacteria propagate to the entire root canal system, including dentinal tubules, ramifications, isthmuses and apical deltas. Bacteria may survive in these locations, even after the complete chemo-mechanical preparation.[12] Therefore it is essential to medicate canals with an effective antibacterial agent after canal preparation. Currently, various root canal medicaments such as calcium hydroxide, camphorated monochlorophenol, chlorhexidine, etc. are in use. Amongst these, according to various studies, the antibacterial efficacy of calcium hydroxide has been well established.[13,14] This is primarily due to its ionic effect and chemical dissolution into calcium and hydroxyl ions. It's high pH (12.6) and hydroxyl ions are responsible for altering bacterial cytoplasmic membrane.[15] However, some studies have shown that it has limited antibacterial efficacy against root canal bacteria.[16,17] It's low solubility and diffusibility makes it difficult to reach inaccessible areas and the buffering capacity of dentin affects its pH and decreases its efficacy.[18] Furthermore, it has been observed that immature teeth treated with Ca(OH)2 show a high failure rate because of occurrence of root fracture and it has been suggested that changes in the physical properties of dentin by the Ca(OH)2 medicament may be responsible for this.[19] Certain drawbacks of present intracanal medicaments have shifted our interest towards a search for better, more potent and safe herbal alternatives, such as curcumin and aloe vera for disinfection of the root canal. Both herbal alternatives have advantages of ease of availability, lack of adverse effects, low cost, better patient tolerance and better antibacterial properties.

The antibacterial efficacy of both curcumin and aloe vera, though researched, require further studies to support their effective use as an intracanal medicaments.[20,21] Under in vivo conditions, mixed endodontic infections may show a different sensitivity or reaction towards the disinfecting agent. In vivo antibacterial studies, which combine physiological and bacteriological dynamics, have a greater clinical relevance than in vitro tests.[22] Hence the present study was conducted to evaluate and compare the in vivo antimicrobial efficacy of curcumin, aloe vera and calcium hydroxide using standard microbiologic culture methods.

In the present study 45 subjects with the age group of 18-55 years were included. Teeth with pulp necrosis with periapical lesions were selected in the study as these teeth have been shown to have a less favourable prognosis than those without periapical lesions.[23] Single canal teeth were included to reduce the percent of errors caused by vitality of other canals & also to provide a single platform for the evaluation of disinfecting agents.[24] All the teeth were disinfected by a single operator for performing the disinfection procedure.[25]

The tooth surface was disinfected using 10% Povidone-iodine and isopropyl alcohol – a simple, effective method used by Oncag et al.[26] These aseptic measures prevented false positive results during the sampling procedures. Access to the pulp chamber was made by round bur without water to prevent contamination of chamber, thereby not affecting the flora of the root canal.[27]

Table 1: Distribution of mean age of cases studied across three study groups.

	Group I (Curcumin) (n=15)		Group II (Aloe vera) (n=15)		Group III (Calcium hydroxide) (n=15)		
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**P-value**
Age (years)	30.47	6.19	35.00	10.06	31.40	7.97	0.292^{NS}

P-value by analysis of variance (ANOVA). P-value<0.05 is considered to be statistically significant. NS-Statistically non-significant.

Table 2: Sex distribution of cases studied across three study groups.

	Group I (Curcumin) (n=15)		Group II (Aloe vera) (n=15)		Group III (Calcium hydroxide) (n=15)		
Sex	**N**	**%**	**N**	**%**	**n**	**%**	**P-value**
Male	9	60.0	8	53.3	8	53.3	0.914^{NS}
Female	6	40.0	7	46.7	7	46.7	
Total	**15**	**100.0**	**15**	**100.0**	**15**	**100.0**	

P-value by Chi-Square test. P-value<0.05 is considered to be statistically significant. NS-Statistically non-significant.

Table 3: Distribution of median microbial colony counts across three study groups.

	Group I (Curcumin) (n=15)		Group II (Aloe vera) (n=15)		Group III (Calcium hydroxide) (n=15)		P-value (Inter-Group)		
Microbial colony counts (CFU/ml)	**Median**	**Min – Max**	**Median**	**Min – Max**	**Median**	**Min – Max**	**Group I vs Group II**	**Group I vs Group III**	**Group II vs Group III**
Access opening (S1)	428	0 – 601	452	0 – 607	410	0 – 520	0.870^{NS}	0.653^{NS}	0.653^{NS}
After 7 days (S2)	102	0 – 150	207	0 – 352	104	0 – 234	0.011*	0.713^{NS}	0.045*
% Change	75.04%	–	42.27%	–	70.52%	–	0.001	0.193^{NS}	0.001
P-value (Intra-Group)	0.003		0.003		0.003				

P-value (Inter-Group) by Kruskal-Wallis H test (non-parametric analysis of variance (ANOVA)). P-value (Intra-Group) by Wilcoxon's signed rank test. P-value<0.05 is considered to be statistically significant. *:-P-value<0.05, ¶:- P-value<0.01, †:-P-value<0.001, NS-Statistically non-significant.

The microbiologic sampling procedure employed by Chu et al was chosen. It has been shown to be efficacious and is widely used.[28] During biomechanical preparation saline was used for irrigation to prevent bias caused by the activity of the irrigants itself.[29]

The Pre-operative and Post-operative samples were transported to microbiology laboratory within 2 hours.[30] Duration and surrounding temperature of the bacterial samples before culture were very important parameters for sustaining live bacterial cells inside the samples.[31] Counting of bacteria was aided by the use of colony count meter. The bacterial colonies in the pre-operative & post-operative samples were compared and analysed by a single operator who was blinded about the disinfection procedures so as to reduce the bias and increase accuracy of results.

Antimicrobial efficacy of the intracanal medicaments was evaluated in terms of reduction in bacterial CFU. The results showed a significant reduction in Post-operative bacterial CFU counts in Group I, Group II and Group III and their median colony count (CFU/ml) in pre-operative & post-operative samples were compared. (Table 3)

In Group I Pre-operative samples the median colony count was 428 (CFU/ml) & in Post-operative samples was 102 (CFU/ml). There was statistically significant reduction in the mean of colony counts from Pre-operative to Post-operative samples (P<0.01). Percentage reduction was 75.04%**.** (Table 3Figures 1 and 2) shows curcumin is having significant antimicrobial efficacy. The results of the present study are in accordance with the study done by- Praveen kumar et al reported that curcumin is having significant antibacterial potential against common endodontic bacteria like Streptococcus mutans, Actinomyces viscosus, Lactobacillus casei, Porphyromonas and Prevotella.[32] Khetarpal et al reported that Curcuma longa showed pre-operative mean colony count of 12.55 and post operative mean colony count was 5.00 that is significant reduction of bacteria.[20]

In Group II - Median colony count (CFU/ml) in Pre-operative & Post-operative samples were 452 (CFU/ml) and 207 (CFU/ml) respectively. There was statistically significant reduction in the colony counts from Pre-operative to Post-operative samples (P<0.01). Percentage reduction was 42.27%. (Refer table 3, Graph 1) The results

of the present study showed significant antibacterial activity of aloe vera.

In Group III –Median colony count (CFU/ml) in Pre-operative & Post-operative samples were 410 (CFU/ml) and 104 (CFU/ml) respectively.There was statistically significant reduction in the microbial colony counts from Pre-operative to Post-operative samples (P<0.01). Percentage reduction was 70.52% (Refer table 3, graph 3).This shows Calcium hydroxide is having significant antimicrobial efficacy against micro-organisms that was also found by Gencoglu et al who evaluated antibacterial efficacy and concluded that calcium hydroxide is effective against anaerobic microorganisms.[14] Sinha et al compared antimicrobial efficacy of calcium hydroxide against facultative anaerobes and obligate anaerobes. Percentage reduction was 95% and 82% for obligate and facultative anaerobes respectively and 61% for Candida spp.[29] The success of Calcium hydroxide as an intracanal dressing is due to its ionic effect observed by the chemical dissociation into calcium and hydroxyl ions and its action on tissue and bacteria.[33]

Intergroup comparison of various medicaments - The median colony count (CFU/ml) for Group I, Group II and Group III, in Pre-operative samples was 428, 452 and 410 and in Post-operative samples was 102, 207 and 104 respectively. This shows that Group I showed highest antibacterial efficacy followed by group III and least antibacterial activity was shown by group II.

Curcumin is more effective than Calcium hydroxide results are in accordance with study of– Shrutisaha et al evaluated antimicrobial efficacy of Calcium Hydroxide and Curcuma Longa Extract as intracanal medicament against E. faecalis and concluded that Curcuma longa has better antimicrobial efficacy than Calcium hydroxide as an intracanal medicament against E. faecalis.[34] Yadav et al compared antimicrobial efficacy of calcium hydroxide, chlorhexidine gel, and curcumin against E. faecalis and concluded that C. longa extract showed mild activity against E. Faecalis while calcium hydroxide paste was the least efficient in eliminating E. faecalis.[35]

Results of the present study reinforced the concept of using medicament in canal is a necessity as medicaments tested were effective in reducing microbial colony count. Curcumin was found to be the most effective intracanal medicament followed by calcium hydroxide and aloe vera.

Limitations of the present study were – less sample size per group, inaccessibility of paper points used to collect samples to the accessory canals. To prove the effectiveness of medicaments further, longer follow-up study and healing of periapical lesion radiographically could be included in the study.

5. Conclusion

Based on the results & within the limitations of the present study it can be concluded that, all three medicaments tested in study i.e. curcumin aloe vera and calcium hydroxide were found to be effective to achieve a thorough disinfection of the root canal system. Antimicrobial efficacy of curcumin showed highest antibacterial efficacy followed by calcium hydroxide and aloe vera as an intracanal medicament.

6. Source of Funding

None.

7. Conflict of Interest

None.

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