Comparative Evaluation Of Anti-Microbial Efficacy Of AH26 Sealer Combined With Amoxicillin And Two Different Herbal Extracts Against Enterococcus Faecalis: An In-Vitro Study

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Abstract :-

Background :

Main aim of endodontic treatment is to make the root canal free from microorganisms and their byproducts. Medicinal herbs, showing anti-microbial property, bio-friendly and lower side effects, can be used in conjunction with sealer during obturation to enhance the success rate of treatment. Hence, the aim of the present in-vitro study was to compare the anti-microbial activity of AH26 sealer when mixed with Amoxicillin, M. longifolia and Asafetida extract on Enterococcus faecalis.

Materials & Method :

In this study 3000 µl of AH26 sealer was mixed and prepared according to manufacturer's instructions and divided into four sections for experimental groups. First group, contained only AH26 sealer. In second, third and fourth groups, Amoxicillin powder, M. longifolia extract and Asafetida powder extract was added at 10% of sealer total weight to AH26, respectively. Positive

and negative control groups contained bacteria or cultivation environment, respectively. Subsequently, after 24 hours incubation, 10µl of the available solution in each tube were transferred to BHI Agar for counting the bacteria colonies. Data were analyzed using ANOVA, post hoc Tukey tests. Statistical significance was set at $p<0.05$.

Result :

There was not statistically significant ($p=0.64$) result in AH26 (Group 1) and AH26 + M. longifolia extract (Group 3) while this difference was highly significant for AH26+ Amoxicillin (Group 2) and AH26 + Asafetida extract (Group 4). However, the lowest mean of $\log_{10}$ CFU/ml is for Group 1- AH26 (2.23) and the highest mean is for Group 2- AH26+ Amoxicillin (5.42).

Conclusion :

It is also concluded that anti-microbial activity of herbal extract (Asafetida) might be compared to those of antibiotics, and when taking into account the likelihood of antibiotic side effects, this herbal extract can be a good alternative.

Keywords :

Enterococcus Faecalis, AH26 sealer, Antibiotics, Amoxicillin, Mentha longifolia, Asafetida

INTRODUCTION

Endodontic therapy aims to make the root canal free from all remnant tissues, debris, microorganisms, and their byproducts. A potential role of poly microorganism in the initiation and continuation of endodontic infections has long been established. [1,2] Nevertheless, literatures shown that during failed root canal treated teeth with periapical lesions *Enterococcus faecalis (E. faecalis)* has been the most frequently identified species. *E. faecalis* is the most resistant microorganism with various survival and virulence factors, including its ability to compete with other microorganisms, excellent adaption to conditions with ample nutrients and low oxygen levels, and the invade dentinal tubules to a depth of more than 1000 micrometer and close to cementum. [3,4]

The presence of these microbes at the time of obturation can drastically lower the success rate of root canal therapy. [5] Hence, some of the limitations of chemo-mechanical preparation may be overcome by using sealers with anti-microbial properties. Nowadays, resin-based sealants, including AH26, are widely used in dentistry because of its positive handling characteristics such as good flow, adapts well to dentin walls and allows sufficient working time. [6] The AH26 sealer, an epoxy-based resin, is composed of two components: powder and resin, which

are combined to create an effective mixture. Literatures evidently acknowledge that filling the canal using antibiotic mixture with sealer is more effective on reduction of *E.faecalis* growth in comparison to sealer alone. [7] Several antibiotics, including doxycycline, amoxicillin, vancomycin, erythromycin, and benzyl penicillin.

On the other hand, due to constant increase in antibiotic resistance and their harmful side effects, there is a growing demand for herbal alternatives. As herbals are nontoxic, biocompatible, bacteriostatic and bactericidal with negligible adverse effects. [8,9]Mentha longifolia (M.longifolia), a perennial flowering plant, is found in the tropics of Central and Southern Europe, Southwest Asia, and North Africa. [10] It has been widely used in traditional medicine for the treatment of gastrointestinal illnesses, eating disorder, emesis, ulcerative colitis, and hepatic diseases. The bactericidal and antioxidant properties have also been assessed for essential oil and extract. [11]

Another herbal plant, Asafetida, is an oleoresin which contains resin (40%-64%) and essential oils (16%-35%). Resin contains fermented acid esters (60%), free fermented acid (1.3%), AzarZinoleal, Farnesiferolols, and coumarone derivatives. [12] It is commonly available in two forms: high-grade lacrimal form, low-grade bulky form. [13] Because of its anti-microbial property, asafetida extract is employed in a variety of fields, including medicine and dentistry.

To date, limited studies have been performed to assess the incorporation of antibiotics to endodontic sealers while no in vivo or in vitro studies have been performed on Mentha Longifolia essential oil solution and asafetida extract combined with sealers to evaluate the anti-microbial effects against E. faecalis. Therefore, the main goal of this invitro study is to compare the anti-microbial activity of combination of amoxicillin, M. longifolia and Asafetida in combination with AH26 sealer.

MATERIALS AND METHOD

The present in-vitro study was carried out based on a direct contact test and included 18 microtubes (6 groups each group of study contained 3 microtubes in order to evaluate the precision and accuracy of data). A volume of 3000 µl of AH26 sealer was mixed and prepared according to manufacturer's instructions (Dentsply, Konstanz, Germany); then divided into four sections each of 750 µl volume equal to half of the micro tube's volume to be used in groups 1 to 4.

In the first group, AH26 sealer was transferred into the microtubes. The second group, Amoxicillin powder was added at 10% of sealer total weight. In third group, M. longifolia extract (National Botanical Research Institute, Lucknow) was added at 10% of sealer total weight. In the fourth group, Asafetida powder extract (National Botanical Research Institute, Lucknow) was added at 10% of sealer total weight. The fifth and sixth study groups, respectively, were the positive control group (which only contained bacteria without the addition of any additional material) and the negative control group (which only contained the culturing environment without any bacteria).

For microbiological procedures, standard suspension of *E. faecalis* (MTCC 2729) was provided by the Cytogene Lab, Lucknow. This suspension was prepared in Cytogene Lab, Lucknow. Bacterial count consisted of 1.5×10^8 CFU/mL (equivalent to 0.5 McFarland). The microorganisms were incubated for 24 hours in brain-heart infusion broth (BHI) at 37°C under aerobic conditions. Subsequently, 10µl of the standard suspension 0.5 McFarland of *E. Faecalis* was added to each micro tube except the negative control group.

After incubation in 100% humidity at 37° C for 60 minutes, the remaining volume of the microtubes were filled with BHI broth environment and gently mixed with shaker unit for 1 minute. 10µl of the available solution in each micro tube was transferred to BHI agar cultivation environment. After incubation for 24 hours at 37° C the number of the bacteria colonies on the plates were counted. The achieved data was analyzed using measurement of ANOVA using SPSS statistical software 23.0 Version and posthoc Tukey analysis was carried out.

RESULTS

The study result has been summarized in table 1. As shown, the lowest mean of $\log_{10}$ CFU/ml is for Group 1- AH26 (2.23) and the highest mean is for Group 2- AH26+ Amoxicillin (5.42). Also, in the pairwise comparisons of groups, the results of Tukey test showed that these changes in AH26 and AH26+ M. longifolia extract were not statistically significant ($p=0.64$), but statically, a significant difference between AH26 and AH26 + Amoxicillin ($p<0.001$), AH26 and AH26 + Asafetida extract ($p<0.001$), AH26+ Amoxicillin and AH26+ M. longifolia extract ($p<0.001$), AH26+ M. longifolia extract and AH26 + Asafetida extract ($p<0.001$), AH26+ M. longifolia extract and AH26 + Asafetida extract ($p<0.001$).

Study groups	Number of Samples	Mean ± SD	p value
AH26 (Group 1)	10	2.23 ± 0.35^{a}	<0.001
AH26+ Amoxicillin (Group 2)	10	5.42 ± 0.53^{a}	
AH26+ M. longifolia extract (Group 3)	10	2.62 ± 0.38^{b}	
AH26 + Asafetida extract (Group 4)	10	4.81 ± 0.50^{c}	

*One-way analysis of variance

There is no statically significant difference between the values with the same superscript (p>0.05)

Table 1: Comparison of the Mean of the Log10 CFU/ml and Standard Deviation (±SD) of E.faecalis in different groups

DISCUSSION

Microorganisms are the main cause of periapical and pulp disease, and the amount of microorganism in the root canal system has a direct correlation with severity of inflammation in these tissues. Therefore, clearing the root canal of bacteria and their byproducts is the foundation of an endodontic procedure with a long-term prognosis. [2,14] A facultative anaerobic bacterium, *E. faecalis* is nowadays been known as the primary cause of endodontic therapy failure. Considering the role of this bacterium in failure of the endodontic treatments (23-70%), it is used for evaluating the anti-microbial specification of different materials. This is due to the various survival and virulence factors possessed by *E. faecalis*, including its ability to compete with other microorganisms and it can survive in extreme conditions due to its ability to form biofilm which makes it more resistant to intracanal medicaments and anti-microbial agents.[15,16] Studies have found that using antibiotics combined with the sealer to seal the canal is more effective in decreasing the growth of *E. faecalis* than using the sealer alone. [7,17]

The herbal alternatives of the antibiotics cause resistance against microorganisms because of its complex structure and its anti-inflammation specifications, but more research is necessary for researchers to determine its exact function. [8,9] Moreover, these herbal mixtures are cheaper than industrial medicines.

In present study, the direct contact method (DCT) was used. Direct contact is a quantitative and repeatable method models the tested microorganisms with endodontic sealers inside the

canal.[18] Using this method, the effect of the sealers in different stages is more valuable in different stages for hardening on microorganisms.

According to the results, the **decreasing order of antibacterial effect is : Amoxicillin > Asafetida > M. Longifolia > AH26.** Kangarlou *et al.* [19] also concluded that amoxicillin significantly improved the antibacterial properties of AH26 sealers. In addition, Baer and Maki [20] reported that the combination of sealer-amoxicillin could provide antibacterial effect against *E.faecalis* even after 7 days, however the sealers without amoxicillin could not inhibit the growth of this microorganism. This contrast could be attributed to different anti-microbial assessment methods used in the present study and these studies.

In the present in vitro study it was found that the antibacterial effect of Asafetida was higher than AH26 sealer and M. Longifolia extract. This could be because of essential herbal essences can penetrate the mitochondrial membrane, and result in more organelles permeability and increase the rate of potassium ion leakage. Potassium ion leakage out of the cell is a velar sign of membrane damage and cell death. Asafetida extract also causes penetration of the cell membrane and disrupts the membrane structure, and ultimately causes the death of bacterial cell.[21.22]

Previous studies by Kavousi et al. [23] has distinguished the antibacterial effect of asafetida plant. Also, in a review study by Iranshahi et al. [24] in 2011, the authors pointed to the anti-inflammatory and anti-microbial effects of asafetida. The anti-microbial effect has been proven for many herbal essences.

Moreover, results also showed that M.longifolia essential oil has slightly higher anti-microbial effect than AH26 sealer against *E.faecalis*. It is because of differences in the number of phytochemicals (the active ingredient of the medicinal plant) and antibacterial activity of M. longifolia essential oil. Study performed by Stanisavljević *et al.*[25] in 2014 stated that the Serbian essential oil of Mentha longifolia had an inhibitory effect at 2% concentration against *E.faecalis* and its diameter of growth inhibition zone was 17.5 mm, while the diameter of growth inhibition zone of compared reference antibiotic (Ampicillin) was 16 mm.

CONCLUSION

Outcome of endodontic therapy also relies on three-dimensional obturation to achieve a hermetic seal. Therefore, anti-microbial combination with sealer is more effective for reducing *E. faecalis* growth in comparison to sealer alone. It is also concluded that anti-microbial

activity of herbal extract (Asafetida) might be compared to those of antibiotics, and when taking into account the likelihood of antibiotic side effects, this herbal extract can be a good alternative. It is suggested that for better determining the effects of the Asafetida in endodontic treatment, this combination could be tested in-vivo and the clinical effects of it could be evaluated statistically and afterwards production could be commercialized as herbal antibiotics.

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