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AN IN VITRO STUDY ON THE ANTIMICROBIAL EFFECT OF PACHYMIC ACID ON E. FAECALIS AND CANDIDA ALBICANS WHEN ADDED TO RESIN BASED ROOT CANAL SEALERS

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ABSTRACT

The aim of this study is to evaluate the antibacterial effect of the Resin based sealer, AH Plus with and without the use of pachymic acid on *Enterococcus faecalis* and *Candida albicans*. The objective of this study is to find out whether the pachymic acid, a biopharmacological agent when added to a resin based sealer (AH Plus) may alter the antibacterial property of the root canal sealer. Twenty grams of Pachymic acid powder (Biopurify Phytochemicals Ltd., Sichuan, China) was dissolved in 100 mL distilled water to obtain 20% pachymic acid solution. Two samples were prepared. One sample being the AH Plus sealer only and mixed according to the manufacturer's instructions. Second sample combining Pachymic acid solution with the AH Plus sealer in 3 concentrations, 30µL, 40µL and 50µL. 6 replica plates containing the BHI agar, was spread with the microbial suspension using a sterile swab. These plates were divided into 2 groups i.e., Group 1 (*E. Faecalis*), Group 2 (*C. Albicans*). For the determination of minimum inhibitory concentration (MIC) plate dilution method was followed. Brain Heart Infusion Agar was the medium used. The seeded plates were incubated at 37°C for 24 hours and the growth is noted down for the two sealers separately. All the experiments were done in triplicates. The results obtained from this study was done measuring the inhibition zone diameter (IZD) and evaluating the mean value. The results obtained showed that both samples showed zones of inhibition of growth of microbes. AH Plus-Pachymic acid combination showed the highest inhibition zone diameter compared to AH Plus sealer alone. This study also shows that the AH Plus-Pachymic acid inhibition zone diameter increases with increasing concentrations, the highest being at 50µL and least at 30µL.

Key words: Pachymic acid, AH Plus, Disc Diffusion, *E. faecalis, C. albicans*, Antimicrobial efficacy.

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INTRODUCTION

The main aim of endodontic therapy should be in eradicating bacteria from the root canal space thereby prevention of further infections of the root canal or the peri-radicular tissues. Pulpal pathology is attributed to microbial cause. Microorganisms are known to colonise not only within the radicular space but also in tortuous areas such as accessory, lateral and furcation canals and also apical ramifications. (Narayanan *et al*., 2010)

The root canal system has numerous portals of entry both apically and laterally into the periodontium

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which thereby forms a relationship with access to the periodontium. A successful endodontic treatment can be therefore obtained only by proper sealing of all connections that acts as a gateway for microorganisms to enter the periodontium and vice versa. The better the seal, the better is the prognosis. (Hegde M *et al*., 2013)

Hence, the basis of root canal treatment should be a complete, three-dimensional obturation of a chemomechanically prepared root canal space followed by placement of a coronal restoration that provides an optimal seal of the access opening. (Vemisetty *et al.,* 2014)

The core materials used to obturate the root canals leave spaces within the canal that are caused by the inability of the core materials to adhere to the root canal. Sealers can therefore be used between dentin and the core materials to fill such spaces that are produced by the core materials. Conventionally, root canal obturation only had the desirable characteristic of adhering the dentin to the core material. Advancement in root canal sealers is being engineered in order to aid in the development of better adhesive qualities like enabling the material to penetrate the dentinal tubules. (Guident, 2011)

Microorganisms may be present not only throughout the pulp chamber but also in areas inaccessible to instrumentation and disinfection, such as lateral canals, apical ramifications, crevices, and dentinal tubules. Therefore, the focus of root canal treatment must be complete, three-dimensional obturation of chemomechanically prepared root canal space followed by placement of a coronal restoration that provides adequate seal of the access opening. (Gomes B. *et al*., 2004)

The root canal sealers are mandatory for three-dimensional obturation. Root canal sealers should adhere dentin and the core material and fill the irregularities between the core material and the root canal dentin. It is stated that, the root canal sealers show antibacterial activity that may contribute to the destruction of intracanal microorganisms.

The most commonly used methods for microbial control include instrumentation, antimicrobial irrigation, intracanal dressing, adequate filling and coronal restoration. Antimicrobial activity plays an important role in the efficacy of an endodontic sealer during root canal filling, and for this reason many studies have dealt with the antibacterial activity of endodontic sealers. (Narayanan *et al*., 2010). This purpose of this study is to evaluate the antibacterial effect of the Resin based sealer, AH Plus with and without the use of pachymic acid on *Enterococcus faecalis* and *Candida albicans*.

MATERIALS AND METHODS

MATERIALS REQUIRED:

(1) Pachymic acid HPLC98% (Biopurify Phytochemicals Ltd., Sichuan, China)
(2) AH Plus (Resin based sealer)
(3) Microbial strains of *E. Faecalis* and *C.Albicans*
(4) Brain Heart Infusion Agar
(5) Petri dishes

Bacterial strain and medium

Enterococcus Faecalis MTCC 2921 Agar medium. Candida Albicans MTCC 10231 Agar medium.

Preparation of Pachymic Acid

Twenty grams of Pachymic acid powder (Biopurify Phytochemicals Ltd., Sichuan, China) is to be dissolved in 100 mL distilled water to obtain 20% pachymic acid solution.

Preparation of Microbial strains

Standard strains of *E. Faecalis* and *C. Albicans* are to be used in this study. Microbes are to be grown aerobically from frozen stock in Brain Heart Infusion (BHI) broth at 37°C for 18-20 hours. Plate dilution method, is followed to determine (MIC). The Medium used is Brain Heart Infusion Agar. The plates are incubated at 37°C for 48 hours and the growth is noted down for different extracts separately. All the experiments were done in triplicates. Extracts were subjected to antimicrobial assay by measuring the zone of inhibition (IZD) using disk diffusion technique.

Antimicrobial activity for the sealers were calculated as the mean value of Zone of Inhibition obtained against Enterococcus Faecalis and Candida Albicans.

Methodology

Bacterial strains were used throughout investigation. All the bacterial cultures were obtained from Microbial Type Culture Collection (MTCC).

The microorganisms were cultivated in brain heart infusion broth for E. Faecalis and C. Albicans to match the turbidity equivalent to 1.0 McFarland standard tube corresponding to 3x 10^8 colony forming units per mL

Two samples are prepared. One sample being the AH Plus sealer alone mixed according to the manufacturer's instructions and the other sample combining Pachymic acid solution with the AH Plus sealer in 3 concentrations, 30µL, 40µL and 50µL. 6 replica plates containing the BHI agar, is to be spread with the microbial suspension using a sterile swab. These plates are then divided into 2 groups i.e.

Group 1 (*E. Faecalis*)
Group 2 (*C. Albicans*)

Disc Diffusion Test

After the preparation of the samples, 3 wells of 6 mm diameter were made with a punch by a sterile pipette

removing the agar at equidistant points. AH Plus sealer discs are used as control and should be placed at the center of the petri dishes in groups 1 and 2. The AH Plus and Pachymic acid combination sealer discs are placed as triplicates around the plates. All plates should be maintained at room temperature for 2 hours for free diffusion of the materials and then incubated at 37°C for 24 hours. The incubation zones around each well were then measured in millimeters. The data obtained is to be tabulated and subjected to statistical analysis.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences [SPSS] for Windows Version 22.0 Released 2013. Armonk, NY: IBM Corp., will be used to perform statistical analyses.

Descriptive Statistics:

Descriptive analysis includes expression of Zone of Inhibition in terms of Mean & SD for *E. Faecalis & C. Albicans* under different concentrations of Pachymic acid added to AH Plus Sealer.

Inferential Statistics

One-way ANOVA test followed by Tukey's post hoc test was used to compare the mean zone of Inhibition between different concentrations of Pachymic Acid with AH plus sealer for *E. Faecalis & C. Albicans* organisms. Independent Student t Test was also used to compare the mean Zone of Inhibition (in mm) between E.Faecalis and *C. Albicans* for different concentrations of Pachymic Acid with AH plus sealer. The level of significance was set at $p<0.05$

RESULTS

Both AH Plus and AH Plus – Pachymic acid combination sealer showed zones of inhibition of growth of microbes. AH Plus – Pachymic acid combination showed the highest inhibition zone diameter compared to AH Plus sealer alone. This study also shows that the AH Plus – Pachymic acid inhibition zone diameter increases with increasing concentrations, the highest being at 50µL and least at 30µL.

Table 1. Comparison of mean Zone of Inhibition (in mm) between different concentrations of Pachymic Acid with AH plus sealer for E.88using One-way ANOVA Test

Organism	Conc.	N	Mean	SD	Min	Max	p-Value
E. Faecalis	30 Conc.	3	12.33	0.58	12	13	<0.001*
	40 Conc.	3	17.00	1.00	16	18	
	50 Conc.	3	19.33	0.58	19	20	
	Control	3	13.67	1.16	13	15	

* - Statistically Significant

Table 2. Comparison of mean Zone of Inhibition (in mm) between different concentrations of Pachymic Acid with AH plus sealer for *C. albicans* using One-way ANOVA Test

Organism	Conc.	N	Mean	SD	Min	Max	p-Value
C. Albicans	30 Conc.	3	13.67	0.58	13	14	<0.001*
	40 Conc.	3	19.67	1.16	19	21	
	50 Conc.	3	21.67	2.08	20	24	
	Control	3	13.33	0.58	13	14	

*-Statistically Significant

Table 3. Multiple comparison of mean difference in Zone of Inhibition (in mm) between different concentrations of Pachymic Acid with AH plus sealer for E. Faecalis organism using Tukey's Post hoc Test

Organism	(I) Conc.	(J) Conc.	Mean Diff. (I-J)	95% CI of the Diff.		p-Value
				Lower	Upper	
E.Faecalis	30 Conc.	40 Conc.	-4.67	-6.93	-2.40	0.001*
		50 Conc.	-7.00	-9.26	-4.74	<0.001*
		Control	-1.33	-3.60	0.93	0.31
	40 Conc.	50 Conc.	-2.33	-4.60	-0.07	0.04*
		Control	3.33	1.07	5.60	0.007*
	50 Conc.	Control	5.67	3.40	7.93	<0.001*

*- Statistically Significant

Table 4. Multiple comparison of mean difference in Zone of Inhibition (in mm) between different concentrations of Pachymic Acid with AH plus sealer for C. Albicans organism using Tukey's Post hoc Test

Organism	(I) Conc.	(J) Conc.	Mean Diff. (I-J)	95% CI of the Diff.		P-Value
				Lower	Upper	

C.Albicans	30 Conc.	40 Conc.	-6.00	-9.29	-2.71	0.002*
		50 Conc.	-8.00	-11.29	-4.71	<0.001*
		Control	0.33	-2.96	3.62	0.99
	40 Conc.	50 Conc.	-2.00	-5.29	1.29	0.28
		Control	6.33	3.04	9.62	0.001*
	50 Conc.	Control	8.33	5.04	11.62	<0.001*

*- Statistically significant

Table 5. Comparison of mean Zone of Inhibition (in mm) between E.Faecalis and C. Albicans for different concentrations of Pachymic Acid with AH plus sealer using Independent Student t Test

Conc.	Organism	N	Mean	SD	Mean Diff	P-Value
30 Conc.	E.Faecalis	3	12.33	0.58	-1.34	0.04*
	C. Albicans	3	13.67	0.58		
40 Conc.	E.Faecalis	3	17.00	1.00	-2.67	0.04*
	C. Albicans	3	19.67	1.16		
50 Conc.	E.Faecalis	3	19.33	0.58	-2.34	0.14
	C. Albicans	3	21.67	2.08		
Control	E.Faecalis	3	13.67	1.15	0.34	0.68
	C. Albicans	3	13.33	0.58		

*- Statistically significant

Fig 1. AH Plus sealer with Pachymic acid combination (30% conc.) as triplicates and AH Plus sealer at the center on E. faecalis

Fig 2. AH Plus sealer with Pachymic acid combination (40% conc.) as triplicates with AH Plus sealer at the center on E. faecalis

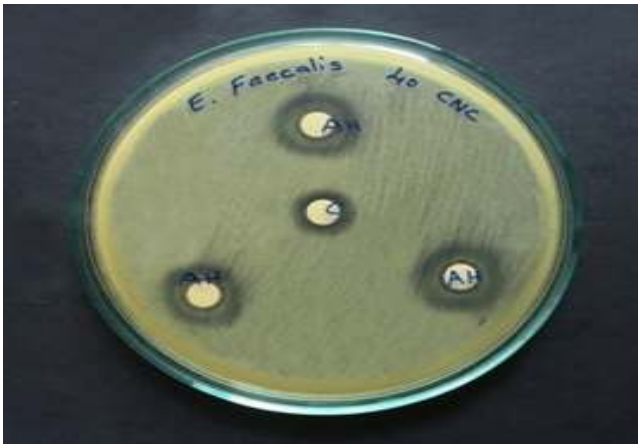


Fig 3. AH Plus sealer with Pachymic acid combination (50% conc.) as triplicates and AH Plus sealer at the center on E. faecalis

Fig 4. AH Plus sealer with Pachymic acid combination (30% conc.) as triplicates and AH Plus sealer at the center on C. Albicans

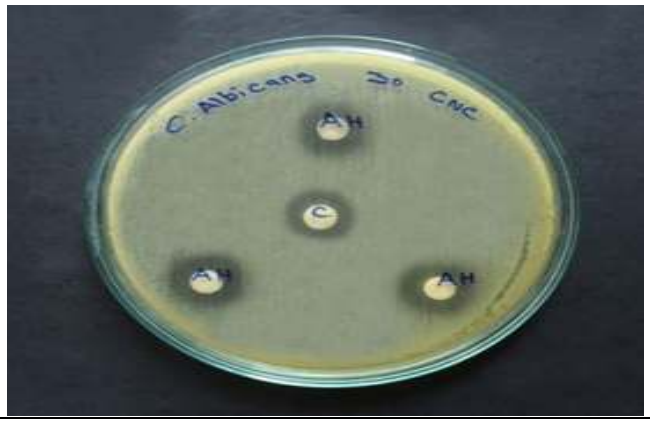
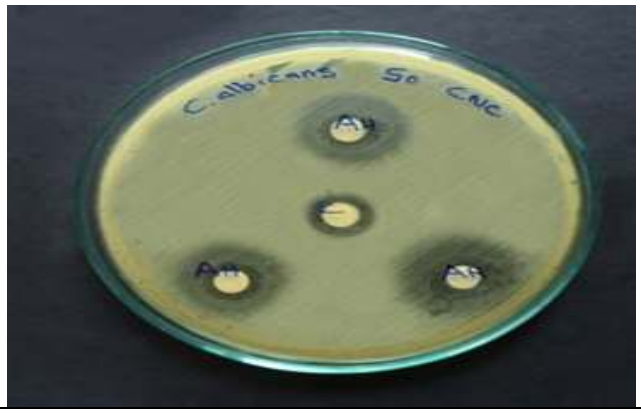
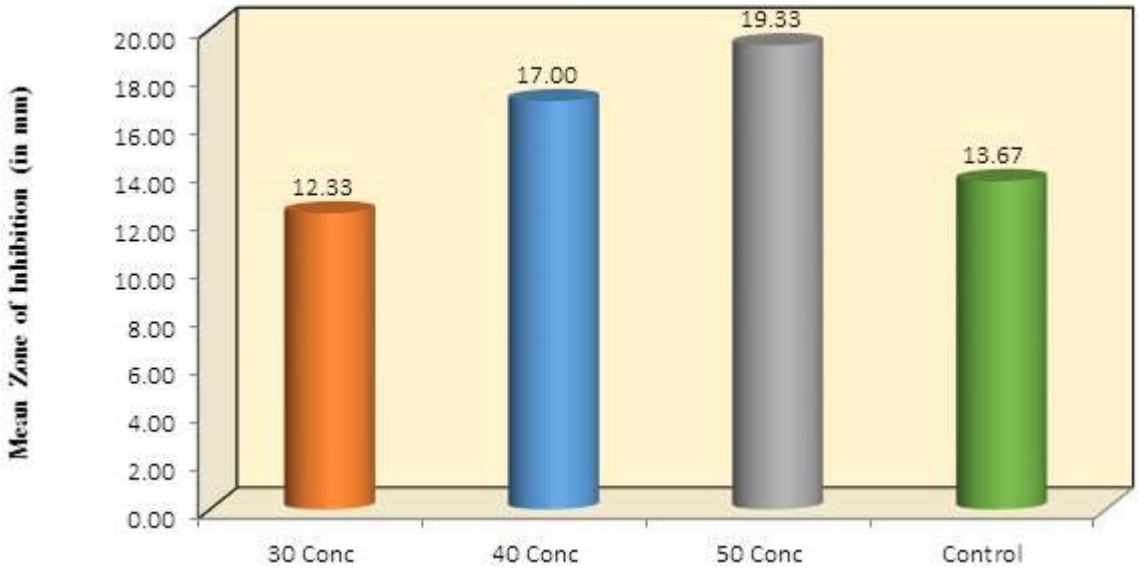


Fig 5. AH Plus sealer with Pachymic acid combination (40% conc.) as triplicates and AH Plus sealer at the center on C. Albicans

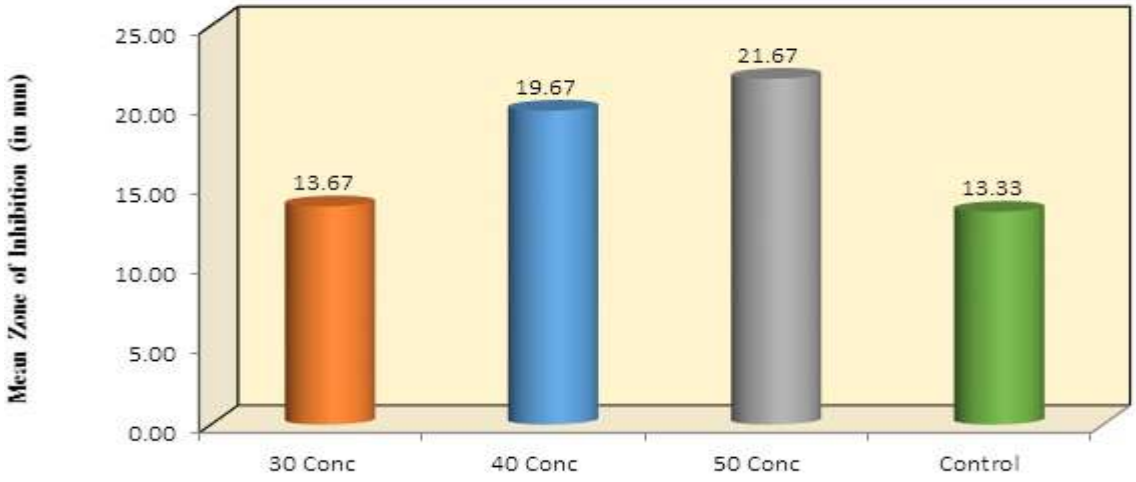
Fig 6. AH Plus sealer with Pachymic acid combination (50% conc.) as triplicates and AH Plus sealer at the center on C. Albicans



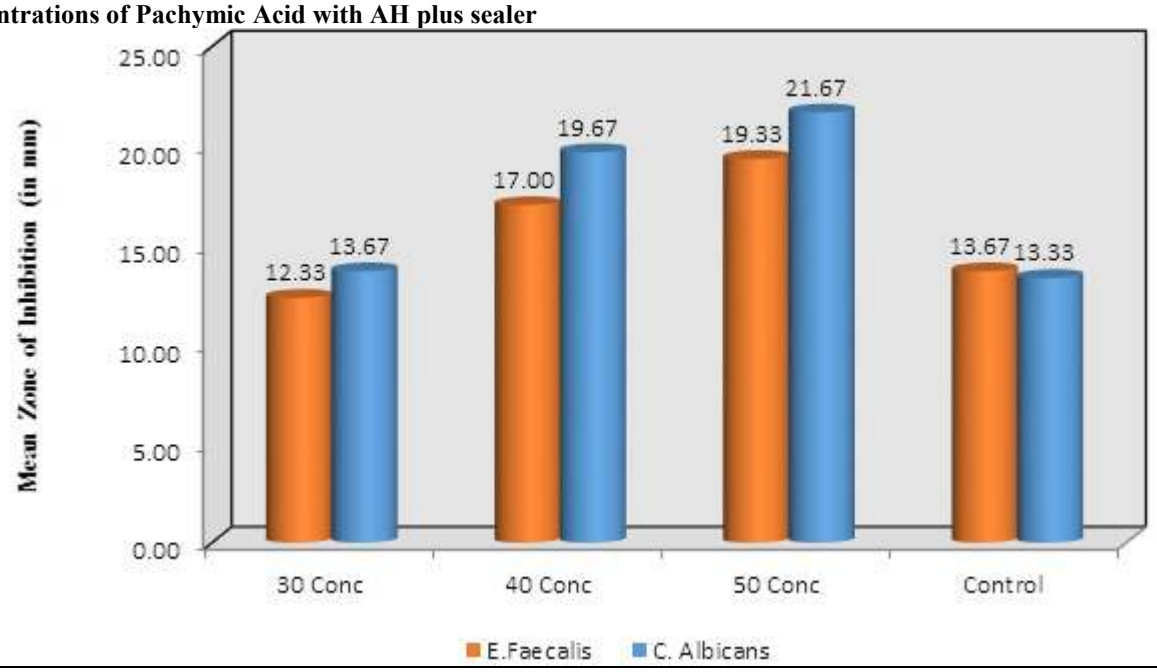
Graph 1. Comparison of mean Zone of Inhibition (in mm) between different concentrations of Pachymic Acid with AH plus sealer for E. Faecalis Organism



Graph 2. Comparison of mean Zone of Inhibition (in mm) between different concentrations of Pachymic Acid with AH plus sealer for Candida Albicans Organism.



Graph 3. Comparison of mean Zone of Inhibition (in mm) between E.Faecalis and C. Albicans for different concentrations of Pachymic Acid with AH plus sealer



DISCUSSION

Numerous studies prove that microbes play a vital role in persistent radicular infections. The microorganisms have the potential to survive in harsh conditions even in the absence of nutrition, blood or oxygen. Most commonly used endodontic sealers include zinc oxide-eugenol, calcium hydroxide, glass ionomer, silicon, resin, and bio-ceramic which have antimicrobial effects depending on their chemical composition. Resin based sealers were first introduced by Schröeder in 1954. Since then, various studies are being performed in order to further enhance the quality of the sealers. (Dalmia *et al*., 2018)

AH plus with epoxy resin base is one of the most commonly used sealers in dentistry. This sealer has a high capacity to seal the root canals. Release of formaldehyde by AH plus sealer during its setting has been confirmed. These sealers do not have formaldehyde in their chemical composition but the chemical reactions between their constituents that occur during the setting phase result in the production and release of formaldehyde which is an effective material for elimination of bacteria. (Gjorgievska *et al*., 2017)

E. faecalis is mainly associated with persistent periradicular lesions after root canal treatment. *E. faecalis* and yeast, mainly *C. albicans*, has been repeatedly identified as the species most commonly recovered from root canals undergoing retreatment, in cases of failed endodontic therapy and canals with persistent infections. *E. faecalis* are gram positive cocci and facultative anaerobes. They are normal intestinal organisms and may inhabit the oral cavity and gingival sulcus. When this bacterium is present in small numbers, it is easily eliminated; but if it is in large numbers, it is difficult to eradicate. (Ashraf *et al*., 2007)

The resin based sealer and the sealer with the incorporation of Pachymic acid were tested using the agar diffusion test. After incubation, the diameter of zones of inhibition around the sealers was measured and the sealer which exhibited the maximum zone of inhibition was considered as having the most efficient antimicrobial activity.

The results obtained in this study showed that AH Plus-Pachymic acid combination performed better than AH Plus sealer when used alone against E.faecalis and C.albicans. This study demonstrated that the addition of Pachymic acid into AH Plus sealer in different concentrations significantly increased the mean inhibition zone diameter. Although, there was no significant statistical difference between the 50% conc. and the 40% conc.

The reason for Epoxy resin based sealer to have antimicrobial property will be due to the release of formaldehyde during polymerization or due to the presence of either bisphenol A- glycidyl ether. The antibacterial activity of AH plus is mostly due to the presence of epoxy resin and amine ingredients (Poggio C *et al*.2017). Tandon *et al*. (2017) demonstrated that freshly mixed AH plus had significant antibacterial effect while set samples did not show any antibacterial activity. Zhang *et al*. (2009), and Kayaoglu *et al*. (2005), showed that freshly mixed AH plus killed E. faecalis effectively.

Pachymic acid is known to have antitumor, anti-inflammatory and antioxidant properties and also has been

reported of being capable of reducing sealer induced cytotoxicity's. (Lee YH *et al*., 2013)

In a study conducted by Cheng *et al*, it was found that Pachymic acid inhibits growth and induces apoptosis of pancreatic cancer. Studies conducted by Arun S *et al*., 2016, have been reported on the addition of pachymic acid to root canal sealers with the results showing increased antimicrobial properties with no cytotoxic reaction.

Lee YH *et al*. (2013) showed that Pachymic acid promotes odontoblastic differentiation through the hemeoxygenase-1 (HO-1) pathway in dental pulp cells. HO-1 is an antioxidant response enzyme that is believed to represent a key role in the protection of cells against environmental stress including inflammatory and oxidative stress. These results suggested that pachymic acid may be applicable for prevention of oral inflammation or to improve dentin mineralization against several stresses. This further highlights the potential therapeutic application of functional chemicals in dental disease treatment. Kim TG *et al*. (2013), evaluated the anti-oxidant property of pachymic acid and it was shown to improve bone disturbance against AH Plus-induced inflammation in MC – 3T3 E1 cells. It was concluded that the property of pachymic acid can mitigate the unfavourable conditions induced by AH Plus stimuli. Therefore, the use of pachymic acid is suggested to prevent the complications of oral diseases such as inflammation and alveolar destruction of the oral cavity.

CONCLUSION

Within the limitations of this study, it can be concluded that Pachymic acid can significantly improve the antimicrobial properties of AH Plus against Enterococcus faecalis and Candida albicans. The efficacy of the AH Plus sealer incorporated with Pachymic acid increased with increasing concentration.

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REFERENCES

Arun S, Sampath V, Mahalakshmi S, Rajkumar K. A comparative evaluation of the effect of the addition of Pachymic acid on Cytotoxicity of 4 different root canal sealers- An *in vitro* study. *Journal of Endodontics*, 43(1), 2017, 96-99.

Ashraf H, SamieeM,Eslami G, Hosseini M. Presence of *Candida Albicans* in Root Canal System of Teeth Requiring Endodontic Retreatment with and without Periapical Lesions. *Iranian Endodontic Journal,* 2007, 2(1), 24–28.

Dalmia S, Gaikwad A, Samuel R, Aher G, Gulve M, Kolhe S. Antimicrobial Efficacy of Different Endodontic Sealers against Enterococcus faecalis: An In vitro Study, *Journal of International Society of Preventive and Community Dentistry*. 2018, 8(2), 104-109.

Gjorgievska ES, Nicholson JW, Coleman NJ, Booth S, Dimkov A, Hurt A. Component release and mechanical properties of endodontic sealers following incorporation of antimicrobial agents. *Biomed Research International,* 2017, 2129807.

Gomes B, Pedroso J, Jacinto R, Vianna M, Ferraz C, Zaia A, de- Souza-Filho F. *In Vitro* Evaluation of the Antimicrobial Activity of Five Root Canal Sealers. *Brazilian Dental Journal,* 2004, 15(1), 30-35.

Gopikrishna AV, Kandaswamy D, Jeyaval RK. Comparative evaluation of the antimicrobial efficacy of five endodontic root canal sealers against Enterococcus faecalis and Candida albicans. *Journal of Conservative Dentistry*. 2006, 9, 2–11.

Guident Online Dental Journal, 2011, Root canal sealers.

Hegde M , Hegde N , Puri A , Kumari S , Sanjeev G , Shetty S. Value Addition Property of Zinc Oxide Eugenol after Electron Beam Radiation. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2013, 4(2), 243.

Kangarlou A, Neshandar R, Matini N, Dianat O. Antibacterial efficacy of AH Plus and AH26 sealers mixed with amoxicillin, triple antibiotic paste and nanosilver. *Journal of Dental Research, Dental Clinics and Dental Prospects,* 2016, 10(4), 220-225.

Kayaoglu G, Erten H, Alaçam T, Ørstavik D. Short-term antibacterial activity of root canal sealers towards *Enterococcus faecalis. International Endodontic Journal*, 2005, 38,483–488.

Kim TG, Lee YH, Lee NH, Bhattarai G, Lee IK, Yun BS, Yi HK. The antioxidant property of pachymic acid improves bone disturbance against AH Plus–induced inflammation in MC-3T3 E1 cells. *Journal of Endodontics* 2013, 39, 461–6.

Lee YH, Lee NH, Bhattarai G. Antinflammatory effect of pachymic acid promoter's odontoblastic differentiation via HO-1 in dental pulp cells. *Oral Disease,* 2013, 19(2),193-199.

Narayanan L, Vaishnavi C. Endodontic microbiology. *Journal of Conservative Dentistry,* 2010, 13(4), 233–239.

Poggio C, Trovati F, Ceci M, Colombo M, Pietrocola G. Antibacterial activity of different root canal sealers against *Enterococcus faecalis*. *Journal of Clinical and Experimental Dentistry*, 2017, 9(6), e743-e748.

Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. *Enterococcus faecalis*: Its role in root canal treatment failure and current concepts in retreatment. *Journal of Endodontics*. 2006, 32, 93–8.

Tandon B, Uppin VM, Hogade SA, Chaudhry S, Bansal S, Bansal S. Antibacterial activity of four root canal sealers against *Enterococcus faecalis* at 1st, 3rd, 5th, and 7th day duration: A In vitro study. *Indian Journal of Dental Science,* 2017, 9, 98–104.

Vemisetty H., Chandra P.V., Reddy J., Kiran R., Nagendra J., Sayini R., Yellamanda J. Evaluation of Push-out Bond Strength of Three Endodontic Sealers with and without Amoxicillin-An *In vitro* Study. *Journal of Clinical and Diagnostic Research,*2014, 8(1), 228-231.

Zhang H, Shen Y, Ruse ND, Haapasalo M. Antibacterial activity of endodontic sealers by modified direct contact test against *Enterococcus faecalis. Journal of Endodontics,* 2009, 35, 1051–1055.