Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(3):472-475

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antimicrobial efficacy of primary root canal filling material against *Enterococcus faecalis*

Samar Ali Al Salameh[1], Mostafa Al Ammori[1] and Shady Azzawy[2]

[1]*Department of Biochemistry and Microbiology, Faculty of Pharmacy, Damascus University, Syria*
[2]*Department of Pediatric Dentistry, Faculty of Dentistry, Damascus University, Syria*

ABSTRACT

Enterococcus faecalis is associated with different root canal infections ,and it is the main cause of chronic periapical lesions . Furthermore it is difficult to eliminate it from root canal in one visit or more ,so The aim of this study was to evaluate the antimicrobial activity of root canal paste on E. faecalis. 42 extracted single rooted primary teeth were manually prepared, sterilized, and then infected with clinical isolates of E. faecalis for 30 days. bacteria were exposed to ZOE and Ledermix pastes as intracanal medication for 1 week. Finally bacterial samples were collected, and colony-forming units were enumerated. none of pastes resulted in complete elimination of bacteria. The antimicrobial effect of ZOE was significantly better than Ledermix at P=.05 . this study has shown that none of the sealers totally inhibited the growth of E. faecalis , and ZOE paste had the greater reduction of the CFU count for E. faecalis cells than Ledermix

Key words: Antimicrobial Efficacy, *E.faecalis*, deciduous teeth, ZOE, Ledermix.

INTRODUCTION

An important and fundamental goal of root canal treatment is to eliminate bacteria from the root canal and prevent reinfection [1], because bacteria or their products are considered to be the primary etiologic factors of periapical lesions and root canal filling failure. Root canal infections have a poly microbial nature hence, anaerobic and facultative anaerobic microorganisms are usually found together in endodontic flare-ups and cases with post-treatment disease [2]. *Enterococcus faecalis* is a Gram-positive cocci and a facultative anaerobe that occur singly, in pairs and in short chains and are capable of surviving in the harsh environment [3]. *E. faecalis* has been found to be the most predominant bacteria in tooth wherein root canal therapy fails, and it is often isolated from persistent cases, including retreatment cases of apical periodontitis [4]. The ability of *E. faecalis* to cause periapical disease and chronic failure of an endodontically treated tooth is due to its ability to bind to the collagen of the dentinal tubule and remain viable within the tubule [5]. As facultative organisms, enterococci are exceedingly hardy. They tolerate a wide variety of growth conditions, including temperatures of 10°C to 45°C and hypotonic, hypertonic, acidic, or alkaline environments [6]. Several studies have shown that enterococci resist various intracanal treatment procedures [7]. This is attributed for their ability to penetrate dentinal tubule [8], withstand high pH values [5] , possess virulence factors [9] and because of biofilm formation [10]. Numerous measures have been described to reduce the numbers of root canal microorganisms, including the use of various instrumentation techniques, irrigation regimens, and intracanal medications [11]. Since the chemomechanical preparation of the root canal reduces endodontic infection, but microorganisms are able to survive within the complex anatomy of the root canal system. So, the antimicrobial intracanal medicaments are used to complement the disinfection of the root canal system [12]. This study was designed to compare the antimicrobial efficacy of two root canal sealers : Zinc oxide and Ougenol and Ledermix against *E. faecalis*.

EXPERIMENTAL SECTION

The present study was conducted in the Department of Biochemistry and Microbiology, in collaboration with the Department of Pediatric Dentistry , Damascus University, Syria.

- **Preparation of samples :**

42 extracted, single canalled human deciduous teeth were collected for therapeutic reasons . Bone, calculus and soft tissues on the root surfaces were slightly removed by means of a periodontal curette. Collected teeth were placed in 5.25% NaOCl for 1 h in order to disinfect the root surfaces and the samples were stored in 0.9% physiological saline. The crowns were cut perpendicular to the long axis of the teeth from cementoenamel junction (CEJ) with a diamond disc in conjunction with physiological saline irrigation and the root lengths were cut and standardized to 10 mm. The root canal was prepared manually using H-file and K-file to size # 30. Under irrigation with 2.5% sodium hypochlorite solution (NaOCl).

- **Smear layer removal from the samples**:

The smear layer was removed by placing it in a 17% EDTA followed by 5.25% NaOCl for 5 minutes.

- **Sterilization of samples:**

Samples were sterilized by autoclaving at 121^0C, 15 psi for 30 minutes.

- **Microorganism**:

A clinical isolates of *E.faecalis* from necrotic root canal of deciduous teeth from Syrian children was used.

- **teeth Contamination**: teeth were inserted into vial , covered with TSB and autoclaved. Then stored in an incubator at 37° C for 24 h after that Vial contaminated with *E.faecalis* (10^9 cfu/ mL) sealed and incubated at 37ºC for 30 days .This incubation period was sufficient for *E. faecalis* to invade the dentinal tubules. culture media were removed with sterile plastic pipettes and substituted one times each 3 days of incubation to ensure the growth of bacterial strain within root canals in the presence of freshly prepared culture media.

After 30 days samples were washed with sterile saline. Wax was used to seal the apex as well as the coronal access cavity. In order to disinfect the outer root surfaces the teeth was immersed in 5.25% NaOCl for 2 min then they immersed in sodium thiosulfate 1% for 1 min.

- **Samples was divided into 3 groups** :

Group 1: contain 10 teeth to count the initial number of bacteria.
Group 2: contain 16 teeth for ZOE paste.
Group 3: contain 16 teeth for ledermix paste.

- **Counting initial number of bacteria :**

Wax was removed ,then the root canals were filled with sterile saline as a transport fluid before sterile absorbent paper points adsorbed the transport fluid for 60 seconds and transferred to a test tube containing 1 ml of saline. All samples were vortexed for twenty seconds and 10-fold dilutions were prepared in saline. Aliquots of 0.1 ml were spread plated onto BHI agar plates, incubated at 37°C for 24 hours, and colony-forming units (CFU) per 1 ml were enumerated .

- **Preparation and Application of the Antimicrobial Agents:**

Root sample was filled with filling past by means of lentulo spiral. Amalgam was used to seal the coronal access cavity; then teeth were implanted in mueller hintone agar and 1 ml left to immersed with tryptic soy broth. All samples were incubated for a week at 37°C under humid conditions.

- **Bacterial Sampling:**

After one week, all of the samples were irrigated with 20 ml sterile saline solution after sealing there's apical foramens with wax. to remove the root canal contents H-file was used with sterile saline . After removing all the paste, The root canals were filled with sterile saline as a transport fluid, before sterile absorbent paper points adsorbed the transport fluid for 60 seconds and transferred it to a test tube containing 1.0 ml of saline. All samples were vortexed for twenty seconds and 10-fold dilutions were prepared in saline. Aliquots of 0.1 ml were spread plated onto BHI agar plates, incubated at 37°C for 48 hours, and colony-forming units (CFU) per 1 mL were enumerated.

Statistical analysis

Differences between group means were detected by analysis of variance, Student's t-test. Data were analyzed with the SPSS 13.0 statistical package. P<0.05 was considered significant.

RESULTS AND DISCUSSION

there was a statistically significant reduction in the mean numbers of colony-forming units (P=.05) after a 1-week application of the pasts (table 1,2) . However, none of them resulted in complete elimination of biofilm bacteria. The antimicrobial effect of ZOE was significantly better than Ledermix at P=.05 (table 3) and figure. 1.

Table. 1 Mean colony forming units after 1-week application of the Ledermix

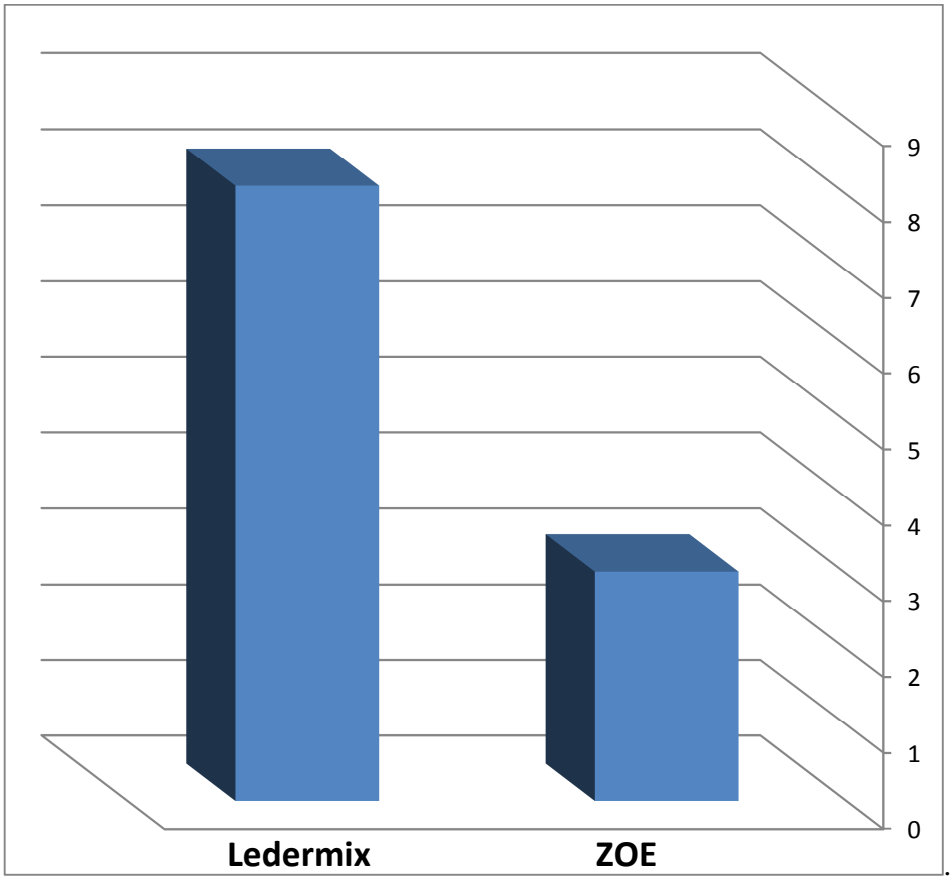
	n	Mean	S.D	t	df	Sig.(2-tailed)
Cfu/ml befor obturation	16	$^{4}10$	0.000	282.538	15	0.000
Cfu/ml after obturation	16	151	139.42464			

Table. 2 Mean colony forming units after 1-week application of the ZOE.

	n	Mean	S.D	t	df	Sig.(2-tailed)
Cfu/ml befor obturation	16	$^{4}10$	0.000	2414.417	15	0.000
Cfu/ml after obturation	16	8	16.55244			

Table .3 Combarazone between the two pastes.

	n	Mean	S.D	t	df	Sig.(2-tailed)
ZOE	16	8	16.55244	4.143	15	0.001
Ledermix	16	151	139.42464			



Figure(1): Combarazone between the two pastes

The golden rule of successful root canal therapy is infection elimination and three dimensional obturation of the canals to preclude subsequent reinfection [13]. However, current techniques of debridement leave parts of root canal space completely untouched by the instruments. *E. faecalis* is associated with persistent apical periodontitis and resists elimination from root canals [14]. *E. faecalis* has the capacity to proliferate in the deeper layers of

dentine [15].. The results showed that none of the sealers totally inhibited the growth of *E. faecalis* , The results of present study also indicated that ZOE paste had an appropriate effect on *E. faecalis* and this is in agreement with the study by Mario et al 2007[16], Hegde et al 2012[1],and Aravind et al 2006 [17], and the antimicrobial effect against *E. faecalis* of ZOE paste was greater than Ledermix paste. The results of present study also indicated that Ledermix had an appropriate effect on *E. faecalis* and this is in agreement with the study by Ávila et al 2013 [18], and Plutzer et al 2009 [19].

CONCLUSION

this study has shown that none of the sealers totally inhibited the growth of *E. faecalis* , and ZOE paste had the greater reduction of the CFU count for *E. faecalis* cells than Ledermix.

Acknowledgement
This study is designed and performed in Damascus University, Faculty of Pharmacy and supported by Faculty of Dentistry

REFERENCES

[1]L Silva; P Nelson-Filho;G Faria;M Souza-Gugelmin;I ITO. *Brazilian dental journal*, **2006**. **17**(2): p. 144-148.
[2]J Siqueira ;I Rôças. Endodontic microbiology. Ames, Iowa: Wiley-Blackwell, **2009**: p. 68Á107.
[3]Mim, C., et al., *Medical microbiology.* Structure, **2003**. **7**: p. 7.
[4]G John;K Kumar;S Gopal;S Kumari;B Reddy. *African Journal of Microbiology Research*, **2015**. **9**(13): p. 898-908.
[5]I Portenier; T Waltimo; M Haapasalo. *Endodontic topics*, **2003**. **6**(1): p. 135-159.
[6]R Halkai; M Hegde; K. Halkai. Short Communications Functional Electrical Stimulation for Neuro Rehabilitation. A New Design Paradigm, **2012**: p. 49.
[7]H Hancock; A Sigurdsson;M Trope;J Moiseiwitsch. *Radiology, and Endodontology*, **2001**. **91**(5): p. 579-586.
[8]C Sedgley; S. Lennan; O Appelbe. International endodontic journal, **2005**. **38**(10): p. 735-742.
[9]C Sedgley;A Molander;S Flannagan;A Nagel;O Appelbe;D Clewell;G dahlen. *Oral microbiology and immunology*, **2005**. **20**(1): p. 10-19.
[10]J Siqueira; I Rôças; D. Ricucci. *Endodontic Topics*, **2010**. **22**(1): p. 33-49.
[11]S Rosenthal; L Spångberg; K. Safavi. Oral Surgery, Oral Medicine, Oral Pathology, *Oral Radiology, and Endodontology*, **2004**. **98**(4): p. 488-492.
[12]P Lana;M Scelza;L Silva;A Mattos-Guaraldi;R Junior. *Brazilian dental journal*, **2009**. **20**(1): p. 32-36.
[13]L Anumula;S Kumar;V Kumar;CH Sekhar;M Krishan;R Pathapati;P Sarath;Y Vadaganadam;R Manne;S Mudlapudi. *ISRN dentistry*, **2012**. **2012**.
[14]B Atila-Pektaş; P Yurdakul;D Gulmez;O Gorduysus. *International endodontic journal*, **2013**. **46**(5): p. 413-418.
[15]J Lucena;E Decker;C Walter;L Boeira;C Lost;R Weiger. *International endodontic journal*, **2013**. **46**(1): p. 53-61.
[16]M Tanomaru-Filho;J Tanomaru;D Barros;E Watanabe;I ITO. Journal of oral science, **2007**. **49**(1): p. 41-45.
[17]V Gopikrishna;D Kandaswamy; R Jeyavel. *Journal of Conservative Dentistry*, **2006**. **9**(1): p. 2.
[18]G Ávila; R Aranda; L MejíaII. Revista Odontológica Mexicana, **2013**. **17**(3): p. 154-158.
[19]B Plutzer Thesis (D.Clin.Dent.) -- University of Adelaide, School of Dentistry, **2009**.