ISSN: 0975-833X

Available online at http://www.journalcra.com

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

International Journal of Current Research
Vol. 13, Issue, 12, pp.19892-19897, December, 2021

DOI: https://doi.org/10.24941/ijcr.42723.12.2021

RESEARCH ARTICLE

COMPARITIVE EVALUATION OF EFFICACY OF CALCIUM HYDROXIDE, TRIPLE ANTIBIOTIC PASTE AND PROPOLIS AS INTRACANAL MEDICAMENT AGAINST ENTEROCOCCUS FAECALIS-AN IN-VITRO STUDY

[1]Dr. Neha Singh, [2]Dr. Richa Singh, [3]Dr. Asheesh Sawhny and [4]Dr. Sharad Sharma

[1]Post graduate student, Rama dental College, Kanpur, U.P.
[2]Reader, Rama dental College, Kanpur, U.P.
[3]H.O.D, Professor, Rama dental College, Kanpur, U.P.
[4]Post graduate student, Rama dental College, Kanpur, U.P.

ARTICLE INFO

Article History:

Received 15th September, 2021
Received in revised form
18th October, 2021
Accepted 20th November, 2021
Published online 29th December, 2021

Keywords:

Intracanal medicament,
E. faecalis,
Bacterial efficacy,
Spectrophotometer,
Bacterial Reduction.

**Corresponding author:*
Dr. Neha Singh

ABSTRACT

Purpose: This study aimed to compare between Calcium hydroxide, Propolis and Triple Antibiotic Paste as root canal medicaments in eradication of *Enterococcus faecalis*. **Material and Methods:** for this study, 45 extracted human teeth were used. After their decapitation, they were initially instrumented to reach 20 K-file, later they were separated into 3 sets according to the medicament used; Group 1: Triple antibiotic paste, Group 2: Propolis paste, Group 3: Calcium hydroxide paste (15 specimens in each group). After sterilization of all samples inoculation was performed with *Enterococcus faecalis* (ATCC29212) over a period of 21 days. Preparation of all samples was done using protaper files till F2 size, irrigation using sodium hypochlorite solution of 2.5% concentration and 17% EDTA, then intracanal medicaments were applied. First sample was collected after inoculation of bacteria into root canals. Second sample was collected after applicated medicament is rinsed. Muller Hilton Yeast agar was used for culturing of samples then went under incubation to count CFU's. **Results:** There was a significant difference between different samples where ($p \leq 0.05$) and there was a continuous decrease of (mean ±SD) value. Pairwise comparisons for all three medicaments showed all samples to be significantly different from each other. **Conclusion:** Antimicrobial activity of Propolis against *E. faecalis* was found to be greater than that of TAP and Calcium hydroxide.

Citation: Dr. Neha Singh, Dr. Richa Singh, Dr. Asheesh Sawhny and Dr. Sharad Sharma, *"Comparitive evaluation of efficacy of calcium hydroxide, triple antibiotic paste and propolis as intracanal medicament against enterococcus faecalis-an in- vitro study", 2021. International Journal of Current Research, 13, (12), 19892-19897.*

INTRODUCTION

Microorganisms are the source for the development of pulpal and periapical conditions. The thing of endodontic treatment is to regulate pulpal and peri-radicular infections and to promote mending. The success of endodontic treatment is directly told by the elimination of microorganisms in infected root canals. Chemo-mechanical debridement removes maturity of bacteria, together with necrotic pulp debris.[1] The bacteria associated with primary endodontic infections are mixed, but are generally gram-negative anaerobic rods, whereas the bacteria associated with secondary infection comprise only one or a many bacterial species – most important of which is Enterococcus facecali.[2]

Enterococcus faecalis is a part of the normal oral foliage and an important pathogen in opportunistic infections in humans. It's infrequently present in primary apical periodontitis, but it's the dominant microorganism in root canal- treated teeth presenting with post treatment apical periodontitis.[3] Eradication of Enterococcus faecalis from the root canal remains a challenge, since it's resistant to a variety of antimicrobial agents. The control and repression of Enterococcus faecalis in the dental procedures is of primary significance in dwindling the penetration of bacteria inside the dentinal tubules and also limiting the conformation of biofilms.[4] Due to complex nature of the root canal system and the presence of numerous inapproachable areas, a combination of mechanical instrumentation and irrigation with intracanal medicaments is necessary to drop the quantum of micro-

organisms in the root canal system.[5] In order to achieve bacteria free root canal system especially in pulpless teeth it's veritably necessary to use intracanal medicaments.[6] Intracanal medicament include the elimination or reduction of microorganisms, rendering canal contents inert, forestallment of post-treatment pain, and to enhance anesthesia.[7] Calcium hydroxide is a gold standard traditionally when used as intracanal medicaments. Although its antibacterial exertion is on wide range of microflora of the root canal. It was demonstrated that the use of calcium hydroxide as a root canal medicament has limitations because it doesn't exclude the whole diapason of microorganisms and it needs long time to give its antimicrobial effect.[8]The antimicrobial exertion of Ca $(OH)_2$ is related to the release of hydroxyl ions in contact with waterless fluids. Hydroxyl ions are largely oxidant free revolutionaries that show extreme reactivity with biomolecules. The murderous effect on microorganisms has been attributed to the following mechanisms- damage to the bacterial cytoplasmic membrane, protein denaturation, and/ or damage to the DNA-yet, it's delicate to establish the main medium involved in the death of bacteria.

Due to the polymicrobial nature of root canal infections, combination of medicines will be more effective mode to treat these infections. Hence, the infected area requires a normal blood force which is no longer the case for teeth with necrotic pulps and for teeth without pulp towel. Thus, original operation of antibiotics within the root canal system may be a more effective mode for delivering the medicine. Microorganisms in dentinal tubules may constitute a force from which root canal infection and re-infection may do.[9] More likely, a combination would be demanded to address the different foliage encountered. The combination that appears to be most promising consists of metronidazole, ciprofloxacin, and minocycline. Maturity of bacteria in the infected root canal dentin are obligate anaerobes. Hence, metronidazole was named as the first choice among antibacterial medicines. Indeed at a high attention, it cannot kill all the bacteria, indicating the necessity for combination of other medicines.

Among herbal medicaments used in endodontics is propolis, which is a resinous hive product collected from various plants accoutrements by honey notions, is considered to be a defensive hedge against the notions adversaries. Propolis was used at the time of Egyptian and Greek civilization, the Greek honored its mending rates. Pharmacological parcels of different propolis medications have been reported as anti-hepatotoxic, antioxidative, neuro-protective,anti-inflammatory, antimicrobial and antiviral. It has also been demonstrated that propolis and some of its active substances have a pronounced anticarcinogenic and antitumor effect. Propolis has numerous implicit uses in oral health like crack mending, storehouse media following avulsion, pulp circumscribing agent, intracanal irrigant, mouth wash, cariostatic agent, treatment of periodontitis and as intracanal medicament.

Recent studies reported that propolis is more effective against resistant microorganisms as well as biocompatible to the peri-radicular tissues than being intracanal medicaments. Still, minimum literature is available on the relative evaluation of the anti-microbial efficacity and bacterial viability of propolis, Triadic antibiotic paste, and calcium hydroxide against E. faecalis. Hence, the purpose of the present study was to compare the antimicrobial efficacity of Calcium hydroxide paste, triadic antibiotic paste and propolis paste as intracanal medicament against Enterococcus faecalis in an uprooted mortal mandibular single embedded premolar using culture study.[10]

MATERIALS AND METHODS

EXPERIMENTAL DESIGN

Teeth selection and Standardization of Working Length: Forty-five single-rooted human mandibular premolars with closed apices, extracted for orthodontic reasons collected from department of Oral and Maxillofacial surgery, Rama dental college, kanpur were used in this study. The teeth were cleaned of superficial debris, calculus, and tissue tags and stored in normal saline to prevent dehydration before use. Each tooth was radiographed to confirm the presence of a single patent canal. The tooth specimens were sectioned below the cementoenamel junction with a diamond disc to obtain a standardized tooth length of 13 mm. The canals were accessed, and initially a size #10 Stainless Steel (SS) K file was inserted into the canal until the file tip was just visible at the apical foramen. The working length (WL) was kept 1mm short of the apical foramen.

Standardization of Apical Canal Dimension: The root canals were prepared using protaper files (Dentsply Maillefer, Switzerland) upto size F2. The canals were irrigated with 2.5% sodium hypochlorite between each instrument and 17% EDTA and 5ml saline as a final irrigant to remove the smear layer. All the roots were then washed and stored in saline.

Sterilization of teeth: All the prepared teeth were packed in suitable autoclave pouches and autoclaved at 121° C and coated with three layers of nail varnish.

Inoculation of enterococcus faecalis: Each root canal was inoculated with 24 hour old cultured broths of bacterial solution of *Enterococcus faecalis* using a sterile endodontic needle in a microbiological safety cabinet. After inoculation, the samples were kept in a closed eppendorf tube and incubated at 37°C for 21 days under aseptic conditions. The canals were re-inoculated with fresh bacterial samples at every 3 days interval to ensure viability of bacteria.

Root canal medication: The canal contents were aspirated after 21 days of incubation, then rinsed with 5 mL saline and patted dry with sterile paper points. The specimens were then randomly divided into three groups (n = 15 each) for intracanal medicaments:

- Group I: Triple antibiotic powder mixed with saline
- Group II: Propolis powder mixed with saline
- Group III: Calcium hydroxide powder mixed with saline

The samples were incubated for 7 days at 37°C (98.6ºF) after sealing with dental wax. The degree of infection was check on the 8th day for the root canal was investigated.

Obtaining the Intracanal medicaments: Calcium hydroxide powder, Triple antibiotic mixture, Propolis powder was mixed with saline to obtain paste like consistency.

Samples Harvesting Method: The dentin chips from the full length of the radicular dentin were harvested using paper points.

The dentin chips was removed from the paper points by placing into a sterile Eppendorf tube containing 1.5 ml of Brain Heart Yeast (BHY) broth in a vortex mixture for 30 seconds. The paper points were removed and the samples were incubated for 24 hours at 37ºC (98.6ºF). Following incubation, each sample was mixed in a vortex mixer for 15 seconds and 1ml ofsolution was pipetted into a cuvette. Optical Density600 (OD600) of each sample was measured to estimate the concentration of *Enterococcus faecalis*.

CULTURE STUDY: After the placement of medicaments, absorbent paper points were used to take samples from the root canals. These absorbent paper points were introduced into the test tube containing sterile Brain Heart Yeast broth and incubated at 37°C for 24 hours. After incubation, the samples were plated onto sterile Muller Hilton agar and incubated for another 24 hours. Colony counting was done to determine the antibacterial efficacy. The number of colonies is directly proportional to the amount of residual bacteria present in the root canals after placement of medicament.

RESULTS

The results obtained were statistically analysed by One-way analysis of variance (ANOVA) using the SPSS version 2.0, which showed the mean, standard deviation and standard error difference between the group I, group II and group III before and after medication respectively. The values obtained were considered statistically significant as the P value < 0.05. There was statistically significant difference among all the tested groups after medication (Table:1,2)

Table:1

		N	Mean	SD	SE	ANOVA	p
Before Medication	Group I	15	0.135	0.002	0.001	0.313	0.733
	Group II	15	0.136	0.003	0.001		
	Group III	15	0.135	0.003	0.001		
	Total	45	0.135	0.003	0.000		
After Medication	Group I	15	0.233	0.012	0.003	136.87	0.001**
	Group II	15	0.313	0.044	0.011		
	Group III	15	0.139	0.020	0.005		
	Total	45	0.228	0.077	0.012		

**Significant at 1%

Table:2

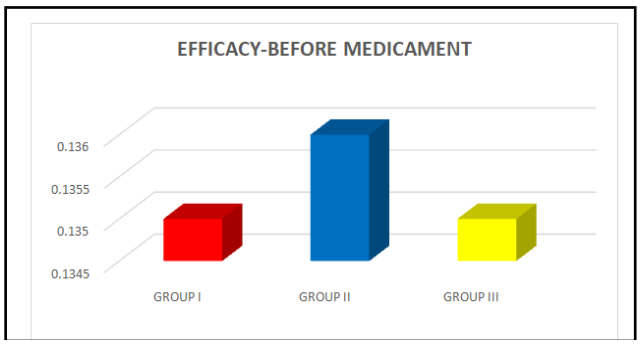
After Medication

Tukey B Post Hoc Test

Group	N	Subset for alpha = .05		
		1	2	3
Group III	15	0.139		
Group I	15		0.233	
Group II	15			0.313

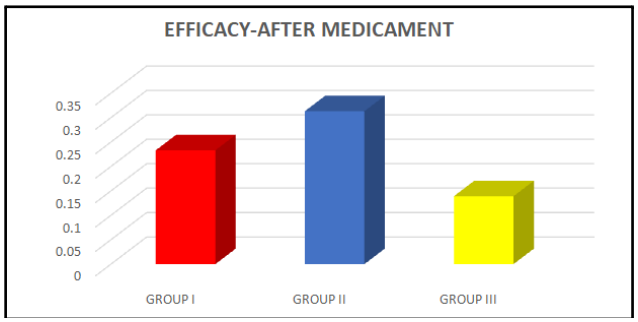
Before medication, the turbidity ofall the samples were more or less same for all the groups when noticed under spectrophotometer for optical density.

The higher level of *Enterococcus faecalis* in the solution indicates increase in the turbidity of the sample solution. The photons of light in the 600nm wavelength gets scattered indicating greater turbidity ofthe sample and then the received photons on the receptor gives the optical density value of that sample.



Graph 1. The mean spectrophotometric values of bacterial samples in each group before medications

When the bacterial growth in the suspended solution decreases, the turbidity ofthe solution also gets decreased which indicates that the photons of the light can passes through the solution and reaches the receptor of the spectrophotometer. Higher the values in the spectrophotometer indicates lower the bacterial count (i.e.) higher the efficacy of the medicament used in this study.



Graph 2. The mean spectrophotometric values of the bacterial samples in each group after medication

The obtained values showed that Group II (Propolis) is better the other groups: group I (Triple antibiotic medicament) and group III (Calcium hydroxide). Thus, Propolis is better in eradicating the gram positive organism, *Enterococccus faecalis* when used as an intracanal medicament.

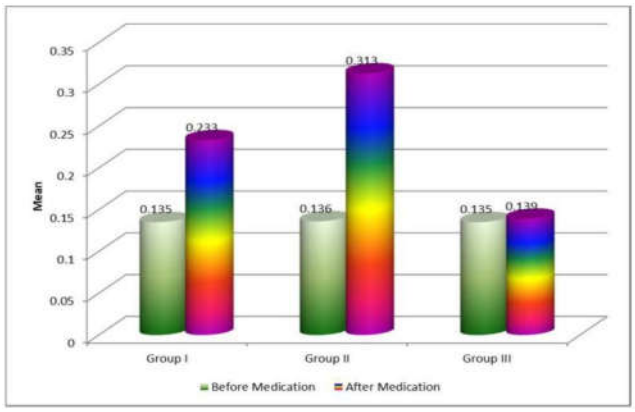
Table: 3

		N	Mean	SD	SE	Paired t	p
Group I	Before Medication	15	0.135	0.002	0.001	31.385	0.001**
	After Medication	15	0.233	0.012	0.003		
Group II	Before Medication	15	0.136	0.003	0.001	15.524	0.001**
	After Medication	15	0.313	0.044	0.011		
Group III	Before Medication	15	0.135	0.003	0.001	0.755	0.463
	After Medication	15	0.139	0.020	0.005		

**Significant at 1%

DISCUSSION

Disinfection ofpulp space is an important step during and after cleaning and shaping.

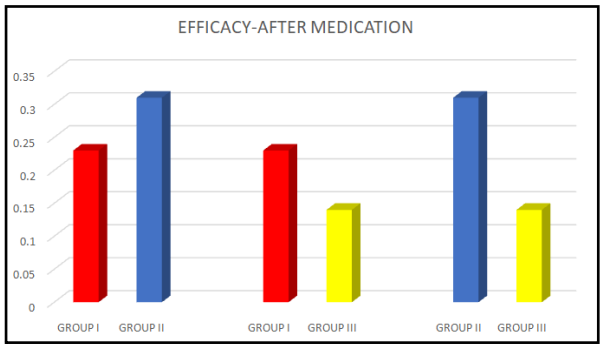


Graph 3. Comparision of spectrophotometric values before and after medication for every sample in each groups

Table 4.

	Mean	Standard deviation	T-value	P-value
GROUP I GROUP II	0.230 0.310	0.012 0.044	-92.14713	<0.00001 S
GROUP I GROUP III	0.230 0.140	0.012 0.020	105.16412	<0.00001 S
GROUP II GROUP III	0.310 0.140	0.044 0.020	182.26536	<0.00001 S

It primarily involves cleaning and shaping the root canal space with endodontic instruments along with irrigants. Still, in certain clinical conditions the polymicrobial nature of the endodontic infection demands the use of an intracanal medicament in addition to the irrigants.[11]



Graph 4. Inter group comparison of spectrophotometric values before and after medication for every sample in each groups.

Therefore, indicating an intracanal dressing to maintain the sanitization process in delicate situations; to reduce endodontic microbiota in primary and secondary infections; control patient exudates; neutralize exertion of the osteoclasts present in the seditious dental resorption; treat large apical periodontitis; favour apical check in apexification; and to treat root perforations.[12] Enterococcus faecalis in dentinal tubules can repel intracanal dressings of calcium hydroxide for over 10 days by forming a biofilm that helps it repel destruction by enabling the bacteria to come 1000 times more resistant to phagocytosis, antibodies, and antimicrobials than non-biofilm producing organisms.[13] Calcium hydroxide, a generally used intracanal medicament, may be ineffective to kill Enterococcus faecalis on its own, if a high pH isn't maintained. Enterococcus faecalis has the capability to form biofilm that can repel calcium hydroxide dressing by maintaining pH homeostasis, but at a pH of 11.5 or lesser, Enterococcus faecalis is unfit to survive.

The frequence of Enterococcus faecalis plant in patient peri-radicular lesions has been shown to be much advanced.[14] The most promising combination in antibiotic that correspond of metronidazole, ciprofloxacin and minocycline. The antimicrobial effect of these medicines collectively showed none of the medicines redounded in complete elimination of microorganism. Metronidazole is a nitro-imidazole emulsion that exhibits a board diapason of exertion against protozoa and anaerobic organism. Ciprofloxacin, a synthetic fluoroquinolone, has a bactericidal mode of action and Minocycline is a semisynthetic outgrowth of tetracycline with an analogous diapason of exertion.[15]

Propolis (Bee cement), a veritably promising material used in herbal drug, is collected by notions from shops or youthful shoots and dinghies of trees. The most recent studies concluded that the effectiveness of Propolis against microorganisms which are largely resistant was plant to be more compatible to the peri-radicular tissues than popular intracanal medicaments. On the other hand, only many studies looked into efficacity of Propolis as intracanal medicament; thus, the end of this study was directed to hold a comparison between the antibacterial effect of Triple antibiotic paste, calcium hydroxide and Propolis in eradication of E. *faecalis*. The result of this study shows that immediate application of all three medicaments could significantly reduce the number of bacteria where ($p<0.001$), the mean value of spectrophotometric values(transmitted light) before application of medicament was found in Triple antibiotic paste (I) (0.135±0.001), Propolis (II) (0.136±0.001) and calcium hydroxide(III) with (0.135±0.001) with almost same values. The result demonstrated that all groups could significantly reduce the number of bacteria from infected root canal where ($p<0.001$), the mean value of spectrophotometric values (transmitted light) after application of intracanal medicament - Triple antibiotic paste (I) was 0.233±0.001 and it was 0.313±00.001 for Propolis (II), and 0.139±0.001 for calcium hydroxide (III) the difference between the three groups is quiet significant.

On comparison of data after application of intracanal medicament (comparing group I and group II) the mean value for Triple antibiotic was 0.233±0.001 and for Propolis was 0.313±0.001 with ($p <0.001$), indicating the test is significant and stated that bacterial efficacy of propolis better than triple antibiotic paste. The results of the current study agree with observations of studies Madhubala MM *et al*. 2011[15] and Hoshino E *et al*. 1996[16] which stated that TAP and Propolis, had the same antibacterial activity against *E. faecalis*. The pharmacological action of minocycline is mainly bacteriostatic with a variety of action in fighting both Gram-positive and Gram-negative bacteria by interfering with protein synthesis of the organism. The pharmacological action of ciprofloxacin is mainly bactericidal action and is extremely more effective against Gram negative bacteria. Lillygrace, E *et al*.2021[17] concluded that Propolis exhibited similar antimicrobial efficacy, which is comparable to triple antibiotic paste. So, propolis can be utilized as an intracanal medicament in young permanent teeth with an open apex. When compared the bacterial reduction for (group I and group III) mean value for Triple antibiotic paste was 0.233±0.001 and for calcium hydroxide was 0.139±0.001 with ($p<0.001$), indicating that test is significant and bacterial efficacy of Triple antibiotic paste is better than Calcium hydroxide. Adl A *et al*. 2012[18] concluded that these results might be attributed to the metronidazole

component of the TAP which is the most pharmacologically effective component in *E. faecalis* eradication; exerting its action through sustained release of its component. Kawashima *et al*. (2009)[19] Calcium hydroxide has been determined as suitable for use as an intracanal medicament as it is stable for long periods, harmless to the body, and bactericidal in a limited area. It also induces hard tissue formation and is effective for stopping inflammatory exudates. Another study by Zapata R *et al*. 2013[20] in which stated that the TAP exhibited a much lower percentage of living bacteria when compared with the 2% chlorhexidine gel and calcium hydroxide in the immediate and second day assessment. TAP was the best in eradicating bacteria when compared with 2% chlorhexidine gel and calcium hydroxide. Comparing bacterial reduction for (group II and group III) mean value for Propolis was 0.313±0.001 and for Calcium hydroxide it was 0.139±0.001 with ($p<0.001$), indicating the quiet significant test and resulting that bacterial efiicacy of Propolis is better than Calcium hydroxide. Ramani *et al.* 2012[21] showed propolis was more effective against *E. faecalis* when compared with chlorhexidine when used as an intracanal medicament. Moreover, when compared with calcium hydroxide, significantly less CFUs were present as documented by Jahromi *et al*. 2012[22] A previous study by Awadeh *et al*. 2008[23] showed that propolis had the highest efficacy against E. faecalis when compared with calcium hydroxide. Despite its excellent properties, calcium hydroxide does not have the same effect over different microorganisms inside root canals. Tanomaru JM *et al.*2003[24] in their study concluded that calcium hydroxide does not have strong effect against *Enterococcus faecalis*, which is a kind of bacteria that is highly resistant to a huge number of antimicrobial agents because of a variety of defense mechanisms, and it's repeatedly found in teeth after failure treatment of root canals. The drawbacks of calcium hydroxide have promoted researchers to look for alternative agents. Calcium hydroxide was ineffective against *E. faecalis* in the studies done by Harrison *et al*.1979[25] and Hemadri*et al*.2011[26] *E. faecalis* is resistant to highly alkaline environment due to the presence of proton pump as a primary resistance mechanism. Nerwich A *et al*. 1993[27] and Love RM 2001[28] concluded in their study that at pH of 11.5 *E. faecalis* cannot survive, however calcium hydroxide medicament result in alkalinity of pH 10.3 within radicular dentin as being reported *in vitro*. This is due to buffering capacity of dentin which provides a decreasing pH from inner to peripheral root dentin. The result of the present study agrees with observation of the investigations held for the comparison between Propolis, calcium hydroxide paste and triple antibiotic paste against *E. faecalis*. Findings implemented that Propolis' effectiveness was greater than triple antibiotic mixture and calcium hydroxide in fighting *E. faecalis*. The reasons for these results may be due to Propolis' main components are phenolic compounds, which include many flavonoids, aromatic acids, and their esters. The pharmacological and the antimicrobial action of Propolis is attributed to its flavonoid content which exhibits its action through inhibiting the synthesis of DNA or RNA in bacteria. Uzel *et al*.2005[29] and. Zohreh *et al*. 2018[30] concluded in their studies.

REFERECES

1. Manzur A, González AM, Pozos A, Silva-Herzog D, Friedman S. Bacterial quantification in teeth with apical periodontitis related to instrumentation and different intracanal medications: a randomized clinical trial. J Endod 2007; Feb 33(2):114-8.
2. Chong BS, Pitt Ford TR. The role of intracanal medication in root canal treatment. Int Endod J 1992 Mar; 25(2):97-106.
3. Stuart CH, Schwartz SA, Beeson TJ, Owatz CB. Enterococcus faecalis: its role in root canal treatment failure and current concepts in retreatment. J Endod 2006 Feb; 32(2):93-98
4. Castilho AL, Saraceni CH, Díaz IE, Paciencia ML, Suffredini IB. New trends in dentistry: plant extracts against Enterococcus faecalis. The efficacy compared to chlorhexidine. Braz Oral Res 2013 Mar-Apr; 27(2):109-115.
5. Athanassiadis B, Abbot PV, Walsh LJ. The use of calcium hydroxide, antibiotics and biocides as antimicrobial medicaments in endodontics. *Australian Dent J* 2007; 52: 64-82.
6. Ørstavik D, Haapasalo M. Disinfection by endodontic irrigants and dressings of experimentally infected dentinal tubules. *Endod Dent Traumatol* 1990; 6: 142-9.
7. Safavi KE, Spangberg LS, Langeland K. Root canal dentinal tubule disinfection. *J Endod* 1990; 16: 207-10.
8. Mahendra M, Agrawal N, Munaga S, Tyagi S. Antimicrobial activity of different biological extracts as intracanal medicament against *Enterococcus faecalis*: An *in vitro* study. Endodontology 2016;28:166-70.
9. Bhutda P.*et al*, Int J Dent Health Sci 2016; 3(5):1183-1188 957
10. Awadeh L, Bertarm AL, Hammad M. Effectiveness of propolis and calcium hydroxide as a short term intracanal medicament against Enterococcus faecalis: a laboratory study. Aust Endod J 2008;34:1–9
11. B. Suresh Chandra and V. Gopikrishna. Grossman's endodontic practice. *13th edition;*324-338.
12. Carlos Estrela.*Endodontic Science* –Volume-2 ; edition-2;747-803
13. Gunnel Svensa¨Ter& Gunnar Bergenholtz.*Biofilms in endodontic infections*. *Endodontic Topics* 2004, 9, 27–36
14. Charles H. Stuart, DDS, Scott A. Schwartz, DDS, Thomas J. Beeson, DDS, AND Christopher B. Owatz, DMD. Enterococcus faecalis: Its Role in Root Canal Treatment Failure and Current Concepts in Retreatment. *Journal of endodontics.*2006;32(2)
15. Rangasamy Vijayaraghavan, Veerabathran Mahesh Mathian, AlagappanMeenakchi Sundaram, Ramachandran Karunakaran, Selvaraj Vinodh. Triple antibiotic paste in root canal therapy. *Journal of pharmacy and bioallied sciences* 2012; 4(2): part-2: 230-233.
16. Hoshino E, Kurihara-Aando N, Sato I, Uematsu H, Sato M, Kota K.In-vitro antibacterial susceptibility of bacteria taken from infected root dentin to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J 1996; 29:125-30.
17. Lillygrace, E., Kethineni, B., Puppala, R., Raichurkar, H. K., Ambati, S., &Saikiran, K. V. (2021). Antimicrobial Efficacy of Triple Antibiotic Paste and Propolis as an Intracanal Medicament in Young Permanent Teeth: An *In Vivo* Study. *International journal of clinical pediatric dentistry*, *14*(2), 243–248. https://doi.org/10.5005/jp-journals-10005-1944
18. Adl A, Shojaee NS, Motamedifar M.A comparison between the antimicrobial effects of triple antibiotic paste and calcium hydroxide against Entrococcus faecalis. Iran Endod J 2012; 7:149–55.

19. Kawashima N, Wadachi R and Suda H. Root canal medicaments. Int Dent J 2009; 59: 5-11.
20. Zapata R, Bramante C, Minotti P, Cavenago B. Antimicrobial activity of Triple antibiotic paste, 2% Chlorhexidine gel, and Calcium Hydroxide on an intraoralinfected dentin biofilm model. J Endod 2013; 39:115-8.
21. Ramani N, Mathew S. Comparative evaluation of antimicrobial efficacy of chlorhexidine digluconate and propolis when used as an intracanal medicament: *Ex vivo* study. J Int Oral Health 2012;4:18-24.
22. ZareJahromi M, Toubayani H, Rezaei M.Propolis: A new alternative for root canal disinfection. Iran Endod J 2012;7:127-33.
23. Awadeh L, Bertarm AL, Hammad M.Effectiveness of propolis and calcium hydroxide as a short term intracanal medicament against Enterococcus faecalis: a laboratory study. AustEndod J 2008;34:1–9.
24. Tanomaru, J. M., Leonardo, M. R., Tanomaru Filho, M., Bonetti Filho, I., & Silva, L. A. (2003). Effect of different irrigation solutions and calcium hydroxide on bacterial LPS. *International endodontic journal*, *36*(11), 733–739. https://doi.org/10.1046/j.1365-2591.2003.00717.x
25. Harrison, J. W., Bellizzi, R., & Osetek, E. M. (1979). The clinical toxicity of endodontic medicaments. *Journal of endodontics*, *5*(2), 42–47. https://doi.org/10.1016/S0099-2399(79)80106-5
26. Hemadri M, Thakurs,Sajjan G. Nisin Vs Calcium Hydroxide – Antimicrobial Efficacy On Enterococcus Feacalis – An In-vitro Study. Int J contemp dent June 2011; 2(3): 55- 61.
27. Nerwich, A., Figdor, D., & Messer, H. H. (1993). pH changes in root dentin over a 4-week period following root canal dressing with calcium hydroxide. *Journal of endodontics*, *19*(6), 302–306. https://doi.org/10.1016/s0099-2399(06)80461-9
28. Love RM. Enterococcus Faecalis – a mechanism for its role in endodontic failure. IEJ 2001. 34: 399-405.
29. Uzel A, Kadriye S, Onçag O, Çogulu D, Gençay O, Salih B. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res 2005; 160: 189-95.
30. Zohreh A, Mandana N, Farzaneh V.Propolis: Chemical Composition and Its Applications in Endodontics. Irani Endod J 2018;13: 285-92

****