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ORIGINAL RESEARCH

An *in vitro* Comparison of Endodontic Medicaments Propolis and Calcium Hydroxide alone and in Combination with Ciprofloxacin and Moxifloxacin against *Enterococcus Faecalis*

[1]Rahul Shrivastava, [2]Vivek Kumar Rai, [3]Aina Kumar, [4]Sonal Sinha, [5]Prerna Tripathi
[6]Khushboo Gupta, [7]Sumit Sabharwal

ABSTRACT

Aim: To evaluate and compare the antimicrobial properties of propolis and calcium hydroxide alone and in combination with ciprofloxacin and moxifloxacin against *Enterococcus faecalis* (*E. Faecalis*).

Materials and methods: The laboratory study was carried out to test the effectiveness of propolis and calcium hydroxidealone as well as in combination with the established endodontic medicaments (moxifloxacin and ciprofloxacin). The various combinations were—group 1: propolis, group 2: calcium hydroxide, group 3: moxifloxacin, group 4: ciprofloxacin, group 5: propolis + moxifloxacin, group 6: propolis + Ciprofloxacin, group 7: calcium hydroxide + ciprofloxacin, group 8: calcium hydroxide + moxifloxacin. The efficacy of these medicaments was tested by checking for the zone of inhibition for the specific strain (ATCC 29212) of *E. faecalis* at different time intervals, i.e. 24, 48 and 72 hours.

Results: Mean zone of inhibition was maximum in group V (21.94 ± 4.26) followed by group VI (18.80 ± 1.93), group I (18.71 ± 4.26), group VIII (15.88 ± 2.59), group III (14.91 ± 1.00), group VII (14.57 ± 2.17), group IV (13.91 ± 1.00) and minimum in group II (12.89 ± 2.14). Mean zone of inhibition was found to be maximum at 72 hours and minimum at 24 hours. At all time intervals, the combination of Propalis with Moxiflocacin showed the maximum antimicrobial efficacy.

Conclusion: On the basis of the results of the present study, it can be concluded that propolis and calcium hydroxide show synergistic effect with moxifloxacin and ciprofloxacin against *E. Faecalis*. Propolis in combination with antibiotics and alone is more effective than calcium hydroxide.

Clinical significance: Since propolis alone and in combination with antibiotics was observed to be more effective than calcium hydroxide, propolis can be considered as an intracanal medicament when compared to traditional calcium hydroxide

Keywords: Calcium hydroxide, Ciprofloxacin, *E. faecalis*, Intracanal medicaments, Laboratory research, Moxifloxacin, Propolis.

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[1-7]Department of Conservative Dentistry and Endodontics Saraswati Dental College, Lucknow, Uttar Pradesh, India

Corresponding Author: Sumit Sabharwal, Postgraduate Student, Department of Conservative Dentistry and Endodontics Saraswati Dental College, Lucknow, Uttar Pradesh, India Phone:01452425650, e-mail: sumit1sabharwal@gmail.com

INTRODUCTION

For successful endodontic treatment, proper diagnosis, thorough chemomechanical preparation, and three-dimensional (3D) obturation of root canal space are paramount. Regardless of these treatment protocols, bacteria can still persist in the complex anatomy of root canal space, thus the ability of intracanal medicament to restrain or eliminate residual bacteria and prevent reinfection may play an increasingly important role in achieving and maintaining a higher success rate of root canal treatment.[1]

The most common species recovered in over one-third of the canals of root filled teeth with persisting periapical lesion is the *Enterococcus faecalis* (*E. Faecalis*). *Enterococcus faecalis* is a Gram-positive, facultative anaerobic, coccoid

bacteria. Medicaments are recommended to eliminate remaining microbes in the root canal, dentinal tubules, accessory canals, canal irregularities (such as fins, transverse anastomoses, apical deltas and other ramifications) and in the periapical/periodontal tissue to reduce periapical inflammation, encourage periapical healing, eliminate apical exudates, control inflammatory root resorption and avert contamination of the canal between appointment.[2]

Earlier strong intracanal antiseptics, such as formacresol and camphorated parachlorophenol were used, but due to their detrimental effects on the connective tissue, their use is limited, nowadays. The excellent biologic and antimicrobial properties of calcium hydroxide have made it, the preferred choice for intracanal dressing of the infected roots canals.[1] Calcium hydroxide has been added to several endodontic sealers to improve their biological properties and to augment their antibacterial activity. When used as an intracanal medicament, the most important property of calcium hydroxide is its strong antimicrobial activity. Its high pH alters the biological lipopolysaccharides in the cell walls of gram-negative species, thereby inactivating the membrane transport mechanisms.[3]

Propolis is a resinous product rich in flavanoid. It has been used as in a number of ways; an anticaries agent, for dentinal hypersensitivity as sealant, storage medium for avulsed tooth, for pulp capping. Propolis is ten times less cytotoxic than calcium hydroxide and has a distinguished antibacterial, antifungal, antiviral, immunomodulatry, antioxidant effect. Recent studies have reported that propolis is more effective against resistant microorganisms and is biocompatible. Antibiotics can be used as an adjunct to endodontic treatment but their ineffectiveness through systemic route of administration has led to the intracanal application, in order to increase their efficacy.[1]

Moxifloxacin and ciprofloxacin are members of the quinolones. Among the drugs commonly used for endodontic infection, ciprofloxacin is indicated due to its efficient action against oral anaerobes, Gram-positive aerobic microorganisms (*Staphylococcus aureus*, *S. epidermidis*, *Sptreptococcus* spp) and Gram-positive enterobacteria (*Escherichia coli*, *Enterobacter* spp and Pseudomonas), which show MIC 90 between 0.015 and 2 μg/ml. All streptococcal species are sensitive to concentrations between 1.0 and 8.0 μg/ml; *S. aureus* and *S. epidermidis* are sensitive to concentrations between 0.25 and 1.0 μg/ml.[4,5] Ciprofloxacin has antimicrobial activity against most Gram-negative bacilli and cocci but limited activity against most Gram-positive organisms. Moxifloxacin is a new fluoroquinolone with expended spectrum of activity, including anaerobes and Gram-positive organisms, especially the multi-resistant ones.[3,6-10] Moxifloxacin has been found to be one of the most active antibiotics against *E. faecalis* with the lowest MIC50 and MIC90. It has proved to be more active than ciprofloxacin which agrees with data that have been reported by several authors.[3,6,8,9,11]

The usage of local antibiotics in endodontic treatment was advocated by Grossman in 1951. Since then numerous antibiotics have been used during endodontic treatment. The antibiotic combination has proved advantageous with the usage of triantibiotic paste.[12]

The laboratory study was carried out to test the effectiveness of propolis and calcium hydroxidealone as well as in combination with the established endodontic medicaments (moxifloxacin and ciprofloxacin).

MATERIALS AND METHODS

The study was carried out in the Department of Conservative Dentistry and Endodontics and Department of Microbiology at Saraswati Dental College, Lucknow, India. Propolis (Hi-Tech Natural product India Ltd.), calcium hydroxide (Vishaldentocare), moxifloxacin (Avelox), ciprofloxacin (Ciplox-500) were used in this study. They were distributed in different groups as presented in Table 1.

The efficacy of these medicaments was tested by checking for the zone of inhibition for the specific strain (ATCC 29212) of *E. faecalis* at different time intervals, i.e. 24, 48 and 72 hours. Specific stain (ATCC 29212) of *E. Faecalis* was procured and grown on brain heart infusion (BHI) broth under favorable temperature overnight and the growth was checked by changes in the turbidity at 24 hours. Blood agar media plate was inoculated with *E. faecalis* (ATCC 29212) by spreading the culture over the surface of the plate with a swab. Intermittent scraping of the culture was done at different sites to make wells for the placement of experimental intracanal medicament. The experimental groups were used in their minimum inhibitory concentration (MIC) against *E. faecalis* (ATCC 29212). Minimum inhibitory concentration of the various antibiotics used in this study was—propalis—340 μg/ml, calcium hydroxide 2500 μg/ml, moxifloxacin 2 μg/ml and ciprofloxacin 8 μg/ml.[2,13]

Table 1: Group distribution

Group 1	Propolis
Group 2	Calcium hydroxide
Group 3	Moxifloxacin
Group 4	Ciprofloxacin
Group 5	Propolis + moxifloxacin
Group 6	Propolis + ciprofloxacin
Group 7	Calcium hydroxide + ciprofloxacin
Group 8	Calcium hydroxide + moxifloxacin

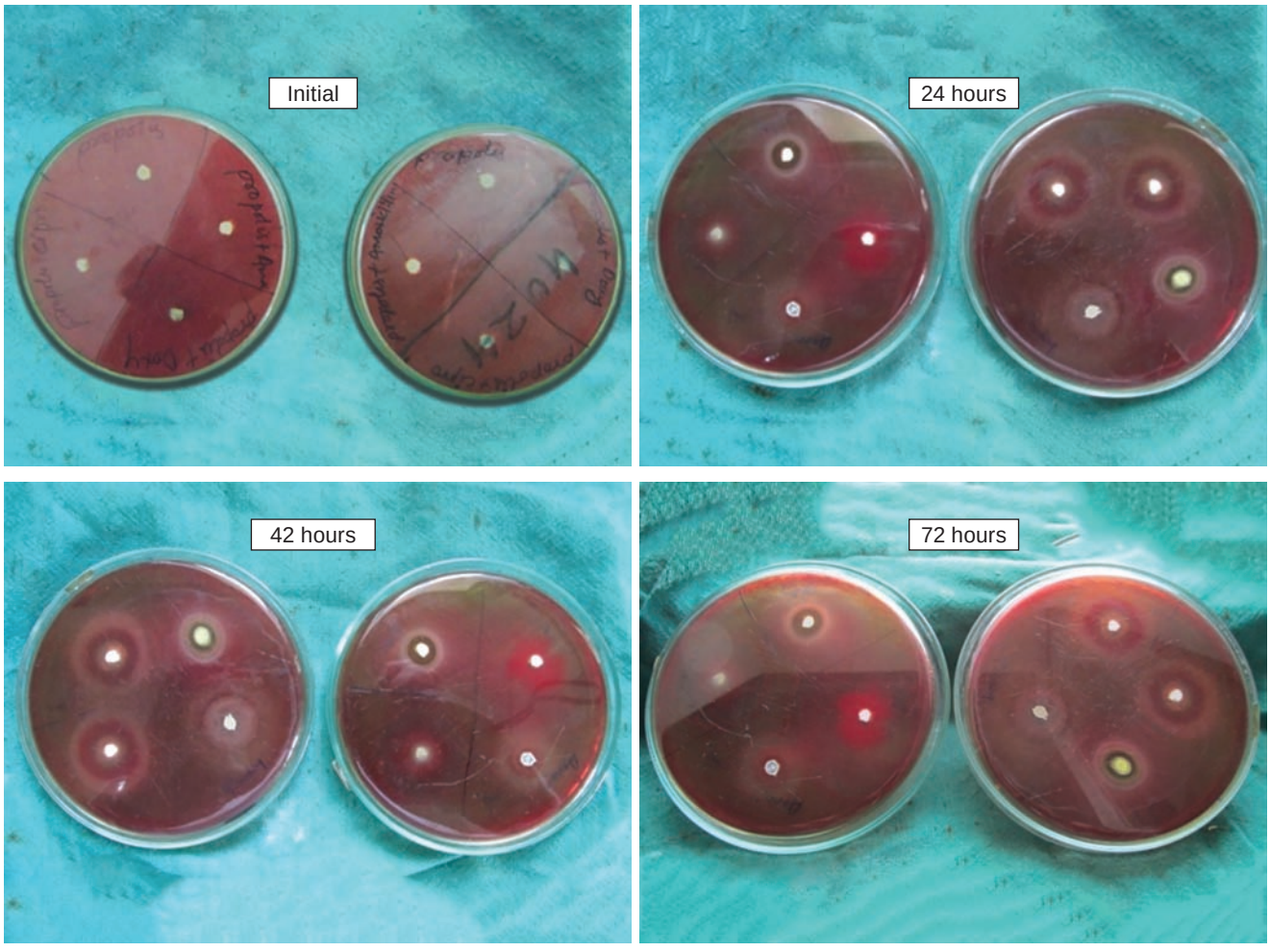

Fig. 1: Zones of inhibition at different time intervals

Propolis, calcium hydroxide and antibiotics were used as a paste in which saline acted as a vehicle. Propolis and calcium hydroxide were mixed separately with saline on a glass slab, with the help of cement spatula, to prepare the paste. For obtaining the antibiotic paste, tablets of ciprofloxacin and moxifloxacin were crushed in mortar and pestle and then mixed with saline on glass slab with cement spatula. Plates were subjected to incubation at 30°C for 24, 48 and 72 hours (Fig. 1) and the diameter of the zones of inhibition were measured in millimeters with HiAntimicrobial Inhibition Zone Scale^{tm}C (Hi-Media Laboratories Pvt Limited, Mumbai) (Fig. 2). The observations were subjected to descriptive and inferential (ANOVA) statistical analysis.

Table 2: Mean zones of inhibition in different groups (Irrespective of time)

Groups	*No. of samples*	*Mean value*	*SD*	*Min.*	*Max.*
I	90	18.71	4.26	12	25
II	90	12.89	2.14	10	25
III	90	14.91	1.00	13	17
IV	90	13.91	1.00	11	16
V	90	21.94	2.55	16	28
VI	90	18.80	1.93	15	23
VII	90	14.57	2.17	11	21
VIII	90	15.88	2.59	11	25

RESULTS

Table 2 shows the mean zones of inhibition in different groups irrespective of time. Zones of inhibitions ranged from 10 (group II) to 28 mm (group VI). Mean zone of inhibition was maximum in group V (21.94 ± 4.26) followed by group VI (18.80 ± 1.93), group I (18.71 ± 4.26), group VIII (15.88 ± 2.59), group III (14.91 ± 1.00), group VII (14.57 ± 2.17), group IV (13.91 ± 1.00) and minimum in group II (12.89 ± 2.14). Table 3 shows the ANOVA applied in different groups. Statistically, intergroup differences were significant ($p < 0.001$). The following order of antimicrobial efficacy of different groups was observed: group V > group I ~ group VI > group VIII ~ group III ~ group VII ~ group IV ~ group II. Table 4 shows the mean zones of inhibition at different time intervals in different groups. Mean zone of inhibition was maximum at 72 hours and minimum at 24 hours, and the intergroup differences were significant. At all the time intervals, group V showed the maximum antimicrobial efficacy.

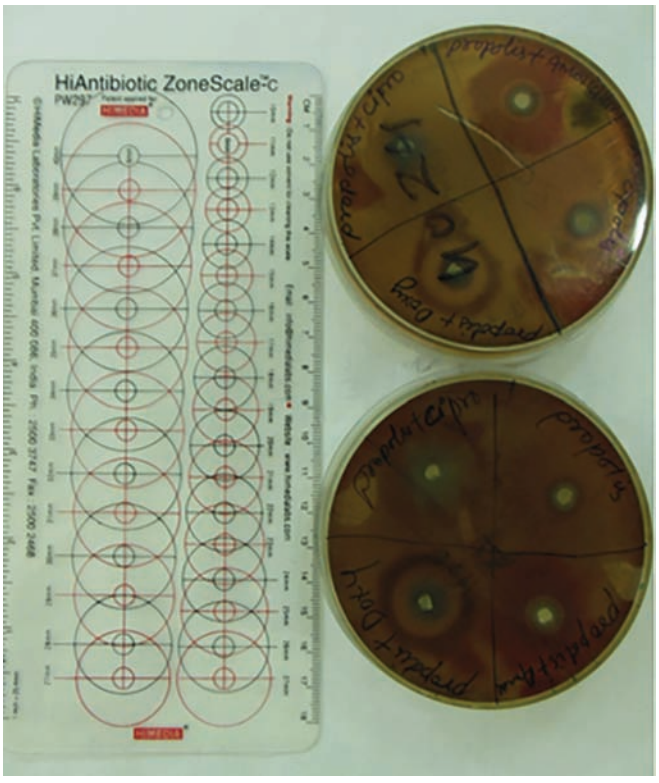

Fig. 2: The HiAntibiotic Zone Scale[tm] C

DISCUSSION

Intracanal medicaments are used to eliminate remnant microbial flora after a thorough chemomechanical preparation of root canal and hasten the healing process. Propolis has been a part of popular medicine since thousands of years and is considered one of the most effective natural products discovered so far.[14,15] Propolis is dark in color. It is produced from material collected from plants by bees and used by them against pathogenic microorganisms. Its anti-inflammatory properties have been described to act mainly against infection, rheumatism, muscular and articular diseases, as well as other types of inflammation.[15-17] The chemical composition of propolis varies widely. Over 200 substances have been identified in the different propolis varieties from various geographical regions, including phenolic acids, flavonoids, esters, aromatic aldehydes, alcohols, amino acids, fatty acids, vitamins and minerals. Special emphasis is given to the flavonoids and phenolic acids which are considered responsible for the biological activity of propolis. Owing to the antimicrobial and anti-inflammatory properties of propolis, it has been suggested as a root canal medicament.[15,18,19]

Calcium hydroxide is one of the main stay of the root canal medicaments. It is a white, odorless powder with low solubility in water, insoluble in alcohol and a high pH. It also has extended clinical action. Moreover, it is biocompatible, has antimicrobial and anti-inflammatory action, and activates the alkaline phosphatase enzyme, which induces mineralized tissue formation and acts in the repair process. It is chemically classified as a strong base, and its association with an adequate vehicle yields an alkaline paste. The success of calcium hydroxide paste as a root canal dressing is related to its dissociation into calcium and hydroxyl ions. The hydroxyl ions alkalinize the environment. To be effective, the hydroxyl ions should be able to diffuse in dentin and remain in pulp tissues in a sufficient concentration to produce the pH level required to destruct bacteria inside the root canal and dentinal tubules[15,20]. Along with various other

Table 3: Analysis of variance of mean zones of inhibition in different groups

	Sum of squares	*Df*	*Mean square*	*F*	*Sig.*
Between groups	5957.47	7	851.07	146.91	< 0.001
Within groups	4124.83	712	5.79		
Total	10082.30	719			

Table 4: Mean zones of inhibition at different time intervals in different groups.

Time interval	*No. of samples*	*Mean value*	*SD*	*Min*	*Max*
Group I					
24 hour	30	13.23	0.97	12	17
48 hour	30	20.10	1.06	18	22
72 hour	30	22.80	1.83	15	25
Group II					
24 hour	30	11.30	1.90	10	20
48 hour	30	13.57	2.30	12	25
72 hour	30	13.80	1.10	12	16
Group III					
24 hour	30	14.90	1.06	13	17
48 hour	30	14.83	0.87	13	16
72 hour	30	15.00	1.08	13	17
Group IV					
24 hour	30	13.90	1.09	11	16
48 hour	30	13.93	0.83	13	15
72 hour	30	13.90	1.09	11	16
Group V					
24 hour	30	19.30	1.15	16	21
48 hour	30	21.70	1.06	20	24
72 hour	30	24.83	1.26	23	28
Group VI					
24 hour	30	16.90	1.03	15	19
48 hour	30	18.73	1.05	16	22
72 hour	30	20.77	1.22	18	23
Group VII					
24 hour	30	12.07	0.91	11	15
48 hour	30	14.83	0.79	14	16
72 hour	30	16.80	1.13	15	21
Group VIII					
24 hour	30	13.13	0.73	11	15
48 hour	30	15.87	1.28	14	19
72 hour	30	18.63	1.65	17	25

studies, Molander A and Tirali et al in their separate studies have found CaOH to have synergistic effect with other endodontic medicaments.[21,22]

Ciprofloxacin, a second generation fluoroquinolone antibiotic, inhibits bacterial DNA synthesis. In dentistry, it is used as a broad spectrum antimicrobial, effective against Gram-negative bacteria (staphylococcus and pseudomonas). Moxifloxacin is a fourth-generation synthetic fluoroquinolone. It differs from earlier antibacterials of the fluoroquinolone class, such as levofloxacin and ciprofloxacin in having greater activity against Gram-positive bacteria and anaerobes. Moxifloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. It functions by inhibiting DNA gyrase, a type II topoisomerase, and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell replication.[12]

First and second generation fluoroquinolones selectively inhibit the topoisomerase II ligase domain, leaving the two nuclease domains intact. This modification, coupled with the constant action of the topoisomerase II in the bacterial cell, leads to DNA fragmentation via the nucleasic activity of the intact enzyme domains. Third and fourth generation fluoroquinolones are more selective for topoisomerase IV ligase domain, and thus have enhanced Gram positive coverage.[12,23]

Antimicrobial property of propolis is due to inhibition of bacterial DNA-dependant RNA polymerases'. Synergism between propolis and antibacterial agents has been observed by Madarova L in 1980. Grange JM and Davey RW found that there was synergism between propolis and antimicrobial drugs against *S. aureus* especially those agents that interfere on bacterial protein synthesis.[24-26] Noori Al-Wahi found propalis to have synergistic properties with honey.[27]

Although calcium hydroxide does not bond to dentin, it does have antibacterial property. Its mechanism of actions are achieved through the ionic dissociation of Ca(+) and OH(–) ions and their effect on vital tissues, the induction of hard-tissue deposition and the antibacterial properties. The lethal effects of calcium hydroxide on bacterial cells are probably due to protein denaturation and damage to DNA and cytoplasmic membranes.[28]

Enteroloccus fecalis appears to be highly resistant to the antimicrobial effect of calcium hydroxide. Evans et al reported that *E. faecalis* was resistant to calcium hydroxide at a pH of 11.1 but unable to survive at higher pH. In radicular dentin, due to its buffering effect, the alkalinity of calcium hydroxide may only reach a pH of 10.3 after intracanal dressing. This could be one of the factors contributing to the resistance of *E. faecalis* to calcium hydroxide. The presence of proton pump in the cell wall of *E. faecalis*, could be another reason for its resistance towards calcium hydroxide.[29]

In the present study, propolis alone and with antibiotic was found to be more effective than calcium hydroxide and calcium hydroxide with antibiotics. Rezende Ceps R et al and Jahromi MZ et al have also found propolis to be more effective than CaOH. Propolis showed the synergistic effect with ciprofloxacin and moxifloxacin at all time intervals.[13,30] In this study, propolis showed maximum efficiency against *E. faecalis* in combination with moxifloxacin followed by ciprofloxacin, which is similar to the results obtained by Krolet et al, Fernandis A et al, Ricardo Oliveria et al, Gheda Helaly et al, Kracko M et al and Stepanovic S et al who found propolis to have synergistic effect with various antibiotics.[31-36]

Calcium hydroxide also showed synergistic effect with antibiotic at all time intervals, which was maximum with moxifloxacin followed by ciprofloxacin at all time intervals.

CONCLUSION

On the basis of the results of the present study, it can be concluded that propolis and calcium hydroxide show synergistic effect with moxifloxacin and ciprofloxacin against *E. Faecalis*. Propolis in combination with antibiotics and alone is more effective than calcium hydroxide. The use of moxifloxacin as an intracanal medicament should be seriously explored. In order to draw more definitive conclusions, a wider and more detailed study needs to be undertaken.

CLINICAL SIGNIFICANCE

Since propolis alone and in combination with antibiotics was observed to be more effective than calcium hydroxide, propolis can be considered as an intracanal medicament when compared to traditional calcium hydroxide.

REFERENCES

1. Ferreira FB, Torres SA, Rosa OP, Ferreira CM, Gracia RB, Marcucci MC, et al. Antimicrobial effect of propolis and other substance against selected endodontic pathogens. Oral Surg Oral Med Oral Pathol 2007;104(5):709-715.
2. Landman D, Mobarakai NK, Quale JM. Novel antibiotic regimens against Enterococcus faecium resistant to ampicillin, vancomycin, and gentamicin. Antimicrob Agents Chemother 1993;37(9):1904-1908.
3. Fuss Z, Weiss EI, Shalhav M. Antimicrobial activity of calcium hydroxide-containing endodontic sealers on Enterococcus faecalis in vitro. Int Endod J 1997;30(6):397-402.
4. Mohammadi Z. Systemic, prophylactic and local applications of antimicrobials in endodontic: an update review. Int Dent J 2009;59(4):175-186.

5. Ferreira MB, Myiagi S, Nogales CG, Campos MS, Lage-Marques JL. Time and concentration dependent cytotoxicity of antibiotics used in endodontic therapy. J Appl Oral Sci 2010;18(3):259-263.
6. Speciale A, Musumeci R, Blandino G, Milazzo I, Caccamo F, Nicoletti G. Minimal inhibitory concentrations and time-kill determination of moxifloxacin against aerobic and anaerobic isolates. Int J Antimicrob Agents 2002;19(2):111-118.
7. Oliphant CM, Green GM. Quinolones: A comprehensive review. Am Fam Physician 2002; 65(3):455-464.
8. Mather R, Karenchak LM, Romanowski EG, Kowalski RP. Fourth generation fluoroquinolones: new weapons in the arsenal of ophthalmic antibiotics. Am J Ophthalmol 2002; 133(4):463-466.
9. Mather R, Stewart JM, Prabriputaloong T, Wong J, McLeod SD. The effect of cataract surgery on ocular levels of topical moxifloxacin. Am J Ophthalmol 2004;138(4):554-559.
10. Andersson MI, MacGowan AP. Development of the quinolones. J Antimicrob Chemother 2003;51(S1).1-11.
11. Pinherio ET, Gomes BP, Drucker DB, Zaia AA, Ferraj CCR, Souza-Filho FJ. Antimicrobial susceptibility of Enterococcus faecalis from canals of root filled teeth with periapical lesions. Int Endod J 2004;37(11):756-763.
12. Somasundaram S, Manivannan K. An Overview of Fluoroquinolones. Annu Rev Res Bio 2013;3(3):296-313.
13. Korstanie JAAH, Willemse JR. Comparative in vitro activity of moxifloxacin against Gram-positive clinical isolates. J Antimicrob Chemother 2000;45(1):31-39.
14. Ghisalbert EL. Propolis: a review. Bee Word 1979;60(2):59-83.
15. Montero JC, Mori GG. Assessment of ion diffusion from a calcium hydroxide-propolis paste through dentin. Braz Oral Res 2012;26(4):318-322.
16. Marcucci MC. Propolis: chemical composition, biological properties and therapeutic activity. Apidologie 1995;26(2): 83-99.
17. Bretz WA, Chiego DJ, Marcucci MC, Cunha I, Custódio A, Schneider LGZ. Preliminary report on the effects of propolis on wound healing in the dental pulp. Z Naturforsch C 1998;53(11-12):1045-1048.
18. Gonsales GZ, Orsi RO, Fernandes Júnior A, Rodrigues P, Funari, S. Antibacterial activity of propolis collected in different regions of Brazil. J Venom Anim Toxins Incl Trop Dis 2006;12(2):276-284.
19. Mori GG, Nunes DC, Castilho LR, de Moraes IG, Poi WR. Propolis as storage media for avulsed teeth: microscopic and morphometric analysis in rats. Dent Traumatol 2010;26(1): 80-85.
20. Mohammadi Z, Dummer PM. Properties and applications of calcium hydroxide in endodontics and dental traumatology. Int Endod J 2011;44(8):697-730.
21. Molander A, Dahien G. Evaluation of the antibacterial potential of tetracycline or erythromycin mixed with calcium hydroxide as intracanal dressing Against Enterococcus Faecalis in vivo. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96(6):744-750.
22. Tirali RE, Gulsahi K, Cehreli SB, Karahan ZC, Uzungolu E, Elhan A. Antimicrobial efficacy of octenidine Hydrochloride, MTAD, chlorhexidene gluconate mixed with calcium hydroxide. J Comtemp Dent Pract 2013;14(3);456-460.
23. Pallo-Zimmerma LM , Byron JK, Graves TK . Fluoroquinolones: then and now. Compend Contin Educ Vet 2010 Jul;32(7):E1-9; quiz E9.
24. Grange JM, Davey RW. Antibacterial properties of propolis (bee glue). J R Soc Med 1990;83(3);159-160.
25. Madarova L. Antibacterial properties of propolis. Ceskoslovenska Stomatologie 1980;80(4):304-307.
26. Fernandes A Júnior, Balestrin EC, Betoni JEC, Orsi Rde O, da Cunha Mde L, Montelli AC. Propolis: anti-Staphylococcus aureus activity and synergism with antimicrobial drugs. Mem Inst Oswaldo Cruz 2005;100(5):563-566.
27. Noori-AL Waili, Al-Ghamdi, Ansari MJ, Salom K, AL-Attal Y. Synergistic effect of honey and propolis toward drug multi-resistant Staphylococcus Aureus, Escherichia Coli and Candida Albicans isolates in single and polymicrobial Cultures. Int J Med Sci 2012;9(9):793-800.
28. Mustafa M, Saujanya KP, Jain D, Sajjanshetty S, Arun A, Laxmi Uppin ,Mahnoor Kadri. Role of calcium hydroxide in endodontic: a review. Global J Medical and Public Health 2012;1(1):66-70.
29. Mimana M, Carnes DL Jr, Walker WA III. pH changes at the surface of root dentin after intracanal dressing with calcium hydroxide. J Endod 2011;27(1):43-45.
30. Rezende GPSR, Costa LRRS, Pimenta FC, Baroni DA. In vitro antimicrobial activity of endodontic pastes with propolis extracts and calcium hydroxide: a preliminary study. Braz Dent J 2008;19(4):301-305.
31. Fernandes Júnior A, Balestrin EC, Betoni JE, Orsi Rde O, da Cunha Mde L, Montelli AC. Propolis: anti-Staphylococcus aureus activity and synergism with antimicrobial drugs. Mem Inst Oswaldo Cruz 2005;100(5):563-566.
32. Krol W, Scheller S, Shani J, Pietsz G, Czuba Z. Synergistic effect of ethanolic extract of propolis and antibiotics on the growth of staphylococcus aureus. Arzneimittelforschung 1993;43(5):607-609.
33. Ricardo de Oliveira Orsi, Sforcin JM, Funari SRC, Fernandes A Junior, Bankova V. Synergistic effect of propolis and antibiotic on the Salmonella Typhi. Braz J Microbiol 2006;37(2): 108-112.
34. Helaly GF, Abd El-Aziz AA, Sonbol FI, El-Banna TE, Louise NL. Dexpanthenol and propolis extract in combination with local antibiotics for treatment of Staphylococcal and Pseudomonal Wound infections. Archives of Clinical Microbiology 2011;4(4:3):1-15.
35. Krocko M, Lavova M, Bezekova J, Canigova M, Gabor M, Duckova V, Trakovická A. Antibiotic resistance of Enterococcus Faecalis Isolated from Gastrointestinal Tract of Broiler chicken after Propolis and Bee Pollen Addition. J Anim Sci Biotec. 2012;45(1):58-63.
36. Stepanović S, Antić N, Dakić I, Švabić-Vlahović M. In vitro antimicrobial activity of propolis and synergism between propolis and antimicrobial drugs. Microbiol Res 2003;158(4): 353-357.