Original Article

Bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400, and polyethylene glycol 1000 against selected microorganisms

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Abstract

Aim: The aim of the present study was to evaluate the bactericidal activity of propylene glycol, glycerine, polyethylene glycol 400 (PEG 400), and polyethylene glycol 1000 (PEG 1000) against selected microorganisms *in vitro*. **Materials and Methods:** Five vehicles, namely propylene glycol, glycerine, PEG 400, PEG 1000, and combination of propylene glycol with PEG 400, were tested for their bactericidal activity. The minimum bactericidal concentration was noted against four standard strains of organisms, i.e. *Streptococcus mutans* American Type Culture Collection (ATCC) 25175, *Streptococcus mutans* ATCC 12598, *Enterococcus faecalis* ATCC 35550, and *Escherichia coli* ATCC 25922, using broth dilution assay. Successful endodontic therapy depends upon thorough disinfection of root canals. In some refractory cases, routine endodontic therapy is not sufficient, so intracanal medicaments are used for proper disinfection of canals. Intracanal medicaments are dispensed with vehicles which aid in increased diffusion through the dentinal tubules and improve their efficacy. Among the various vehicles used, glycerine is easily available, whereas others like propylene glycol and polyethylene glycol have to be procured from appropriate sources. Also, these vehicles, being viscous, aid in sustained release of the medicaments and improve their handling properties. The most commonly used intracanal medicaments like calcium hydroxide are ineffective on many microorganisms, while most of the other medicaments like MTAD (Mixture of Tetracycline, an Acid, and a Detergent) and Triple Antibiotic Paste (TAP) consist of antibiotics which can lead to development of antibiotic resistance among microorganisms. Thus, in order to use safer and equally effective intracanal medicaments, newer alternatives like chlorhexidine gluconate, ozonized water, etc., are being explored. Similarly, the five vehicles mentioned above are being tested for their antimicrobial activity in this study. **Results:** All vehicles exhibited bactericidal activity at 100% concentration. **Conclusion:** Propylene glycol was effective against three organisms namely *S. mutans E. faecalis* and *E. coli* and its bactericidal activity was at 50%, 25% and 50% respectively. PEG 1000 was effective against *S. mutans* and *E. coli at 25%.* Hence propylene glycol was effective on more number of organisms of which *E. faecalis* is a known resistant species. PEG 1000 was bactericidal at a lower concentration but was effective on two organisms only.

Key words: *Bactericidal activity, broth dilution, minimum bactericidal concentration, minimum inhibitory concentration, polyethylene glycol 400 and polyethylene glycol 1000, propylene glycol*

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INTRODUCTION

Last three decades have been very challenging, especially due to the global menace of developing antibiotic resistance.[1] Newer drugs are being discovered with millions and billions being invested in drug research.[2] Instead, the need for developing newer alternatives or unexplored properties of existing agents

which can enhance the activity of pre-existing drugs or antibiotics is definitely a viable option.[3] For instance, in the field of Endodontics, both pediatric and adult, intracanal medicaments are commonly used for proper disinfection of the root canals, especially in refractory cases. Proper disinfection is the mainstay for successful endodontic therapy and it is achieved through the collective effects of biomechanical preparation, irrigation, and intracanal medication.[4-6]

Furthermore, previous studies have shown that bacteria in infected root canals and also periapical tissues (especially furcation area in primary molars) reside deep within dentine, cementum, and periapical tissues too.[7,8] In order to reach these areas effectively, enhanced penetration is attained by means of intracanal medicaments used along with carriers or vehicles, for example, propylene glycol.[9]

One of the properties which have been under-investigated is whether these vehicles or the so-called "excipients" in Pharmaceutics have antimicrobial property or activity on their own. If they really possess antimicrobial activity, they can be used as an effective alternative for disinfection of root canals, with reduced probability of development of antibiotic resistance. In view of the above-mentioned facts, we planned to study the antimicrobial activity of the vehicles propylene glycol, glycerin, polyethylene glycol 400 (PEG 400), polyethylene glycol 1000 (PEG 1000), and a combination of propylene glycol and PEG 400, as this might shed some light on the ideal vehicle for intracanal medicaments. Polyethylene glycol is also referred to as "Macrogol" and the number denotes its molecular weight. Higher the number, higher is its viscosity.[10] The combination of propylene glycol with Macrogol was first used in dentistry *in vivo* by Takushige *et al*., whereas PEG 1000 was used *in vitro* recently by Carreira *et al*.[11,12] To the best of our knowledge and literature search, no study of the bactericidal activity of these vehicles and their comparison has been conducted till date.

MATERIALS AND METHODS

This study was carried out in Dr Prabhakar Kore Basic Science Research Centre, KLE University, Belgaum. It was approved by the Institutional Review Board of KLE University (Ref No. KLEU/Ethic/14-15/D-73) and it is a part of an ongoing *ex vivo* study. The susceptibility of the test organisms to propylene glycol, glycerine, PEG 400, PEG 1000, and propylene glycol with PEG 400 was assessed using broth dilution assay, as minimum inhibitory concentration (MIC) can be readily converted to the minimum bactericidal concentration (MBC). Triplicates were performed for each of the standard strains.

- Culture media: Brain Heart Infusion (BHI) broth
- Test organisms: Four micro-organisms were selected for the study: *Streptococcus mutans* American Type Culture Collection (ATCC) 25175, *Staphylococcus aureus* ATCC 12598, *Enterococcus faecalis* ATCC 35550, and *Escherichia coli* ATCC 25922 [Figure 1]. All microorganisms were previously subcultured in appropriate media and under gaseous conditions to confirm their purity at 35°C for 48 h prior to testing of the vehicles
- Inoculum preparation: The growth method or the log phase method was performed as follows. At least three to five well-isolated colonies of the same morphological type were selected from an agar culture plate. The top of each colony was touched with a loop, and the growth was transferred into a tube containing 4–5 ml of BHI broth. The broth culture was incubated at 35°C for 2–6 h until it achieved the turbidity of the 0.5 McFarland standard. The turbidity of actively growing broth culture was adjusted with broth to obtain a final turbidity optically comparable to that of the 0.5 McFarland standard, done visually by comparing the inoculum tube and the standard against a white card with contrasting black lines
- Broth dilution method: A total of 10 tubes were taken and nine dilutions of the vehicle were done with BHI for MIC and MBC. In the initial tube, only 200 µl of vehicle was added. For further dilutions, 200 µl of BHI broth was added to the next nine tubes separately. In the second tube,

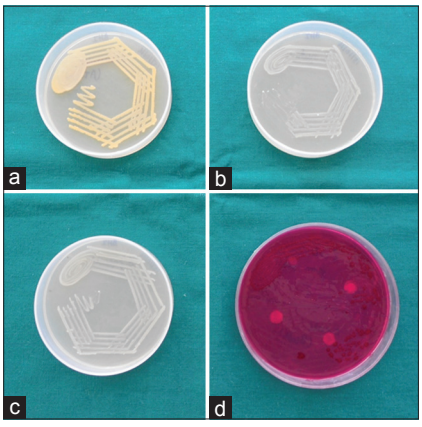

Figure 1: Standard strains against which bactericidal activity of the five vehicles was checked: (a) *Streptococcus mutans* ATCC 25175; (b) *Staphylococcus aureus* ATCC 12598; (c) *Enterococcus faecalis* ATCC 35550; (d) *Escherichia coli* ATCC 25922

200 µl of vehicle was added which already contained 200 µl of BHI broth. This was considered as 10^{-1} dilution. From the 10^{-1} diluted tube, 200 µl was transferred to the second tube to make 10^{-2} dilution. The serial dilution was repeated up to 10^{-8} dilution for each vehicle. From the maintained stock cultures of the required organisms, 5 µl was taken and added to 2 ml of BHI broth. In each serially diluted tube, 200 µl of the above culture suspension was added. The last tube contained only the media and the culture suspension, i.e. the growth control. The tubes were kept for incubation for 24 h at 37°C in bacteriological incubator and observed for turbidity [Figure 2]

- MBC: After recording the lowest concentration inhibiting the growth of organisms as MIC, all the tubes not showing visible growth were subcultured on BHI agar along with the control tube, i.e., growth control, and incubated overnight. The amount of growth was noted; no growth indicated the whole inoculum was killed and this highest dilution showing 99.99% inhibition was recorded as MBC [Figure 3][13]
- Triplicates were performed for each of the standard strains. The experimental data were collected and statistically analyzed using Fisher's exact test.

RESULTS

The MBC results for the vehicles are shown in Table 1. The results showed that all vehicles did exhibit bactericidal activity on the selected microorganisms at different concentrations. Out of all the vehicles, PEG 1000 was the most effective antimicrobial vehicle while glycerine was the least effective on the basis of its MIC. Propylene glycol was the second most effective against all microorganisms except *Sta. aureus*.

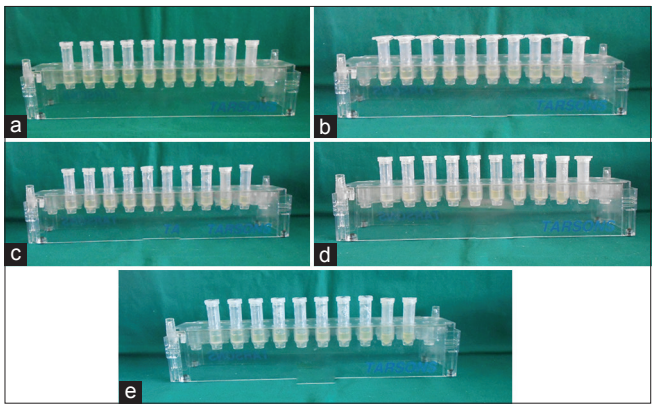

Figure 2: Susceptibility of *Eschericia coli* ATCC 25922 to (a) Propylene glycol, (b) Glycerine, (c) PEG 400, (d) PEG 1000 and (e) Propylene glycol with PEG 400 assessed using Broth dilution assay

Among all the ATCC strains of microorganisms, *Str. mutans* and *Es. coli* were the most susceptible to the vehicles, *En. faecalis* exhibited intermediate susceptibility, and *Sta. aureus* was the most resistant to all the vehicles.

The combination of propylene glycol and PEG 400 did not show any synergistic antimicrobial activity and, in fact, its efficacy decreased against *Str. mutans*, *En. faecalis,* and *Es. coli*, in comparison to propylene glycol alone.

PEG 1000 was most effective bactericidal agent against *Str. mutans* and *Es. coli* among all the five vehicles and the difference was statistically significant (Fisher's exact test: $P = 0.003$ and $P = 0.004$, respectively). For *Sta. aureus* and *En. faecalis*, none of the five vehicles showed statistically significant difference in their bactericidal activity (Fisher's exact test: $P = 1$ and $P = 0.326$, respectively).

DISCUSSION

In endodontic therapy, few cases do not respond to the conventional therapy in the pediatric and adult

Table 1: Bactericidal activity of vehicles against ATCC strains of *Str. mutans, Sta. aureus, En. faecalis* and *Es. coli*

Vehicles	*Str. mutans* (%)	*Sta. aureus* (%)	*En. faecalis* (%)	Es. coli (%)
PG	50	100	25	50
Glycerine	100	100	100	100
PEG 400	100	100	100	100
PEG 1000	25	100	100	25
PG+PEG 400	100	100	100	100

PEG=Polyethylene glycol, PG=Propylene glycol

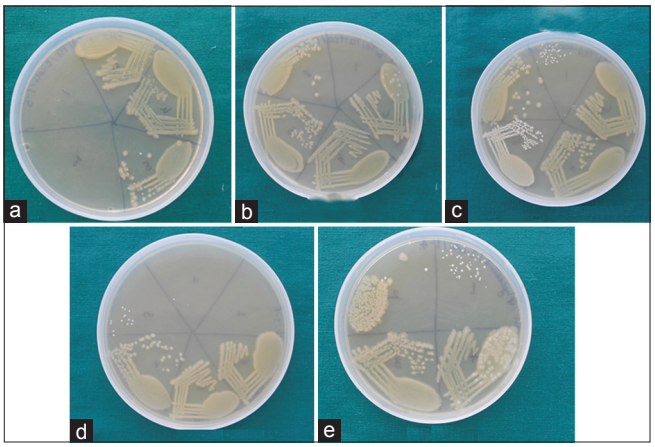

Figure 3: Minimum Bactericidal Concentration (MBC) of *Eschericia coli* ATCC 25922 to (a) Propylene glycol, (b)glycerine, (c) PEG 400, (d) PEG 1000 and (e) Propylene glycol with PEG 400 was assessed after being subcultured on BHI agar

Endodontics. This difficulty to treat such refractory cases may be due to many reasons such as anatomic variation, presence of biofilms, and development of antibiotic resistance.[9] To overcome the problem of the global menace of developing antibiotic resistance, the use of alternative medications and substances or a combination of pharmaceutical excipients is suggested to increase the spectrum of antimicrobial action.[3] The most difficult to tackle is facultative anaerobes, as they are the ones that develop resistance most of the time.[14]

The use of intracanal medicaments becomes mandatory in Endodontics as many non-vital and abscessed teeth lack blood circulation. As a result of this, systemic antibiotics fail to reach the site of infection and, hence, such infections cannot be treated.[15] Also, local drug delivery and sustained release along with better diffusion into the surrounding periradicular tissues may prove to be an added advantage.[9,16] The vehicles used to dispense the intracanal medicaments have a direct influence on the release, time of onset of action of the medicament, penetration of the intracanal medicaments into dentinal tubules, and also the dissociation of drugs. The *in vitro* bactericidal activity of a variety of glycols, especially monopropylene, dipropylene, and triethylene, has been investigated more extensively. The bactericidal activity of PEG 400 had been studied almost three decades ago by Vaamonde *et al*. in 1982 and Chirfe *et al*. in 1983,[17] whereas the antimicrobial effect of PEG 1000 has been studied only recently.[12] The antimicrobial activity of PEG 1000 might be related to the hydrophilic property of PEG. Removal of water possibly does not allow microbial growth, as a certain amount of water is essential for the multiplication and development of microbes.[12] It is interesting to note that PEG 1000, like propylene glycol, also allowed greater dentinal penetration when combined with intracanal medicaments.[9] After a thorough review of literature, we have not come across a single article which explores and compares the bactericidal activity of these pharmaceutical excipients.

Also, after a series of *in vitro* and *in situ* studies, the Cariology Research Unit of the Niigata University School of Dentistry developed the concept of 3M-MP, in which 3M stands for triple mix of antibiotics and MP stands for Macrogol, i.e. polyethylene glycol, and propylene glycol, respectively.[11,18] This group of investigators have carried out extensive research on the most ideal antibiotics to be used for disinfection of dentinal tubules, but there is only one such study regarding penetration of propylene glycol as vehicle for intracanal medicament.[9] Propylene glycol is a known antimicrobial and is an effective preservative.[19] A recent article by Carreira *et al*. published in 2007 has thrown some light on the phenomenon of synergism in bactericidal activity, which was observed in Ciprofloxacin–PEG 1000 association, Metronidazole–PEG 1000 association, and Ciprofloxacin–Metronidazole–PEG 1000 combination. Also, PEG 400 produced severe plasmolysis, cell wall collapse, and finger-like extrusions in *Klebsiella pneumonia*.[20] Promising results have been shown by PEG-coated nanoparticles which were found to be most effective in killing *Es. coli*, *Sta. aureus*, and multi-drug resistant clinical isolates of *Shigella* spp. and *Vibrio cholera*.[21] Of all the vehicles being used in Endodontics, camphorated monochlorophenol (CMCP) is effective but tissue toxic, whereas distilled water possesses no antimicrobial activity.[22]

In our study, all vehicles exhibited antimicrobial activity at 100% concentration. We chose broth dilution over agar well diffusion, as the diffusion of vehicles through agar would be difficult due to them being viscous. Of them, propylene glycol exhibited maximum activity against *En. faecalis*, which was otherwise the least susceptible to the remaining vehicles. In 1965, Olitzky reported that propylene glycol is a known antimicrobial with marked germicidal activity.[23] Glycerine exhibited activity only at 100% concentration and, therefore, was the least effective antimicrobial. This is in accordance with the results observed by Antony *et al*. in 1997.[24] Other studies demonstrated bactericidal activity at 30% and 25% concentration but at a longer incubation time, i.e. after 48 h and 7 days, which is contrary to the findings of the present study.[25]

PEG 400, glycerine, and propylene glycol combination with PEG 400 showed similar bactericidal activity at 100% concentration only, against all the selected organisms. PEG 1000 showed bactericidal activity at the lowest concentration, i.e. at 25% against *Str. mutans* and *Es. coli*, but it exhibited bactericidal activity against *Sta. aureus* and *En. faecalis*, the most resistant of the selected standard strains, at 100% concentration only.

Str. mutans and *Es. coli* were the most susceptible organisms. Susceptibility of *Str. mutans* and *Es. coli* to 25% of PEG 1000 is in accordance with the findings by Carreira *et al*., but the results of *Sta. aureus* and *En. faecalis* vary and are not in agreement, which might be due to the selection of different standard strains. The results of the antimicrobial activity of PEG 400 and glycerine are contrary to the findings of Gomes *et al*.,

which could be because they had used agar diffusion method to test the antimicrobial activity. The negative results may be due to their inability to diffuse through agar due to their viscous nature and not necessarily due to the absence of antimicrobial activity.[12,26] Both PEG and propylene glycol possess low toxicity and are well-recognized vehicles for drugs.[10,27] Also, they result in better handling properties of the resulting paste.[22] Combination of propylene glycol with PEG 400 exhibited a non-synergistic effect. PEG 400 has an inherent advantage that it does not interact with other components of the paste and exerts its antibacterial activity because it has a low water activity.[28]

CONCLUSION

The purpose of conducting this study was to test the bactericidal activity of the above-mentioned vehicles and to determine the vehicle with the maximum bactericidal activity. This will aid in enhancing the diffusion, antimicrobial activity, and release of the intracanal medicaments for a longer period of time.[9] Hence, the most effective antimicrobial vehicle has its applications in Endodontics as an intracanal medicament,[4] in Pedodontics for lesion sterilization and tissue repair,[11,18] in chronic periodontitis for local drug delivery,[16] and also in the emerging field of Regenerative Endodontics.[29] The use of vehicles with good bactericidal activity might reduce the usage of antibiotics and toxic substances like CMCP and encourage the use of innovative substances like chlorhexidine gluconate as intracanal medicaments which do not develop resistance and are more biocompatible.[30]

Within the limitations of this study, of all the vehicles, PEG 1000 followed by propylene glycol were found to have better antimicrobial and bactericidal activities. Also, it had better handling properties of the resulting paste and diffusion through dentinal tubules.[9,12] The use of combination of propylene glycol with PEG 400 is not justified. PEG 1000 has greater bactericidal activity on *Str. mutans* and *Es. coli*.

Further studies should be carried out to verify and compare the diffusion of all the vehicles through dentinal tubules and against clinical isolates *in vitro*. Due to the synergistic action or even nullifying effect of combination of these vehicles with various intracanal medicaments, for instance, commonly used antibiotics, *in vitro* studies should be carried out to find the best medicament and vehicle combination, followed by *in vivo* studies.

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REFERENCES

1. Dhawane BS, Konda SG, Shaikh BM, Chobe SS, Khandare NT, Kamble VT, Bhosale RB. Synthesis and *in vitro* antimicrobial activity of some new 1-thiazolyl-2-pyrazoline derivatives. Int J Pharm Sci Rev Res 2010;1:44-8.
2. Light DW, Lexchin JR. Pharmaceutical research and development: What do we get for all that money? BMJ 2012;345:e4348.
3. Siedenbiedel F, Tiller JC. Antimicrobial polymers in solution and on surfaces: Overview and functional principles. Polymers 2012;4:46-71.
4. Gomes BP, Ferraz CC, Vianna ME, Berber VB, Teixeira FB, Souza-Filho FJ. *In vitro* antimicrobial activity of several concentrations of sodium hypochlorite and chlorhexidine gluconate in the elimination of Enterococcus faecalis. Int Endod J 2001;34:424-8.
5. Peters OA, Peter CI. Cleaning and shaping of root canal. In: Hargreaves KM, Cohen S, editors. Chen's Pathways of Pulp. 10th ed. Haryana, India: Elsevier; 2010. p. 283-348.
6. Young GR, Parashos P, Messer HH. The principles of techniques for cleaning root canals. Aust Dent J 2007;52(Suppl):S52-63.
7. Ando N, Hoshino E. Predominant obligate anaerobes invading the deep layers of root canal dentin. Int Endod J 1990;23:20-7.
8. Peters LB, Wesselink PR, Buijs JF, van Winkelhoff AJ. Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 2001;27:76-81.
9. Cruz EV, Kota K, Huque J, Iwaku M, Hoshino E. Penetration of propylene glycol into dentine. Int Endod J 2002;35:330-6.
10. Rowe RC, Sheskey PJ, Quinn ME. Handbook of Pharmaceutical Excipients. 6th ed. Italy: Pharmaceutical Press and American Pharmacists Association and RPS Publishing; 2009. p. 519-22.
11. Takushige T, Cruz EV, Asgor Moral A, Hoshino E. Endodontic treatment of primary teeth using a combination of antibacterial drugs. Int Endod J 2004;37:132-8.
12. Carreira Cde M, dos Santos SS, Jorge AO, Lage-Marques JL. Antimicrobial effect of intracanal substances. J Appl Oral Sci 2007;15:453-8.
13. Cockerill FR, Wikler MA, Alder J, Dudley MN, Eliopoulos GM, Ferraro MJ, *et al.* CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Second Informational Supplement. CLSI document M100-S22. Wayne PA: Clinical and Laboratory Standards Institute; 2012. p. 136-8.
14. Gaetti-Jardim Júnior E, Landucci LF, Lins SA, Vieira EM, de Oliveira SR. Susceptibility of strict and facultative anaerobes Isolated from endodontic infections to metronidazole and beta-lactams. J Appl Oral Sci 2007;15:539-45.
15. Peedikayil FC. Antibiotics: Use and misuse in pediatric dentistry. J Indian Soc Pedod Prev Dent 2011;29:282-7.
16. Kalsi R, Vandana KL, Prakash S. Effect of local drug delivery in chronic periodontitis patients: A meta-analysis. J Indian Soc Periodontol 2011;15:304-9.
17. Chirfe J, Herszage L, Joseph A, Bozzini JP, Leardini N, Kohn ES. *In vitro* antibacterial activity of concentrated

polyethylene glycol 400 solutions. Antimicrob Agents Chemother 1983;24:409-12.
18. Prabhakar AR, Sridevi E, Raju OS, Satish V. Endodontic treatment of primary teeth using combination of antibacterial drugs: An *in vivo* study. J Indian Soc Pedod Prev Dent 2008;26(Suppl 1):S5-10.
19. Kinnunen T, Koskela M. Antibacterial and antifungal properties of propylene glycol, hexylene glycol, and 1,3-butylene glycol *in vitro*. Acta Derm Venereol 1991;71:148-50.
20. Bozzini JP, Kohn ES, Joseph A, Herszage L, Chirife J. Submicroscopical changes in Klebsiella pneumoniae cells treated with concentrated sucrose and polyethylene glycol 400 solutions. J Appl Bacteriol 1986;60:375-9.
21. Bhattacharya D, Samanta S, Mukherjee A, Santra CR, Ghosh AN, Niyogi SK, *et al*. Antibacterial activities of polyethylene glycol, tween 80 and sodium dodecyl sulphate coated silver nanoparticles in normal and multi-drug resistant bacteria. J Nanosci Nanotechnol 2012;12:2513-21.
22. Ganesh MR, Chaurasia VR, Masamatti VK, Mujeeb A, Jhamb A, Agarwal JH. *In vitro* evaluation of antibacterial efficacy of calcium hydroxide in different vehicles. J Int Soc Prev Community Dent 2014;4:56-60.
23. Olitzky I. Antimicrobial properties of a propylene glycol based topical therapeutic agent. J Pharm Sci 1965;54:787-8.
24. Anthony DR, Gordon TM, del Rio CE. The effect of three vehicles on the pH of calcium hydroxide. Oral Surg Oral Med Oral Pathol 1982;54:560-5.
25. Barr M, Tice LF. A study of the inhibitory concentration of various sugars and polyols on the growth of microorganisms. J Am Pharm Assoc Am Pharm Assoc (Baltim) 1971;46:219-21.
26. Gomes BP, Ferraz CC, Vianna ME, Rosalen PL, Zaia AA, Teixeira FB, *et al*. *In vitro* antimicrobial activity of calcium hydroxide pastes and their vehicles against selected microorganisms. Braz Dent J 2002;13:155-61.
27. Li BQ, Dong X, Fang SH, Gao JY, Yang GQ, Zhao H. Systemic toxicity and toxicokinetics of a high dose of polyethylene glycol 400 in dogs following intravenous injection. Drug Chem Toxicol 2011;34:208-12.
28. Ambrose U, Middleton K, Seal D. *In vitro* studies of water activity and bacterial growth inhibition of sucrose-polyethylene glycol 400-hydrogen peroxide and xylose-polyethylene glycol 400-hydrogen peroxide pastes used to treat infected wounds. Antimicrob Agents Chemother 1991;35:1799-803.
29. Vijayaraghavan R, Mathian VM, Sundaram AM, Karunakaran R, Vinodh S. Triple antibiotic paste in root canal therapy. J Pharm Bioallied Sci 2012;4 (Suppl 2):S230-3.
30. Komorowski R, Grad H, Wu XY, Friedman S. Antimicrobial substantivity of chlorhexidine- treated bovine root dentin. J Endod 2000;26:315-7.

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