ORIGINAL RESEARCH

Comparative Evaluation of Microbial Reduction Using Silver Diamine Fluoride, *Azadirachta indica* (Neem) and Sodium Hypochlorite as Root Canal Irrigants after Biomechanical Preparation in Uniradicular Canals: An *In Vivo* Study

Shruti Raj[1], Anshul Sharma[2], Kavita Dhinsa[3], Sonali Saha[4], Gunjan Yadav[5], Prerna Sharma[6]

Abstract

Background: It is difficult to completely eradicate microorganisms from the infected root canal system. Intracanal irrigants seem necessary for eradication of infected tissues and microorganisms in addition to mechanical debridement. Continuous use of chemical antimicrobial agents leads to serious side effects. Therefore, the need arises for alternative agents to overcome the disadvantages of their chemical counterparts.

Aim: To evaluate and compare the antimicrobial efficacy of three endodontic irrigants against clinically isolated bacteria found in root canals containing necrotic pulp.

Materials and methods: Preirrigation sample were collected using sterile paper points and sent for microbial count. Chemomechanical preparation was performed, and the root canals were irrigated with 5 mL of test samples. After 3 days, the patient was recalled, and a postirrigation sample was collected and sent for microbial count.

Result synthesis: Both 3.8% silver diamine fluoride (SDF) and 3% sodium hypochlorite (NaOCl) showed a superior capacity to sterilize the root canals compared to the neem group.

Conclusion: The use of SDF as an endodontic irrigant is feasible as it effectively removes the microbes present in the canal.

Clinical significance: Silver diamine fluoride has not been shown to be cytotoxic or carcinogenic, unlike NaOCl, suggesting it could be used as a potential endodontic irrigant. However, few studies have evaluated the antimicrobial efficacy of SDF as an endodontic irrigant.

Keywords: Neem, Silver diamine fluoride, Sodium hypochlorite.

International Journal of Clinical Pediatric Dentistry (2024): 10.5005/jp-journals-10005-2862

Introduction

Endodontic therapy deals with the successful preservation of teeth that are pulpally and periapically involved, aiming to maintain functions, esthetics, and arch integrity.[1,2] However, complete disinfection of root canals cannot be achieved by mechanical instrumentation alone due to the complexity of radicular configuration.[3]

Various intracanal irrigants, such as sodium hypochlorite (NaOCl) and normal saline (NaCl), are commonly used.[4]

Sodium hypochlorite has excellent antimicrobial and tissue-dissolving properties.[5] However, it requires careful handling as several factors are associated with its safety concerns.[6]

Herbal alternatives to irrigating solutions have been sought to eliminate side effects and limit the increase in antibiotic-resistant strains. Herbal irrigants offer advantages such as reduced toxicity, minimal microbial resistance, and ease of access.[7]

According to Briseño-Marroquín et al., among various herbal extracts, neem extract has shown maximum antimicrobial and antifungal efficacy.[8]

Briseño-Marroquín et al. indicated that silver, specifically as ammoniacal silver nitrate, effectively sterilized the infected part of coronal and radicular dentin.[8]

Silver diamine fluoride (SDF), an anticariogenic agent (noncytotoxic, noncarcinogenic), is deemed to be very effective. It hinders the growth of cariogenic biofilm formed on dentine surfaces and hence prevents caries progression and dentin demineralization.[9] An SDF solution with a concentration of 3.8% w/v was developed as an irrigating solution.[10] However, there is a paucity of literature comparing the antimicrobial efficacy of SDF as an irrigating solution with other irrigants.[9]

[1–6]Department of Pedodontics and Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, Uttar Pradesh, India

Corresponding Author: Sonali Saha, Department of Pedodontics and Preventive Dentistry, Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, Uttar Pradesh, India, Phone: +91 9889234995, e-mail: drsonalisaha24@gmail.com

How to cite this article: Raj S, Sharma A, Dhinsa K, *et al.* Comparative Evaluation of Microbial Reduction Using Silver Diamine Fluoride, *Azadirachta indica* (Neem) and Sodium Hypochlorite as Root Canal Irrigants after Biomechanical Preparation in Uniradicular Canals: An *In Vivo* Study. Int J Clin Pediatr Dent 2024;17(6):625–629.

Source of support: Nil

Conflict of interest: None

Materials and Methods

The present study was carried out in the Department of Pedodontics and Preventive Dentistry Sardar Patel Post Graduate Institute of Dental and Medical Sciences, Lucknow, Uttar Pradesh, India.

For this purpose, 76 patients requiring endodontic treatment with relevant history, clinical, and radiological examinations were selected based on the inclusion criteria—healthy patients, teeth with sufficient tooth structure to support rubber dam isolation, single-rooted teeth with necrosed pulp, abscess or sinus openings, radiolucencies present periapically or in the furcation region involving at least two-thirds of the root structure. Patients under antibiotic coverage for 2 weeks before sample collection, those with systemic complications, irreplacable teeth, perforated pulpal floors, grade III mobility, more than one-third pathological root resorption, or teeth with well-defined large periapical radiolucency with a corticated border suggestive of a radicular cyst were not included in the study.

Around 66 single-rooted permanent teeth were randomly allocated to one of the three groups, respectively, among the irrigants being tested (22 each)—group I—3.8% SDF, group II—*Azadirachta indica* (neem), group III—3% NaOCl. Additionally, 10 teeth were included as negative controls and irrigated with 0.9% NaCl.

After radiographic examination, complete isolation was achieved with a rubber dam. Surfaces were disinfected using iodine tincture both before and after initial access cavity preparation with a high-speed air turbine and a round diamond bur. Cavity walls were refined using a safe-end cutting diamond bur. The canals were irrigated with 0.9% sterile saline solution, followed by the introduction of a sterile 20K file into the canal, filing to a length 1 mm short of the radiographic root apex in a smooth motion. Biomechanical preparation was completed in a single appointment for all cases. Teeth were irrigated using one of the experimental irrigating solutions.

Sample collection was conducted before and after treatment completion for both aerobic and anaerobic microorganisms, referred to as pretreatment and posttreatment samples. The pretreatment sample was taken after access opening, while the posttreatment sample was collected after irrigating with the experimental irrigants. Sterile paper points were used to dry the canals for a minimum of 60 seconds, ensuring they reached the same level to absorb fluid from the canal. These paper points were then transferred into two separate tubes for culturing under aerobic and anaerobic conditions.

Peptone broth was used as a transport medium for aerobic culturing techniques, and 5% Columbia sheep blood agar plates were used for anaerobic cultures. The contaminated broth was inoculated onto 5% Columbia sheep blood agar culture plates.

After collecting the posttreatment samples, a standard operating protocol was followed. The teeth were treated with calcium hydroxide as an intracanal medicament, followed by obturation with gutta-percha using the lateral compaction technique and zinc oxide eugenol as a sealant. Final restoration was completed using composite resin (tetric N-ceram). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 21.

Results

On intergroup comparison, no significant difference was found among the pretreatment samples (S1) of the study groups for aerobic colony counts. However, in the posttreatment samples (S2), the mean colony count was significantly reduced in group I (SDF) and group III (NaOCl) compared to group II (neem) and group IV (NaCl).

On intragroup comparison, a significant reduction was observed in colony counts in group I (SDF) and group III (NaOCl) from pretreatment samples (S1) to posttreatment samples (S2). However, this reduction was found to be insignificant in group II (neem) and group IV (NaCl) (Table 1).

On intergroup comparison, no significant difference was found among the pretreatment samples (S1) of the study groups for anaerobic colony counts. However, in the posttreatment samples (S2) after irrigation with the study solutions, mean colony counts were significantly reduced in group I (SDF) and group III (NaOCl) compared to group II (neem) and group IV (NaCl). Based on this observation, the antimicrobial efficacy was highest in SDF, followed by sodium hypochlorite, neem, and saline, respectively.

On intragroup comparison, statistically significant reductions were observed in all groups from the pretreatment samples (S1) to the posttreatment samples (S2) (Table 2).

On intergroup comparison of percentage reduction in aerobic colony counts, group I (SDF) and group III (NaOCl) showed

Table 1: Intergroup and intragroup comparison of pretreatment and posttreatment mean colony counts of aerobic microorganisms

			Aerobic				
			Sample 1 colony counts (pretreatment) (×10⁴)		*Sample 2 colony counts (posttreatment) (×10⁴)*		
		N	*Mean*	*Standard deviation (SD)*	*Mean*	*SD*	*p[a] value of intragroup comparison*
Aerobic	Group I (SDF)	22	566.82	609.07	48.77	58.24	<0.001, S
	Group II (neem)	22	983.64	753.69	842.32	735.49	0.120, NS
	Group III (NaOCl)	22	675.91	497.19	48.86	51.38	<0.001, S
	Group IV (saline)	10	732.0000	757.39026	704.0000	740.30324	0.007, S
	p[b] value		0.187, NS		<0.001		
	Post hoc pairwise comparison[c]		Group I * Group II—0.151, NS Group I * Group III—0.944, NS Group I * Group IV—0.908, NS Group II * Group III—0.397, NS Group II * Group IV—0.738, NS Group III * Group IV—0.996, NS		Group I * Group II—<0.001, S Group I * Group III—0.999, NS Group I * Group IV—0.003, S Group II * Group III—<0.001, S Group II * Group IV—0.872, NS Group III * Group IV—0.003, S		

*,S, significance; NS, nonsignificance; [a]Paired *t* test; [b]One way ANOVA; [c]Turkey's test

significantly greater reductions compared to group II (neem) and group IV (saline).

On intergroup comparison of percentage reduction in anaerobic colony counts, group I (SDF) showed the maximum reduction, followed by group III (NaOCl), group II (neem), and group IV (NaCl) in descending order (Table 3).

Discussion

Microorganisms are considered the primary cause of pulpal and periapical diseases due to the high number of them present in infected root canals.[11] The primary objective of root canal treatment is the complete removal of pulpal tissues to achieve thorough disinfection of root canals and eliminate microbes.[12] The goal is to maintain aseptic conditions both before and after obturation and to remove the source of infection from the radicular system.[13] Biomechanical preparation is the primary step for the elimination of microbes. However, completely removing microorganisms from the root canal system can be challenging.[12] Therefore, the use of irrigating solutions plays an important role in the removal of necrotic tissue,[14] thereby facilitating the removal of microorganisms and dentin chips from the root canal by the flushing action.[3]

Various irrigants are available in the field of dentistry, such as chlorhexidine gluconate, NaOCl, etc.[15]

Sodium hypochlorite shows a higher dissolving effect on the necrotic tissue than as compared to the vital tissue.[16] It shows a lack of tooth discoloration and bioavailability.[17]

According to Berber et al., NaOCl is most efficient when used at concentrations of 0.5, 2.5, and 5.25%. However, higher concentrations may potentially damage the ultrastructure of dentin, thereby affecting the apical and periapical tissues.[18] Also stated by Tennert et al., NaOCl has been proven to be a potent antimicrobial agent as a root canal irrigant at a concentration of 3%.[19] Therefore, in the present study, a 3% concentration of NaOCl was chosen to assess its efficacy on the microorganisms present in the root canal, as it is less toxic and commercially available.[20]

Herbal extracts have been utilized in dentistry as anti-inflammatory, antioxidant, antimicrobial, and analgesic agents.[21] In a study conducted by Hedge and Kesaria, the antimicrobial efficacy of NaOCl (2%), propolis, neem leaf extract, turmeric, and licorice was assessed. They concluded that neem leaf extract exhibited inhibition against *Enterococcus faecalis* and *Candida albicans*.[22]

On the other hand, in recent times, SDF has been recognized as an effective anticariogenic agent. It has the capacity to remineralize

Table 2: Intergroup and intragroup comparison of pretreatment and posttreatment mean colony counts of anaerobic microorganisms

			Anaerobic				
			Sample 1 (pretreatment) colony counts (×10⁴)		*Sample 2 (posttreatment) colony counts (×10⁴)*		
		N	*Mean*	*SD*	*Mean*	*SD*	*p-value of intragroup comparison*[c]
Anaerobic	Group I (SDF)	22	222.18	124.17	18.22	11.48	<0.001, S
	Group II (neem)	22	314.00	248.26	291.91	248.88	<0.001, S
	Group III (NaOCl)	22	185.45	110.31	95.91	76.54	<0.001, S
	Group IV (saline)	10	390.0000	326.01977	378.0000	326.38764	0.005, S
	p[b] value		0.026, S		<0.001, S		
	Post hoc pairwise comparison[c]		Group I * Group II—0.422, NS Group I * Group III—0.927, NS Group I * Group IV—0.128, NS Group II * Group III—0.147, S Group II * Group IV—0.707, NS Group III * Group IV—0.041, S		Group I * Group II—<0.001, S Group I * Group III—0.494, NS Group I * Group IV—<0.001, S Group II * Group III—0.003, S Group II * Group IV—0.604, NS Group III * Group IV—0.001, S		

*,S, significance; NS, nonsignificance; [b]One way ANOVA; [c]Turkey's test

Table 3: Intergroup comparison of percentage reduction in mean colony counts of aerobic and anaerobic microorganisms

		Percentage reduction in colony counts from S1 (pretreatment) to S2 (posttreatment)			
		Aerobic		*Anaerobic*	
	N	*Mean*	*SD*	*Mean*	*SD*
Group I (SDF)	22	92.2663	2.11299	92.2346	1.15518
Group II (neem)	22	22.4127	30.98872	11.4947	17.25432
Group III (NaOCl)	22	93.5871	3.66394	47.8076	22.56136
Group IV (saline)	10	8.2270	8.40892	3.5611	6.94242
p[b] value		<0.001, S		<0.001, S	
Post hoc pairwise comparison[c]		Group I * Group II—<0.001, S Group I * Group III—0.994, NS Group I * Group IV—<0.001, S Group II * Group III—<0.001, S Group II * Group IV—0.142, NS Group III * Group IV—<0.001, S		Group I * Group II—<0.001, S Group I * Group III—<0.001, S Group I * Group IV—<0.001, S Group II * Group III—<0.001, S Group II * Group IV—0.542, NS Group III * Group IV—<0.001, S	

*,S, significance; NS, nonsignificance; [b]One way ANOVA; [c]Turkey's test

the tooth surface and increase its hardness, thereby possessing cariostatic action.[23]

Apart from this, saline has also been used as a neutral irrigant to remove debris from the canal, thereby providing antimicrobial efficacy to the root canal. Hence, NaCl was used as a control in the study.[24]

All the studies have used these antimicrobial agents *in vitro* investigations, but few have reported *in vivo* investigations. Therefore, our study was conducted *in vivo*.

The study was conducted on single-rooted permanent teeth, following the protocols of Sonarkar et al. and Podar et al. According to these authors, single-rooted teeth have wider and straighter canals, which allows for the assessment of disinfection protocols without procedural errors.[25,26]

In our study, microbial samples were collected using paper points from inside the root canals, not from the dentinal tubules or deeper areas. This approach was chosen to minimize variability in the quantity and type of microbial isolation from the root canal system. The method of sample collection was consistent with that conducted by Sivakumar et al. and Maru et al.[10,27]

The transport media used was peptone broth, known for supporting rapid microbial growth. Contaminated broth was inoculated onto 5% Columbia sheep's blood agar culture plates. These agar plates are commonly used for both aerobic and anaerobic microorganisms because they can be incubated in an atmosphere containing 3–10% carbon dioxide.[26]

Nasr et al. stated that the microbial culture method is effective for assessing antimicrobial effectiveness because it allows quantification of the amount of microorganisms present in the sample.[28]

The results showed that on intragroup comparison for both aerobic and anaerobic microorganisms, there was a statistically significant reduction in the percentage reduction of microbial colony counts from pretreatment to posttreatment samples in the neem group, SDF group, NaOCl group, and saline group. This finding aligns with the studies conducted by Maru et al. and Shah et al.[10,20] As recommended by Gomes-Filho et al. SDF can be used as an alternative root canal irrigating because it possesses bactericidal potential and is compatible with the root canal at low concentrations.[29]

Conversely, Weber et al. stated that NaOCl was more effective than SDF because silver ions from SDF can cause grey discoloration in the root canals, which may lead to lower acceptance by patients.[30]

A significant percentage reduction in microbial colony counts from pretreatment to posttreatment samples with NaOCl was observed. This finding is consistent with Khandelwal et al., who highlighted that the antimicrobial effect, tissue dissolution ability, and appropriate biocompatibility of less concentrated solutions are crucial characteristics of this irrigant.[31]

Contrary to the results of our present study, Sivakumar et al. stated that NaOCl is cytotoxic, chemically unstable, and interferes with the adherence of restorative materials to the dentinal surface. They further reported that NaOCl causes significant changes in dentinal structure and a loss in dentin's mechanical characteristics.[27]

Prasad et al. reported that despite its antioxidant and antibacterial properties, the inhibitory activity of neem leaf extract varies depending on the organism and solvent used. Additionally, its bitter taste may contribute to lower patient acceptance.[32]

Divya and Sujatha stated that flushing debris and removing pulpal tissue from the root canal enhances NaCl's efficiency as an irrigant.[33]

Jain et al. and Afshan et al. stated that saline primarily possesses the ability to remove root canal debris rather than exhibiting significant antimicrobial properties.[4,24]

On intergroup comparison, the SDF group showed a significantly higher percentage reduction in microbial colony counts compared to NaOCl, which in turn was significantly higher than the neem and saline groups. This finding aligns with Shah et al.[20]

Also stated by Mathew et al., SDF solution can be used as an endodontic irrigating solution because it efficiently eliminates microorganisms present in the circumpulpal dentin and canal. Additionally, it inhibits bacterial cell wall synthesis, cell division, and unwinding of DNA, thereby reducing the microbial count in the root canal.[13] SDF has an advantage over other irrigants in that besides having similar antibacterial characteristics; it can interact with teeth and SDF. It also causes the formation of fluorapatite. This increases the antibacterial activity of SDF, increases longevity and enables prevention of reinfection inside the root canal. [34]

Jose et al. stated that the presence of chlorine in hypochlorite solution is responsible for its antimicrobial action. Chlorine inhibits bacterial cell wall synthesis and causes irreversible oxidation of sulfhydryl groups present in bacterial enzymes.[35]

Conversely, as stated by Maru et al., NaOCl can cause toxicity upon contact with periradicular tissues, leading to discomfort for the patient due to its taste and smell.[10] Thus, making NaOCl inferior to SDF.

Conclusion

To conclude, within the limitations of the study, it became evident that SDF can serve as a superior alternative root canal irrigant in dentistry. Further investigation is warranted to explore its potential benefits in greater detail.

Clinical Significance

Silver diamine fluoride has been proven to be an anticariogenic agent in pediatric dentistry. Additionally, it is noncytotoxic and noncarcinogenic compared to chlorhexidine or NaOCl. SDF has also demonstrated biocompatibility as an endodontic irrigant; however, further studies are needed to expand the literature on this topic.

References

1. Paudel KR, Jaiswal A, Parajuli U, et al. Different pharmacological solutions in intracanal irrigation. Nepal Med Coll J 2011;13(2):111–114.
2. Saha S, Samadi F, Jaiswal JN, et al. Antimicrobial activity of different endodontic sealers: an *in vitro* evaluation. J Indian SocPedodPrev Dent 2010;28(4):251–257. DOI: 10.4103/0970-4388.76151
3. Ambhore S, Allwani V, Rathod J. Influence of routinely used root canal irrigants vs herbal extracts on fracture resistance of endodontically treated teeth. Glob J Res Anal 2018;7(2):48–51.
4. Afshan T, Parwez A, Prasanna PL, et al. Comparison of antimicrobial efficacy of herbal root canal irrigants (*Azadirachta indica*, Morinda citrifolia) against Enterococcus faecalis. World J Dent 2020;11(3):206–210. DOI: 10.5005/jp-journals-10015-1733
5. Kandaswamy D, Venkateshbabu N. Root canal irrigants. J Conserv Dent 2010;13(4):256–264. DOI: 10.4103/0972-0707.73378
6. Jaiswal N, Sinha DJ, Singh UP, et al. Evaluation of antibacterial efficacy of chitosan, chlorhexidine, propolis and sodium hypochlorite on Enterococcus faecalis biofilm: an *in vitro* study. J ClinExp Dent 2017;9(9):1066–1074. DOI: 10.4317/jced.53777
7. Kulkarni MU, Desai N. An *in vitro* evaluation of antimicrobial efficacy of 5% sodium hypochlorite, 2% chlorhexidine, and herbal extracts of neem and Aloe vera in disinfection of gutta-percha cones. Saudi Endod J 2019;9(3):181–185. DOI: 10.4103/sej.sej_110_18

8. Briseño-Marroquín B, Ismael Y, Callaway A, et al. Antibacterial effect of silver diamine fluoride and potassium iodide against E. faecalis, A. naeslundii and P. micra. BMC Oral Health 2021;21(1):175. DOI: 10.1186/s12903-021-01531-1
9. Chu CH, Mei L, Seneviratne CJ, et al. Effects of silver diamine fluoride on dentine carious lesions induced by Streptococcus mutans and Actinomycesnaeslundii biofilms. Int J Paediatr Dent 2012;22(1):2–10. DOI: 10.1111/j.1365-263X.2011.01149.x
10. Maru V, Padawe D, Naik S, et al. Assessment of bacterial load using 3.8% SDF as an irrigant in pulpectomized primary molars: a randomized controlled trial. Int J ClinPediatr Dent 2022;15(Suppl 1):47–51. DOI: 10.5005/jp-journals-10005-2130
11. Ercan E, Ozekinci T, Atakul F, et al. Antibacterial activity of 2% chlorhexidine gluconate and 5.25% sodium hypochlorite in infected root canal: *in vivo* study. J Endod 2004;30(2):84–87. DOI: 10.1097/00004770-200402000-00005
12. Panchal V, Gurunathan D, Muralidharan NP. Comparison of antibacterial efficacy of cinnamon extract, neem extract as irrigant and sodium hypochlorite against Enterococcus faecalis: an *in vitro* study. Indian J Dent Res 2020;31(1):124–128. DOI: 10.4103/ijdr.IJDR_177_18
13. Mathew VB, Madhusudhana K, Sivakumar N, et al. Antimicrobial efficiency of silver diamine fluoride as an endodontic medicament – an ex vivo study. Contemp Clin Dent 2012;3(3):262–264. DOI: 10.4103/0976-237X.103615
14. Esmail KM, Kamel WH, El-dein MN, et al. Comparative evaluation of natural herbal extracts as root canal irrigation versus routine chemical root canal irrigation. Al-Azhar J Dent 2020;7(1):125–134. DOI: 10.21608/adjg.2019.9646.1126
15. Metkari S, Rao D, Banga K. Effect of different instrumentation and irrigation methods on apical microbial extrusion: an ex vivo study. Biomed Pharmacol J 2021;14(4). DOI: 10.13005/bpj/2315
16. Agarwal V, Rao MR, Dhingra K, et al. An *in vitro* comparison of antimicrobial effcacy of three root canal irrigants-BioPure MTAD, 2% chlorhexidine gluconate and 5.25% sodium hypochlorite as a final rinse against E. faecalis. J Contemp Dent Pract 2013;14(5):842–847. DOI: 10.5005/jp-journals-10024-1413
17. Somayaji SK, Ballal NV, Shobha KL, et al. Comparison of antimicrobial efficacy of triphala, *withania somnifera* and sodium hypochlorite against *Enterococcus faecalis* biofilm—an *in vitro* study. Int J Pharm Pharm Sci 2014;6(2):31–36.
18. Berber VB, Gomes BP, Sena NT et al. Efficacy of various concentrations of NaOCl and instrumentation techniques in reducing Enterococcus faecalis within root canals and dentinal tubules. Int Endod J 2006;39(1):10–17. DOI: 10.1111/j.1365-2591.2005.01038.x
19. Tennert C, Drews AM, Walther V, et al. Ultrasonic activation and chemical modification of photosensitizers enhances the effects of photodynamic therapy against Enterococcus faecalis root-canal isolates. Photodiagnosis Photodyn 2015;12(2):244–251. DOI: 10.1016/j.pdpdt.2015.02.002
20. Shah SN, Awinashe MV, Mohammed A, et al. Antimicrobial efficacy of sodium hypochlorite, neem, tulsi, and aloe vera as a root canal irrigants against E. faecalis. Int J Health Sci 2022;6(3):809–815. DOI: 10.53730/ijhs.v6nS3.5476
21. Kumar G, Jalaluddin M, Rout P, et al. Emerging trends of herbal care in dentistry. J Clin Diagn Res 2013;7(8):1827–1829. DOI: 10.7860/JCDR/2013/6339.3282
22. Hedge V, Kesaria DP. Comparative evaluation of antimicrobial activity of neem, propolis, turmeric, liquorice and sodium hypochlorite as root canal irrigants against E. faecalis and C. albicans - an *in vitro* study. Endodontology 2013;25(2):38–45.
23. Suzuki T, Nishida M, Sobue S, et al. Effects of diamine silver fluoride on tooth enamel. J Osaka Univ Dent Sch 1974 14:61–72.
24. Jain P, Yeluri R, Garg N, et al. A comparative evaluation of the effectiveness of three different irrigating solutions on microorganisms in the root canal: an *in vivo* study. J ClinDiagn Res 2015;9(12):ZC39–ZC42. DOI: 10.7860/JCDR/2015/13133.6991
25. Sonarkar SS, Singh S, Podar R, et al. An *in vivo* comparison of the antibacterial efficacy of photoactivated disinfection, diode laser, and 5% sodium hypochlorite in root canal disinfection. J Conserv Dent 2018;21(2):205–209. DOI: 10.4103/JCD.JCD_227_17
26. Podar R, Kulkarni GP, Dadu S, et al. *In vivo* antimicrobial efficacy of 6% Morinda citrifolia, *Azadirachta indica*, and 3% sodium hypochlorite as root canal irrigants. Eur J Dent 2015;9(4):529–534. DOI: 10.4103/1305-7456.172615
27. Sivakumar JS, Soundappan SP, Pandian AK, et al. Comparative evaluation of antimicrobial efficacy of three different endodontic irrigants against Candida albicans: an *in vitro* study. J Oper Dent Endod 2021;6(1):45–50. DOI: 10.5005/jp-journals-10047-0109
28. Nasr M, Diab A, Roshdy NN, et al. Assessment of antimicrobial efficacy of nano chitosan, chlorhexidine, chlorhexidine/nano chitosan combination versus sodium hypochlorite irrigation in patients with necrotic mandibular premolars: a randomized clinical trial. J Med Sci 2021;9(4):235–242. DOI: 10.3889/oamjms.2021.7070
29. Gomes-Filho JE, Silva FO, Watanabe S, et al. Tissue reaction to silver nanoparticles dispersion as an alternative irrigating solution. J Endod 2010;36(10):1698–1702. DOI: 10.1016/j.joen.2010.07.007
30. Weber MT, Alkhafaji Y, Pioch A, et al. Quantification of bacterial DNA from infected human root canals using qPCR and DAPI after disinfection with established and novel irrigation protocols. Materials 2022;15(5):1911. DOI: 10.3390/ma15051911
31. Khandelwal R, Mantri SP, Paul B, et al. Antimicrobial efficacy of octenidine hydrochloride, green tea, sodium hypochlorite and chlorhexidine gluconate as retreatment endodontic irrigant Against E. faecalis, Candida & mixed culture–in-vitro study. J Evol Med Dent Sci 2021;10(32):2629–2633. DOI: 10.14260/jemds/2021/538
32. Prasad SD, Goda PC, Reddy KS, et al. Evaluation of antimicrobial efficacy of neem and Aloe vera leaf extracts in comparison with 3% sodium hypochlorite and 2% chlorhexidine against E. faecalis and C. albicans. J NTR Univ Health Sci 2016;5(2):104–110. DOI: 10.4103/2277-8632.185436
33. Divya S, Sujatha S. Evaluation of antimicrobial effect of triphala versus conventional root canal irrigants in primary teeth - an *in vivo* study. Res J Pharm Tech 2019;12(2):655–659. DOI: 10.5958/0974-360X.2019.00116.1
34. Sauvik G, Shubharata P, Pabale SL, et al. Stretching new boundaries of caries prevention with silver diamine fluoride: a review of literature. Int J Pedod Rehabil 2018;3:1–4. DOI: 10.4103/ijpr.ijpr_32_17
35. Jose J, Shoba K, Aman S, et al. Comparative evaluation of antimicrobial activity of green tea extract, garlic extract, neem leaf extract and sodium hypochlorite as root canal irrigants against *E. faecalis* and *C. albicans*—an *in vitro* study. Int J Curr Microbiol App Sci 2015;4(10):384–391.