Research Article

JRAD Journal of Research and Advancement in Dentistry

J Res Adv Dent 2020;10:2:92-97.

Comparative Evaluation of the Cytotoxicity of 5.25% Sodium Hypochlorite, Triphala, 2% Chlorhexidine on Human Red Blood Corpuscles: An In-Vitro Study

Ekta Singh Suneja[1*] Parvinder Singh Baweja[2] Pooja Sood[3] Bhuvanesh Tandon[4] Abhinav Sagar[5] Bharat Suneja[6]

[1]Professor and Head, Department of Conservative Dentistry & Endodontics, BJS Dental College, Ludhiana, Punjab, India.
[2]Professor, Department of Conservative Dentistry & Endodontics, BJS Dental College, Ludhiana, Punjab, India.
[3]Reader, Department of Conservative Dentistry & Endodontics, BJS Dental College, Ludhiana, Punjab, India.
[4]Reader, Department of Conservative Dentistry & Endodontics, BJS Dental College, Ludhiana, Punjab, India.
[5]PG Student, Department of Conservative Dentistry & Endodontics, BJS Dental College, Ludhiana, Punjab, India.
[6]Professor and Head, Department of Pedodontics, BJS Dental College, Ludhiana, Punjab, India.

ABSTRACT

Background: The aim of this study was to analyze the cytotoxicity of various volumes of irrigants i.e, 5.25% Sodium hypochlorite, Triphala, 2% Chlorhexidine by checking for hemolysis of human red blood corpuscles.

Material & Methods: A total volume of 100 ul of diluted red blood corpuscles obtained through centrifugation was added to three irrigating solutions (5.25% sodium hypochlorite, Triphala, 2% Chlorhexidine) of six test tubes each. Individual irrigants per group were added in increasing volume starting from the second test tube keeping first as the control. After incubation for 3 minutes hemoglobin content was measured using an automated hemoanalyzer. Data obtained was analyzed statistically.

Results: 5.25% NaOCl was most cytotoxic at cellular level compared to other irrigants and it should be used with high caution.

Conclusion: Triphala can be a valuable herbal alternative as a root canal irrigant.

Keywords: Triphala, Irrigant, Herbal.

INTRODUCTION

Successful endodontic treatment depends on effective mechanical and chemical cleaning of the root canal system and subsequently filling it in three dimension[1].Endodontic therapy is primarily based on the removal of potentially noxious stimuli from the complex root canal system[2]. The complexity of the root canal system is, however, one of the determining factors in the failure of root canal treatment even in properly treated teeth, due to irregular characteristic of the root canals system[3,4].The persistence of residual pulp tissue, infected dentin or bacteria in the root canal system is responsible for treatment failure[5]. Irrigation dynamics plays a significant role during root canal treatment and the effectiveness depends on the working mechanism of the irrigant and the ability to bring the irrigant in contact with the microorganisms and tissue debris in the root canal system[4]. Several irrigating solution have been used during root canal treatment.

Sodium hypochlorite is the most commonly used irrigant in endodontics. It has an antimicrobial as well as solvent activity for both necrotic and vital

Received: Oct. 12, 2019: **Accepted**: Jan. 5, 2020
*Correspondence Dr. Ekta Singh Suneja.
Department of Conservative Dentistry & Endodontics, BJS Dental College, Ludhiana, Punjab, India.
Email: Not Disclosed

pISSN 2278-0076
eISSN 2321-9270

tissues.[2] Severe irritations of the periradicular tissue usually resulted if concentrated solution is forced beyond the apex of the tooth[5]. Chlorhexidine is a cationic biguanide that possesses broad antibacterial activity in combination with relatively low toxicity. Nearly 2% has been suggested as an irrigant for the root canal system, which lacks the ability to dissolve the organic and inorganic material[6].

An endodontic irrigant should be non toxic when it comes in contact with vital tissues and non caustic to the periodontal tissues. A potential complication of irrigation is the forced extrusion of the irrigant and debris through the apex. Tissue cytotoxicity is therefore a major concern when choosing an endodontic irrigant for root canal treatment. Various methods have been employed to evaluate the cytotoxicity of endodontic irrigants. **Pashley *et al*.1985**[7]evaluated the cytotoxicity on red blood corpuscles, **Faria *et al*.2007**[8]and **Zhang *et al*.2003**[9] evaluated the cytotoxicity on L929 fibroblasts and **Barnhart *et al*.2005** [10] on gingival fibroblasts. Triphala [three (tri) fruit(phala)] is one of the well known Indian ayurvedic herbal formulation consisting of dried and powdered fruits of three medicinal plants namely Terminalia Bellerica, Terminalia Chebula and Emblica Officinalis with tannic acid being its main constitiuent. Triphala shows bacteriostatic or bacteriocidal effect on gram-positive and gram negative pathogens. Compared to commonly used chemical root canal irrigants, it is safe and is composed of compounds with proper physiologic effects in addition to its anti-oxidative and anti-inflammatory properties. The most important advantages of triphala include: easy availability, low cost, long-term substantivity,less toxicity and absence of microbial resistance. Triphala's fruit is rich in citric acid, shows the property of chelation and helps in the removal of smear layer from the walls of the root canal.Dimethylsulfoxide (DMSO) was used as a solvent for triphala and it is a clean, safe, highly polar, aprotic solvent that helps in bringing out the pure properties of all the components of the herb being dissolved. **J. Prabhakar et al** [11] conducted a study on the antimicrobial efficacy of herbal root canal irrigants and found that Triphala exhibited significant antimicrobial activity. Therefore, the aim of this study was to analyze the cytotoxicity of various volumes of 5.25% sodium hypochlorite, 2% chlorhexidine gluconate and Triphala by checking for hemolysis of human red blood corpuscles.

MATERIAL & METHODS

RBCs were chosen as the convenient cell type as it is readily available and whose intracellular hemoglobin content can be easily measured. Fresh blood was drawn from a human volunteer and collected in EDTA bottles, its hemoglobin content was measured . Blood was spun in centrifuge, plasma removed and packed cell volume of RBCs obtained. RBCs were washed with saline and centrifuged several times to remove white cells and any traces of plasma. 1ml of packed cell volume added to 4 ml of saline to increase volume of blood to 5ml. Its hemoglobin content was measured using Siali's method. 100 µl of this diluted RBCs is added to 18 test tubes with six test tubes in each group Containing 5 ml of saline and mixed by shaking.

Group I : 5.25% Sodium hypochlorite

Group II : 2% Chlorhexidine

Group III :Triphala

Group I (5.25% Sodium Hypochlorite) : The first test tube was kept as a control no irrigant was added. In the second test tube 10 µl of the irrigant was added. 20 µl was added to third test tube. 30 µl was added to fourth test tube. 40µl was added to fifth test tube. 50µl was added to sixth test tube.

Group II (2% Chlorhexidine) : The first test tube was kept as a control and no irrigant was added. In the second test tube 10 µl of the irrigant was added. 20 µl was added to third test tube. 30 µl was added to fourth test tube. 40µl was added to fifth test tube. 50µl was added to sixth test tube.

Group III (Triphala) : The first test tube was kept as a control and no irrigant was added. In the second test tube 10 µl of the irrigant was added. 20 µl was added to third test tube. 30 µl was added to fourth test tube. 40µl was added to fifth test tube. 50µl was added to sixth test tube. After incubation of three minutes all the test tubes were subjected to calorimetric assay to analyse for hemolysis and hemoglobin content of each test tube of every group was measured.

RESULTS

Table 1: Comparison at 0 ul volume among the groups

Groups	Mean	SD	Anova	p value
Group 1 (Sodium Hypochlorite 5.25%)	2.8	0	0	1
Group 2 (Chlorhexidine 2%)	2.8	0		
Group 3 (Triphala)	2.8	0		

Table 2: Comparison at 10 ul volume among the groups

Groups	Mean	SD	Anova	p value
Group 1 (Sodium Hypochlorite 5.25%)	2	0.31	3.38	0.17
Group 2 (Chlorhexidine 2%)	2.7	0.24		
Group 3 (Triphala)	2.7	0.33		
Tukey HSD Post-hoc Test.				
Group 1 vs Group 2: Diff=0.7000, 95%CI=-0.5992 to 1.9992, p=0.208				
Group 1 vs Group 3: Diff=0.7000, 95%CI=-0.5992 to 1.9992, p=0.208				
Group 2 vs Group 3: Diff=0.0000, 95%CI=-1.2992 to 1.2992, p=1				

Table 3: Comparison at 20 ul volume among the groups

Groups	Mean	SD	Anova	p value
Group 1 (Sodium Hypochlorite 5.25%)	1.1	0.17	14.34	0.02*
Group 2 (Chlorhexidine 2%)	2.3	0.19		
Group 3 (Triphala)	2.7	0.24		
Tukey HSD Post-hoc Test.				
Group 1 vs Group 2: Diff=1.2000, 95%CI=-0.0992 to 2.4992, p=0.06				
Group 1 vs Group 3: Diff=1.6000, 95%CI=0.3008 to 2.8992, p=0.02*				
Group 2 vs Group 3: Diff=0.4000, 95%CI=-0.8992 to 1.6992, p=0.49				

Table 4: Comparison at 30 ul volume among the groups.

Groups	Mean	SD	Anova	p value
Group 1 (Sodium Hypochlorite 5.25%)	0.7	0.03	57.94	0.004*
Group 2 (Chlorhexidine 2%)	2	0.22		
Group 3 (Triphala)	2.3	0.16		
Tukey HSD Post-hoc Test.				
Group 1 vs Group 2: Diff=1.3000, 95%CI=0.6397 to 1.9603, p=0.0033*				
Group 1 vs Group 3: Diff=1.6000, 95%CI=0.9397 to 2.2603, p=0.0003*				
Group 2 vs Group 3: Diff=0.3000, 95%CI=-0.3603 to 0.9603, p=0.2834				

Table 5: Comparison at 40 ul volume among the groups.

Groups	Mean	SD	Anova	p value
Group 1 (Sodium Hypochlorite 5.25%)	0	0	79.54	0.003*
Group 2 (Chlorhexidine 2%)	1.5	0.13		
Group 3 (Triphala)	2.2	0.28		
Tukey HSD Post-hoc Test.				
Group 1 vs Group 2: Diff=1.5000, 95%CI=0.7552 to 2.2448, p=0.003*				
Group 1 vs Group 3: Diff=2.2000, 95%CI=1.4552 to 2.9448, p=<0.01*				
Group 2 vs Group 3: Diff=0.7000, 95%CI=-0.0448 to 1.4448, p=0.06				

Table 6: Comparison at 50 ul volume among the groups

Groups	Mean	SD	Anova	p value
Group 1 (Sodium Hypochlorite 5.25%)	0	0	86.15	0.002*
Group 2 (Chlorhexidine 2%)	1.2	0.22		
Group 3 (Triphala)	2.2	0.19		
Tukey HSD Post-hoc Test.				
Group 1 vs Group 2: Diff=1.2000, 95%CI=0.4957 to 1.9043, p=0.009*				
Group 1 vs Group 3: Diff=2.2000, 95%CI=1.4957 to 2.9043, p=<0.01*				
Group 2 vs Group 3: Diff=1.0000, 95%CI=0.2957 to 1.7043, p=0.02*				

DISCUSSION

Ideally an endodontic irrigant should be systemically non toxic when they come in contact with vital tissues , non caustic to periodontal tissues with little potential to cause anaphylactic reaction and act as an antimicrobial agent[12]. Root canals have complex anatomy because of which approximately 50 % of canal walls remain uninstrumented during preparation which results in insufficient debridement of necrotic tissue remnants[13]. Drug induced hemolysis is a relatively rare, but serious toxicity liability. It occurs by two mechanisms, toxic hemolysis and allergic hemolysis. The toxic hemolysis referred to the direct toxicity of the drug, its metabolite, or an excipient in the formulation. It evaluates hemoglobin release in the

plasma (as an indicator of red blood cell lysis) following test agent exposure. The US FDA recommended it for haemolytic potential[14].So This method was used in this *in vitro* study.

The irrigating solutions used in this study were isotonic and thus excluded an osmotic pressure gradient, so the observed hemolysis and loss of cellular protein was due to the oxidizing effect of the solutions on the cell membrane of red blood corpuscles. Based on the classic studies of pulp tissue dissolution of Sodium hypochlorite by Grossman and Meiman and because of their wide – spectrum antimicrobial efficacy Sodium hypochlorite is considered the ideal irrigant in endodontics [15] Controversy exists regarding the toxic and irritating potential of sodium hypochlorite[16]. Sodium hypochlorite is very caustic, non specific agent whose action is not limited to necrotic tissue but is cytotoxic to all except heavily keratinized epithelia[17].

One of the most serious complications of sodium hypochlorite is the passage of some solutions through the foramina which sometimes occurs when the needle is wedged tightly into canal or in the case of blunderbuss canals, perforations and in case of improper techniques . **Pashley et al** [17] evaluated the cytotoxicity of various dilutions of sodium hypochlorite on red blood cells and found that 5.25% sodium hypochlorite in 1:1000 dilution caused complete hemolysis of Red blood cells in vitro. Studies by **Spangberg et al** [18] reported the irritating effect of Sodium hypochlorite at high concentration. He recommended 0.5% to be an acceptable non cytotoxic solution. Hypochlorite preparations are sporicidal, virucidal and show far greater tissue dissolving effects on necrotic than on vital tissues. Various concentrations of sodium hypochlorite from 0.5% to 5.25% have been tried out[19]. The higher the concentration the better will be the antimicrobial effect and the tissue dissolving capacity. So, higher concentration of sodium hypochlorite was used for the study. Chlorhexidine is recommended as an endodontic irrigant because of its antimicrobial activity, presumed non toxicity and intracanal substantivity[20].At higher concentrations, chlorhexidine results in extensive cell damage, coagulation of cytoplasm and precipitation of proteins and nucleic acids[21]. The concentration often used in endodontic therapy is 2% as it is more effective in less time when compared to other concentrations of chlorhexidine ranging from 0.002%to 2%. Chlorhexidine was reported to be toxic to human gingival cells and red blood cells in culture and the toxic potency is dependent on the length of exposure and the composition of the exposure medium. Our results were in accordance with Giannelli *et al* [22].They attributed its toxic effect to be consisted in the induction of apoptotic and autophagic/necrotic cell deaths and involved disturbance of mitochondrial function, intracellular Ca2 + increase and oxidative stress. In addition, its lytic properties could be the reason for hemolysis. Chlorhexidine has been recommended as an Endodontic irrigant because of its antimicrobial activity and presumed non toxicity and intracanal substantivity [23]. They showed that 0.12% chlorhexidine solution inhibited macrophage adherence however at lower intensity than 5.25 % sodium hypochlorite.

Morever a recent study by Yu-Chao et al on showed that sodium hypochlorite and chlorhexidine to be cytotoxic to human PDL cells in a time and dose dependent manner by using fluorescent assay [24]. Mechanisms proposed by which chlorhexidine is cytotoxic to human PDL cells is by inhibiting double stranded nucleic acid content, protein synthesis and mitochondrial activity.Previous studies have shown that chlorhexidine was cytotoxic to human fibroblasts via inhibition of protein synthesis [25]. However studies have shown sodium hypochlorite to posses the property to dissolve pulp which chlorhexidine lacks[26]. Hence making Sodium Hypochlorite the irrigant of choice. Chlorhexidine has been recommended as an alternative in patients allergic to sodium hypochlorite or in teeth with incomplete root formation and crestal perforations to prevent inflammatory response in proximity to the epithelial attachment[27].

Triphala is equally effective as Sodium hypochlorite in the present study against both C. albicans and E. faecalis biofilm. The studies conducted by **Pujar et al. and Prahakar et al.**[28] have also proved the efficacy of Triphala against E. faecalis biofilm. Antimicrobial activity of Triphala is attributed to its formulation which contains three different medicinal plants in equal proportions – Terminalia bellerica, Terminalia chebula, Emblica officinalis resulting in an additive or synergistic positive effect. The strong antioxidant activity may

be partially responsible for many of the biological properties.

CONCLUSION

From the present study, 5.25% NaOCl is most cytotoxic at cellular level compared to other irrigants and it should be used with high caution.Triphala is well known in India as one of the most versatile medicinal plants having a wide spectrum of biological activity. Several studies have proven that triphala extract is effective against E.faecalis and C.albicans & as a irrigating solution· The major advantages of using herbal alternatives are easy availability, cost-effectiveness, increased shelf-life, low toxicity. Considering factors such as high antimicrobial efficacy with least cytotoxicity even on fragile RBC, Triphala at higher concentration could be potential alternative to conventional root canal irrigants and might be an adjunctive to the mechanical debridement in endodontic procedures.

CONFLICTS OF INTEREST

The authors declare they have no potential conflict of interests regarding this article.

REFERENCES

1. Schilder H. Cleaning and shaping the root canal. Dent Clin North Am 1974;18:269-96.
2. Yesilsoy C, Whitaker E, Cleveland D, Phillips E, Trope M. Antimicrobial and toxic effects of established and potential root canal irrigants. J Endod 1995;21:513-5.
3. Nair PN. On the causes of persistent apical periodontitis: A review. Int Endod J 2006;39:249-81.
4. Gulabivala K, Patel B, Evans G. Effects of mechanical and chemical procedures on root canal surfaces. Endod Topics 2005;10:103-22.
5. Witton R, Henthorn K, Ethunandan M, Harmer S, Brennan PA. Neurological complications following extrusion of sodium hypochlorite solution during root canal treatment. Int Endod J 2005;38:843-8.
6. Zamany A, Safavi K, Spångberg LS. The effect of chlorhexidine as an endodontic disinfectant. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003;96:578-81.
7. Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-8
8. Faria G, Celes MR, De Rossi A, Silva LA, Silva JS, Rossi MA. Evaluation of chlorhexidine toxicity injected in the paw of mice and added to cultured l929 fibroblasts. J Endod 2007;33:715-22.
9. Zhang W, Torabinejad M, Li Y. Evaluation of cytotoxicity of MTAD using the MTT-tetrazolium method. J Endod 2003;29:654-7.
10. Barnhart BD, Chuang A, Lucca JJ, Roberts S, Liewehr F, Joyce AP. An *in vitro* evaluation of the cytotoxicity of various endodontic irrigants on human gingival fibroblasts. J Endod 2005;31:613-5.
11. Prabhakar, J & Senthilkumar, Manjula & Priya, M.S. & Krishnan, Mahalakshmi & Sehgal, P.K. & Sukumaran, V.G.. (2010). Evaluation of Antimicrobial Efficacy of Herbal Alternatives (Triphala and Green Tea Polyphenols), MTAD, and 5% Sodium Hypochlorite against Enterococcus faecalis Biofilm Formed on Tooth Substrate: An In Vitro Study. Journal of endodontics. 36. 83-6. 10.1016/j.joen.2009.09.040.
12. Kandaswamy D , Venkateshbabu N , Root canal irrigants J .Conserv.Dent 2010 :13:256-264 .
13. Nadja naenni ,Cand , Kaya Thoma Mathias Zehnder .Soft –tissue dissolution capacity of currently used and potential endodontic irrigants .The American association of endodontists Vol.30.No 11 Nov 2004 785-787.
14. FDA. Guidance for Industry - Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients. Pharmacology/ Toxicology. May 2005. p. 1-12
15. Grossman LI ,meiman B W . Solution of pulp tissue by chemical agents . J . Am . Dent Assoc 1941:28:223-25 .

16. Kaufman AY, Keila S. Hypersensitivity to sodium hypochloriteJ Endod. 1989 May;15(5):224-6.

17. Pashley EL, Birdsong NL, Bowman K, Pashley DH. Cytotoxic effects of NaOCl on vital tissue. J Endod 1985;11:525-8.

18. Spangberg L. Biologic effects of dental materials Part III . Toxicity and antimicrobial effect of endodontic antiseptics in vitro . Oral Surgery Oral medicine Oral Pathology 1973; 36 ,856-71 .

19. Vianna ME, Gomes BP, Berber VB, Zaia AA, Ferraz CC, de Souza-Filho FJ. *In vitro* evaluation of the antimicrobial activity of chlorhexidine and sodium hypochlorite. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004;97:79-84.

20. Leonard MR ,Tanomaru Filho , M.Silva CAB ,Nelson Filho N , Bonifacio KC. In vivo antimicrobial activity of 2% chlorhexidine used as a root canal irrigating solution. journal of endodontics. 1999; 25,167-171 .

21. Sirtes G, Waltimo T, Schaetzle M, Zehnder M. The effects of temperature on sodium hypochlorite short-term stability, pulp dissolution capacity, and antimicrobial efficacy. J Endod 2005;31:669-71.

22. M.Gianelli et al Toxicology invitro 22(2008) 308-317

23. L .A Okino , E-L Siqueira M Santos A.C Bombana and J A P Figueiredo . Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel . International Endodontic Journal 2004,37,38-41

24. Yu – Chao Chung , Fu – Mei Huang, Kuo –wei Tai and Ming –Yung Chou Tai chung Taiwan . The effect of sodium hypochlorite and chlorhexidine on cultured human pdl cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001 : 92 : 446-450 .

25. Pucher JJ , Daniel OC . The effect of chlorhexidine digluconate on human fibroblasts in vitro . J . Periodontol 1993 : 62 : 526-32

26. L .A Okino , E-L Siqueira M Santos A.C Bombana and J A P Figueiredo . Dissolution of pulp tissue by aqueous solution of chlorhexidine digluconate and chlorhexidine digluconate gel . International Endodontic Journal 2004,37,38-41

27. Fuss Z . Trope M . Root perforations classification and treatment choices based on prognostic factors . Endodontics and Dental Traumatology 1996 , 12 ,225-64

28. Prabhakar, J & Senthilkumar, Manjula & Priya, M.S. & Krishnan, Mahalakshmi & Sehgal, P.K. & Sukumaran, V.G.. (2010). Evaluation of Antimicrobial Efficacy of Herbal Alternatives (Triphala and Green Tea Polyphenols), MTAD, and 5% Sodium Hypochlorite against Enterococcus faecalis Biofilm Formed on Tooth Substrate: An In Vitro Study. Journal of endodontics. 36. 83-6. 10.1016/j.joen.2009.09.040.

29. Chainani S, Siddana S, Reddy CV, Thippeswamy M, Maurya M, Rudraswamy S. Antimicrobial activity of Triphala on Lactobacilli and Candida albicans: An in vitro study. J Orofac Sci 2015;7:104-7.