



## EVALUATION OF THE ANTIBACTERIAL EFFECT OF APPLE CIDER VINEGAR, BLACK TEA AND SODIUM HYPOCHLORITE IRRIGANT SOLUTIONS ON INFECTED ROOT CANAL MICROORGANISMS OF PRIMARY TEETH: AN INVITRO STUDY

Mohamed Abdel Nasser Alyamany<sup>1\*</sup>, Alaa Eldeen Abd Allah<sup>2</sup>, Alaa Nabil Abass<sup>3</sup>  
Amani Mohammed Tawfik Ahmed<sup>4</sup>

### ABSTRACT

**Objective** of this study was to assess the antibacterial effects of apple cider vinegar, black tea and sodium hypochlorite irrigant solutions on infected root canals microorganisms of primary teeth. **Subjects and methods:** This study was carried out on Sixty four xtracted primary molars. Crown was rebuilt up after caries removal and root canals were standardized mechanically prepared .Molars were packed and sterilized in autoclave, then inoculated with E fecalis. Molars were randomly divided into four equal groups of sixteen molar each. Group A: sixteen molars were irrigated with apple cider vinegar as an irrigant solution. Group B: sixteen molars were irrigated with black tea as an irrigant solution. Group C: sixteen molars were irrigated with sodium hypochlorite as an irrigant solution. Group D: sixteen molars were irrigated with normal saline as an irrigant solution. Microbiological evaluation was based on taking microbial sample by paper point from root canal before, immediate after and forty eight hour after irrigation. **Results:** apple cider vinegar and black tea showed acceptable antibacterial effects against E. faecalis after immediate irrigation with non significant difference. After forty eight hour black tea had significant decrees in antibacterial activity. **Conclusion:** Apple cider vinegar and Black tea could be used as irrigant solution.

**KEYWORDS:** Apple cider vinegar, Black tea, sodium hypochlorite, E fecalis.

### INTRODUCTION

Irrigation is a key part of successful root canal treatment because it's the only way to impact those areas of the root canal wall not touched by mechanical instrumentation such as lateral accessory canal and complex internal canal anatomy. Majority of root canal failures are caused by persistent microorganisms in the root canal even after proper

treatment. Studies have shown that 45.8% of the failures are caused by *Enterococcus faecalis* <sup>(1-4)</sup>.

The ideal root canal irrigant should be have broad antimicrobial spectrum, have high efficacy against microorganisms organized in biofilms, have ability to dissolve necrotic pulp tissue remnants, have ability to inactivate endotoxin, reduces friction between the instrument and dentine, cools the file

1. Masters Candidate, Ministry of Health
2. Associate Professor, Pedodontics and Oral Health Department, Faculty of Dental Medicine, Boys, Cairo, Al-Azhar University
3. Lecturer of Pedodontics and Oral Health, Faculty of Dental Medicine, Boys, Cairo, Al-Azhar University.
4. Assistant Professor of Microbiology, Faculty of Medicine, Girls, Cairo, Al-Azhar University.

• **Corresponding author:** [dentistmohamed89@gmail.com](mailto:dentistmohamed89@gmail.com)

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and tooth, have ability to prevent the formation of a smear layer during instrumentation or to dissolve it once it has formed. Systemically non-toxic non-irritant to vital tissues, and with little potential to cause an anaphylactic reaction<sup>(5)</sup>.

Recently, many new antimicrobial agents were used from various sources to combat microbial resistance. Apple cider vinegar (ACV) is a type of organic vinegar made from apple must. It has been used for therapeutic purpose for thousands of years for its various medicinal properties. Since it show antibacterial activity, blood pressure reduction, antioxidant activity, reduction in the effects of diabetes, prevention of cardiovascular disease, and increased vigor after exercise. ACV has been used for cleaning and treating nail fungus, head lice, warts, ear infections and inhibiting the growth of foodborne pathogenic microorganisms in food. The organic acids in vinegar are mainly acetic acid and malic acid which pass into cell membranes of microorganism leading to bacterial cell death<sup>(6-9)</sup>.

Black tea is the most common beverage in the world. It has great antioxidant and antibacterial properties which are generally attributed to its metabolites such as flavonoid components (theaflavins, bisflavanols and theaflavic acids) and polyphenolic compounds (catechins, caffeine and theobromine and theophylline). Catechins compound has anti-cariogenic properties. These include a direct bactericidal effect against *Streptococcus mutans* and *S. sobrinus*, preventing bacterial adherence to teeth, inhibition of glucosyl transferase, thus limiting the biosynthesis of sticky glucan<sup>(10-12)</sup>.

Sodium hypochlorite (NaOCl) is the most commonly used irrigating solution. It is potent antimicrobial agent killing the bacteria instantly. It also has the ability to dissolve the organic components of dentin such as pulpal remnants and collagen. The only limitation of hypochlorite is that its inability to remove the smear layer<sup>(13)</sup>.

Normal saline is isotonic to the body fluids. It is universally accepted as the most common irrigating solution in all endodontic and surgical procedures. It is also found to have no side effects, even if pushed into the periapical tissues. However, saline should not be the only solution to be used, it is preferably used in combination with other solutions<sup>(14)</sup>.

## SUBJECTS AND METHODS

### Study design:

- Intervention randomized controlled invitro study.
- Sixty four extracted human primary molar were collected from Pediatric Dental Outpatients Clinic, Faculty of Dental Medicine, Al-Azhar University due to orthodontic serial extraction procedures and retained primary molars.
- A sample size of 16 in each group has a 80% power to detect a difference between means of 0.74 with a significance level (alpha) of 0.05 (two-tailed).. In 80% (the power) of those experiments, the P value will be less than 0.05 (two-tailed) so the results will be deemed "statistically significant". In the remaining 20% of the experiments, the difference between means will be deemed "not statistically significant. Report created by Graph Pad Stat Mate 2.00.

### a. Inclusion criteria :

Primary teeth with no root resorption or resorption less than one third of root. Restorable primary teeth.

### b. Exclusion criteria :

Primary teeth with root resorption more than one third of root. Non restorable teeth . Teeth with previous pulp therapy.

### Interventions:<sup>(15-18)</sup>

The crown was rebuilding up after caries removal. Each root canal was standardized mechanically prepared. The molars were packed in

separate pouch and sterilized by autoclave at 121°C for 30 minutes. Five molars were tested for proper sterilization. The remaining teeth were inoculated by *Enterococcus faecalis* and incubated for 24 hours then divided into four groups as follow: Group A: sixteen molars were irrigated with apple cider vinegar as an irrigant solution. Group B: sixteen molars were irrigated with black tea as an irrigant solution. Group C sixteen molars were irrigated with sodium hypochlorite as an irrigant solution. Group D: sixteen molars were irrigated with normal saline as an irrigant solution and serve as positive control group. Microbial sample was taken by paper point from root canal of teeth before irrigation and transferred to bile esculin specific media for *E. faecalis* then another microbial sample was taken from the same root canal immediate after irrigation and another sample was taken 48 hours after irrigation.

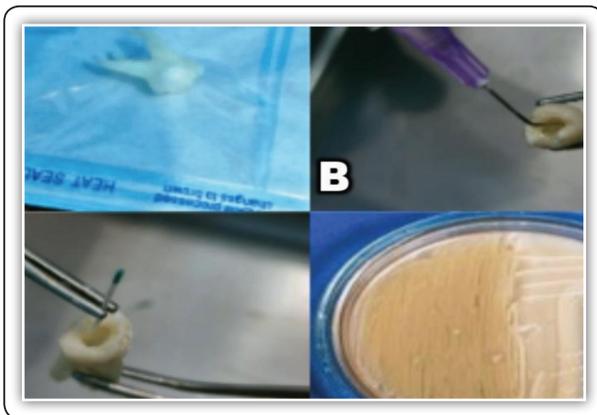


FIG (1) A: Molar packed in sterilization pouch B: Irrigation with subjected solution. C: Taking sample D: Transferred sample to bile esculin media

**Ethical considerations**

The study was approved by the pedodontics scientific Committee and department council (Ethical code 631/2148), Faculty of Dental Medicine, Boys, Cairo, Al-Azhar University

**Statistical analysis**

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0.

(Armonk, NY: IBM Corp) Qualitative data were described using number and percent. The significance of the obtained results was judged at the 5% level.

**RESULTS**

**The antibacterial effect of irrigant solution after immediate irrigation:**

Apple cider vinegar had significant decreased in colonial numbers between before and immediate after irrigation was about 50%. Black tea had significant decreased in colonial numbers between before and immediate after irrigation was about 55%. Sodium hypochlorite had significant decreased in colonial numbers between before and immediate after irrigation was about 97%.

**The antibacterial effect of irrigant solution after 48 hours of irrigation:**

Apple cider vinegar had significant decreased in colonial numbers between before and 48h after irrigation was about 75%. Black tea had significant decreased in colonial numbers between before and 48h after irrigation was about 35% with increase in number of bacterial colony than immediate after. Apple cider vinegar had significant decreased in colonial numbers between before and 48h after irrigation was about 99%.

**TABLE (1)** The mean, standard deviation (SD) values of different groups for each time period.

Group	Time	Mean	SD
Apple cider vinegar	Before	2224.0	153.7
	Immediate after	1002	68.28
	48h after	666	46.24
Sodium hypochlorite	Before	2064	128.86.7
	Immediate after	52	21.5
	48h after	12	10.33
Black tea	Before	2088	220.34
	Immediate after	626	65.35
	48h after	1393	195.85

## DISCUSSION

Endodontic infections are due to existence of oral microorganisms which are present not only inside of the root canal system but also penetrating the dentinal tubules. Moreover, some of them show resistance to endodontic antibacterial protocols. For example, *Enterococcus faecalis* which is the most commonly found microorganism in resistant lesions after root canal therapy<sup>(19-21)</sup>.

There are many disinfectant solutions, with particular advantages and disadvantages. For example, sodium hypochlorite (NaOCl) shows antimicrobial activity against a wide range of micro-organisms including *E. faecalis*, and also shows relatively good tissue solubility. However this irrigants several disadvantages such as cytotoxicity, tissue burning, bad taste and odor and discoloration of patient's clothes<sup>(22-24)</sup>.

Therefore, the use of solutions that are compatible to host tissues and show less tissue toxicity, especially if they also have anti-inflammatory properties is needed.

The result of present study shows Regarding Percentage Reduction in Bacterial Colonies, after immediate irrigation, Apple cider vinegar was 50%, Black tea was 55%, Sodium hypochlorite was 97%, and Saline was 0%. While After 48 hours, Apple cider vinegar was 75%, Black tea was 35%, Sodium hypochlorite was 99.5%, and Saline was 0%. Sodium hypochlorite was higher in Percentage Reduction in Bacterial Colonies followed by Apple cider vinegar. Immediate: Sodium hypochlorite group showed a lower CFU (/ml) than Black tea group than other groups. After 48 hours: Sodium hypochlorite group showed a lower CFU (/ml) than Apple cider vinegar group than other groups. Sodium hypochlorite and Apple cider vinegar decreases CFU ( $\downarrow 99.42 \pm 0.50$  and  $\downarrow 70.05 \pm 0.29$  respectively) than other groups.

The result of present study shows that Apple cider vinegar has an antibacterial effect on *E. faecalis* with statistically significant difference in decreasing number of *E. faecalis* before, immediate

after and 48 hours after irrigation due to the presence of acetic acid and malic acid which causes loss of cell integrity.

This result agrees with Mohanty et al. who compared the Antimicrobial efficacy of apple cider vinegar against *Enterococcus faecalis* and *Candida albicans*: An in vitro study with 5% sodium hypochlorite. The antimicrobial activity of apple cider vinegar is almost similar to 5% sodium hypochlorite against *E. faecalis*<sup>(25)</sup>.

Result also agreed with study by Taghreed et al. evaluated the antibacterial effect of Apple Vinegar as a root canal irrigant using Endovac irrigation System. Endovac irrigation system was effective in eradication of *E. faecalis* from the root canals using either NaOCl or Apple vinegar<sup>(26)</sup>.

On other hand Showkat et al. evaluated the antimicrobial efficacy of chitosan, chlorhexidine, apple cider vinegar and sodium hypochlorite on *E. faecalis* biofilm in primary teeth. In this study apple cider vinegar showed poor antibacterial efficacy comparing to other material in study<sup>(27)</sup>.

The result of present study shows that black tea has an antibacterial effect on *E. faecalis* with statistically significant difference in decreasing number of *E. faecalis* before and immediate after irrigation. Also has statistically significant difference in decreasing number of *E. faecalis* before and 48 hours after irrigation, but it has an increased number of bacteria than immediate after. Which may refer to rapidly losing antibacterial efficacy of black tea.

This result agree with Abd Allah et al ,who said that that the black tea beverage had a highly significant effect on reducing the cariogenic bacterial counts in the immediately post and after 1 h of tea drinking samples<sup>(28)</sup>.

Result also agreed with Hacıoglu et al who reported that herbal teas (including black tea) have antimicrobial activities against gram-positive and -negative bacteria and yeast when they were used alone<sup>(29)</sup>.

The result of present study disagreed with Hos-sain et al, In his study, he systematically tested the methanolic extract of Bangladeshi black tea against a range of human pathogens(including *E. faecalis*) and demonstrated that black tea extract showed noticeable inhibitory activity against almost all of the tested bacteria for 24 and 48 hours and this may be due to different testing protocol<sup>(30)</sup>.

## CONCLUSION

From the results of the present study, the following conclusions could be drawn:

1. Apple cider vinegar and black tea showed acceptable antibacterial effects against *E. faecalis*.
2. Possible use of Apple cider vinegar and Black tea as a herbal irrigants inside the canal as an alternative to NaOCl to avoid its detrimental properties.

## REFERENCES

1. Wong R. Conventional endodontic failure and retreatment. *Dent Clin North Am* 2004;48:265-89.
2. Basmadjian C, Farge P, Bourgeois M, Lebrun T. Factors influencing the long-term results of endodontic treatment: a review of the literature. *Int Dent J* 2002 ;52:81-6.
3. Ferreira B, Alfredo E, Porto M, Silva T, Sousa D. Histological analysis of the cleaning capacity of nickel-titanium rotary instrumentation with ultrasonic irrigation in root canals. *Aust Endod J*2004 ;30:56-8.
4. Rodrigues R, Antunes H, Neves M, Sequeira F ,Rocas N. Infection control in retreatment case ,in vivo effect of two instrumentation system. *J Endod* 2015 ;41:1600-5.
5. Zehnder M. Root Canal Irrigants. *J Endod.* 2006 May; 32(5):389-98.
6. Yagnik D, Serafin V, Shah A. Antimicrobial activity of apple cider vinegar against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* ; Down regulation of cytokine and microbial protein expression. *J Bacteriol Parasitol* 2018;8:1-2.
7. Saqib A. Antimicrobial activity of apple cider vinegar. *Mapana J S* 2017;16:11-2.
8. Mohanty S, Ramesh S. Antimicrobial efficacy of apple cider vinegar against *Enterococcus faecalis* and *Candida albicans*: An in vitro study. *JAPER*2017; 7:137-8.
9. Estrela C, Holland R, Bernabe P, Souza V, Estrela C. Antimicrobial potential of medicament used in healing process in dogs' teeth with apical periodontitis. *Braz Dent J* 2004;15:181-5.
10. Bracken M, Triche E, Belanger K, Hellenbrand K. Association of maternal caffeine consumption with decrements in fetal growth. *Am J Epidemiol* 2003 1;157:456-66.
11. Arora D, Kaur G, Kaur H. Antibacterial activity of tea and coffee: their extracts and preparations. *INT J FOOD PROP*2009;12:286-94.
12. Mugha T, Tahir A, Qureshi S, Nazir T. Antibacterial activity of black tea against streptococcus mutans and synergism with antibiotic. *JAP* 2010;2:60-7.
13. Weber CD, McClanahan SB, Miller GA, Diener-West M, Johnson JD. The effect of passive ultrasonic activation of 2% chlorhexidine or 5.25% sodium hypochlorite irrigant on residual antimicrobial activity in root canals. *Journal of endodontics.* 2003;29(9):562-4.
14. Barrette WC, Hannum DM, Wheeler WD, Hurst JK. General mechanism for the bacterial toxicity of hypochlorous acid: abolition of ATP production. *Biochemistry.* 1989;28(23):9172-8.
15. Kumar S, Seby T, Asokan S, Jhon B, Priya G. Comparative evaluation of antimicrobial efficacy of diode laser, sodium hypochlorite and their synergistic effect against *Enterococcus faecalis* contaminated root canal as in vitro study. *Int J Clin Pediatr Dent* 2017 ; 10: 14–17.
16. warren N, van der vyver P, Botha F. A comparison of the efficacy of various disinfection protocols in endodontic treatment: an in vitro study. *S A D J March* 2015; 70 (2) 60 - 4.
17. Vijaykumar S, GunaShekhar M, Himagiri S. In vitro effectiveness of different endodontic irrigants on the reduction of *Enterococcus faecalis* in root canals. *J Clin Exp Dent.* 2010;2(4): 169-72.
18. Thomas S, Asokan S, John B, Priya G, Kumar S. Comparison of Antimicrobial Efficacy of Diode Laser, Triphala, and Sodium Hypochlorite in Primary Root Canals: A Randomized Controlled Trial *Int J Clin Pediatr Dent.* 2017; 10(1): 14–17.
19. Gomes BP, Pinheiro ET, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Microbial analysis of canals of root-filled teeth with periapical lesions using polymerase chain reaction. *J Endod.* 2008;34(5):537-40.
20. Siqueira JF, Jr., Rocas IN. Clinical implications and microbiology of bacterial persistence after treatment procedures. *J Endod.* 2008;34(11):1291-301 e3.

21. Razmi H, Bolhari B, Dashti NK, Fazlyab M. The Effect of Canal Dryness on Bond Strength of Bioceramic and Epoxy-resin Sealers after Irrigation with Sodium Hypochlorite or Chlorhexidine. *Iranian endodontic journal*. 2016;11(2):129.
22. Mohammadi Z, Shalavi S, Giardino L, Palazzi F, Asgary S. Impact of Ultrasonic Activation on the Effectiveness of Sodium Hypochlorite: A Review. *Iranian endodontic journal*. 2015;10(4):216.
23. Giardino L, Estrela C, Generali L, Mohammadi Z, Asgary S. The in vitro Effect of Irrigants with Low Surface Tension on *Enterococcus faecalis*. *Iranian endodontic journal*. 2015;10(3):174.
24. Mothana RA, Lindequist U, Gruenert R, Bednarski PJ. Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from the island Soqatra. *BMC Complement Altern Med*. 2009;9:7.
25. Mohanty S, Ramesh S, Muralidharan NP. Antimicrobial efficacy of apple cider vinegar against *Enterococcus faecalis* and *Candida albicans*: An in vitro study. *J Adv Pharm Edu Res* 2017;7(2):137-141.
26. Taghreed S, Mohsen M, Rokaya E, Mohamed M. Evaluation of the Antibacterial Effect of Apple Vinegar as a Root Canal Irrigant Using Endovac Irrigation System. *ADJ-for Girls*, 2019; 6: 53:59.
27. showkat I, Chaudhary S, Sinha A, Ghaus M, Priya N. evaluation the antibacterial efficacy of chitosan, chlorohexidine, apple cider vinegar and sodium hypochlorite on *E.fecalis* biofilm in primary teeth :an invitro study. *E JPMR*, 2020,7(5), 667-673.
28. Abdalla A, Ibrahim M, Al-atrouny M. Effect of Black Tea on Some Cariogenic Bacteria. *J World Applied Sciences* 2011;12 (4): 552-558.
29. Hacıoğlu M, Dosler S, Tan A S, Otuk G. Antimicrobial activities of widely consumed herbal teas, alone or in combination with antibiotics: an invitro study.
30. Hossain M, Nibir Y, Zerín S, Ahsan N. Antibacterial Activities of the Methanolic Extract of Bangladeshi Black Tea against Various Human Pathogens. *Dhaka Univ. J. Pharm. Sci.* 2014 ;13(1): 97-103.