Future Dental Journal

Volume 7 | Issue 2 Article 5

2021

Antibacterial Effect of Azadirachta Indica (Neem Extract) and Chlorhexidine as Cavity Disinfectants in Primary Teeth in a Group of Egyptian Children (A Randomized Clinical Trial)

Maha Samy Elhabbak
Future University in Egypt, maha.elhabbak@fue.edu.eg

Norhan El-Dokky
Future University in Egypt, norhan.eldokky@fue.edu.eg

Manal Elsheikh
Cairo University, manal.elshiekh@dentistry.cu.edu.eg

Eman Alaa
Future University in Egypt, eman.alaa@fue.edu.eg

Recommended Citation

Elhabbak MS, El-Dokky N, Elsheikh M, Alaa E. Antibacterial Effect of Azadirachta Indica (Neem Extract) and Chlorhexidine as Cavity Disinfectants in Primary Teeth in a Group of Egyptian Children (A Randomized Clinical Trial). *Future Dental Journal*. 2022; 7(2):99-102. doi: https://doi.org/10.54623/fdj.7025.

Future Dental Journal Volume 7, Issue 2 (2021) 99—102

Contents lists available at Arab Journals Platform

Future Dental Journal

Journal homepage: https://digitalcommons.aaru.edu.jo/fdj/

Antibacterial Effect of Azadirachta Indica (Neem Extract) and Chlorhexidine as Cavity Disinfectants in Primary Teeth in a Group of Egyptian Children (A Randomized Clinical Trial)

Maha Samy Elhabbak,[a,*] Norhan El-Dokky,[a] Manal Elsheikh,[b] Eman Alaa,[a]

a Future University in Egypt
b Cairo University, Egypt

ARTICLE INFO

Discipline:
Pediatric Dentistry

Keywords:
Cavity disinfection, Neem, Phytotherapy, Chlorhexidine

* Corresponding author.
E-mail address:
maha.elhabbak@fue.edu.eg
(Maha Samy Elhabbak).

ABSTRACT

Introduction: The prevention and control of caries requires the eradication of cariogenic microorganisms that produce acids responsible for reducing the pH and starting the demineralization process. With the increasing incidence of drug resistance in the prevalent pathogens and an associated risk with chemotherapeutic agents, it is essential to find an alternative to existing drugs.

Aim: The aim of this study was to investigate the effect of neem extract and 2% chlorhexidine (CHX) as cavity disinfectants in the reduction of total viable count in Atraumatic Restorative Treatment (ART) in children.

Subjects and Methods: The study included two test groups, Group I (2% chlorhexidine) and Group II (neem extract). Nine patients with at least one tooth with a carious lesion suitable for ART were selected for each group; samples of dentin were collected using sterile spoon excavators at three stages from each tooth: pre-excavation, post-excavation and post-disinfection of the cavities. These dentinal samples were subjected to microbiological analysis for Total Viable Count (TVC). The data collected were statistically analysed using ANOVA and Shapiro-Wilk tests.

Results: The results of present study showed that there was a statistically significant reduction in TVC when compared between pre and post excavation in both groups.

Conclusion: Natural antibacterial agents like neem could be effectively used as cavity disinfectant which will help in minimizing secondary caries and rendering a long term restorative success

1. INTRODUCTION

Dental caries is considered as a major public health problem which affects the adult and the child. It is a multifactorial disease which is caused mainly by oral bacteria, fermentable substrate, food, time and other secondary factors. It is a complex process initiated by accumulation of dental plaque followed by demineralization of tooth hard structure, enamel and dentin, by organic acids produced. Once initiated it begins to progress causing pain and discomfort. Moreover, the child with dental caries can suffer from premature tooth loss with its consequences of compromised chewing and damage to the permanent teeth [(1)].

The carious dentin is classified into two main layers; the superficial layer (infected dentin) which is abundant by carious bacteria and decalcified dentin that is supposed to be removed to avoid its progression and the deep layer (affected dentin) which is different from the other layer in the hardness, color and moisture with less bacterial invasion [(2)].

At present, the recommendations are toward preserving more tooth structure and avoidance of pulp approximation and exposures. This can be achieved by maintaining the affected carious dentin by selective removal of caries followed by appropriate sealing of the cavity leading to starvation of the microorganisms and cessation of carious process. Controlling of caries nesseciates the eradication of cariogenic microorganisms that produce acids responsible for reducing the pH and starting the demineralization process. Chlorhexidine is considered as one of the most efficient chemotherapeutic agents against S. mutans and dental caries. Consequently, it has been widely accepted as a positive control for studies on cavity disinfectants[(3)].

With the increasing incidence of drug resistance in the prevalent pathogens and an associated risk with chemotherapeutic agents, it is essential to find an alternative to existing drugs. The herbals are considered to be from the best alternatives as they possess effective pharmaceuticals properties [(4)].

Azadirachta indica, commonly known as neem, is a fast-growing tree, cultivated in several parts in India. In recent years, it gained worldwide attraction and prominence due to its medicinal significance. Every part of the tree has been widely used in the traditional systems of medicine in India and alternative medicine. It has been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders. Later on, it became a focus of attention of the modern medicine [(5)].

Literature search revealed limited randomized controlled trials studied the antibacterial role of neem extract as a disinfectant herbal agent on primary teeth. Thus this study was conducted to evaluate the effect of neem extract and chlorhexidine as cavity disinfectants after ART.

2. SUBJECTS AND METHODS

This is a Randomized clinical trial. The study proposal was approved by the ethical committee of research of Faculty of Oral and Dental Medicine, Future University in Egypt (code 22/11-2019). Informed consent explaining the rationale of the study was read and signed by the parents of the children selected for the study.

This study was conducted in the outpatient clinics of Department of Pedodontics and Preventive Dentistry at Faculty of Oral and Dental Medicine, Future University in Egypt.

Children aged 4-7 years suitable for ART with at least one cavitated dentinal lesion in primary anterior tooth, were selected irrespective of sex, race and socioeconomic status.

2.1 Eligibility criteria:

Inclusion criteria:

- Children who showed at least one asymptomatic cavitated carious lesion of incisor or canine.
- Children with age range between 4 and 7 years.
- Child and parent cooperation and willingness to participate in the study. Exclusion criteria:
- Symptomatic tooth.
- Fistula, abscess and swelling of the soft and periodontal tissues adjacent to tooth.
- Pain in the same quadrant.

2.2 Sample size calculation:

Based on a previous study [3] sample size of 5 children in each group has a 80% power to detect a difference between means of 18.52 with a significance level (alpha) of 0.05 (two-tailed) and 95% confidence intervals.

2.3 Randomization and allocation

Dr. ***(E.A)*** performed randomization using computer software (random.org) for the assignment of the patients in each group. Eighteen patients were randomly divided between the both groups with 1:1 ratio consisting of nine patients each.

According to the allocation sequence generated by the computer software, the numbers were written in small folded opaque papers and put in opaque sealed envelopes. All those papers were ready before conducting any procedure. On the scheduled date the patient was presented to the clinic and asked to pick his/her number from the pile of envelopes and was assigned accordingly. Only Dr. ***(E.A)*** had access to the randomization table and was able to tell which group each number (patient) was allocated. Lesions were allocated to the first group (Chlorhexidine gluconate 2%) or to the second group (Neem extract).

2.4 Chlorhexidine gluconate

Commercially available 2% chlorhexidine gluconate (Cerkamed, Poland) was used in this study.

2.5 Preparation Neem extract

The neem ethanolic extract was prepared in the Pharmaceutical and Drug Industries Research division, Phytochemistry Department- National Research Center, Egypt. The leaves of the plant were washed with distilled water. The leaves were dried in an oven at 40°C for 48 h and then powdered. An ethanolic extract was obtained by dissolving 500 g of the powder in 5000 ml of 70% ethyl alcohol. The contents were then filtered using Whatman filter paper no. 1, and the filtrate was evaporated for dryness.

2.6 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC):

For finding a specific and reliable concentration a pilot study was performed by the microbiologist in the laboratory. Sterile BHI broth one ml was taken in test tubes to which 100 microlitres of the fresh bacterial inoculums were added. Then the extract was added in the concentrations of 500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.95 and 0.97 mg/ml to each tube.

The tubes were checked for turbidity and the lowest dilution showing turbidity was taken as MIC. Subcultures were done on MSA agar from each of the tubes and the plates incubated for 24 hours at 37°C [6]. The lowest dilution that did not grow any colony was taken as MBC which was 31.25 mg/ml.

2.7 Dentin sampling procedure

Proper isolation was done using a rubberdam (Optradam). The superficial parts of the necrotic dentin was removed with an excavator and then discarded. A baseline dentin sample was obtained using a sharp, sterile spoon excavator which was sufficient enough to cover the surface of the excavator and was immediately transferred to a transport medium (a sterile eppendorf tube containing one ml BHI broth).

Excavation of caries was continued until firm dentin was reached [7]**.** Then a second dentin sample was collected from the dentin using another sterile excavator and immediately transferred to another sterile tube of transport medium.

Approximately one ml of the disinfecting testing agent CHX or neem extract was syringed out into the cavity for one minute and then the cavity was washed with distilled water by a syringe and air dried. A third dentin sample was then collected using another sterile spoon excavator and immediately transferred to another tube of transport medium [8]. The tooth was then restored with glass ionomer cement (GC Equia Forte®). Then the three dentin samples from each tooth collected were transferred to the microbiology laboratory within 2 hours for microbial analysis [3].

2.8 Microbiological Procedure

The samples collected were subjected to microbiological processing and were cultivated so that the total number of viable bacteria could be detected.

The samples were shaken by a vortex for 15 seconds to disperse bacterial aggregates and 10 µl aliquots of each dilution were taken using an inoculation loop onto each solid media and spread on the surface of the agar. Blood agar was used for determining bacterial total viable count (TVC). The plates were then incubated aerobically for 48 hours at 37°C in an electric incubator

After incubation, microbial count on blood agar was performed by a single examiner. Bacterial count was measured as Colony forming units (CFU) as following: CFU per ml= Colonies count x 100 / dilution factor [9].

Statistical analysis

The mean and standard deviation values were calculated for each group in each test. Viable counts of antibacterial activity were transformed to their

https://digitalcommons.aaru.edu.jo/fdj/vol7/iss2/5
DOI: https://doi.org/10.54623/fdj.7025

log10 values. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests.

Repeated measure ANOVA test was used to compare between more than two groups in related samples. Paired sample t-test was used to compare between two groups in related samples. Independent sample t-test was used to compare between two groups in non-related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

3. RESULTS

The first test group (2% CHX) showed a mean TVC pre-excavation, post-excavation and post-disinfection of 7.48 ± 0.95, 7.01 ± 1.30, 2.86 ± 0.68 respectively in CFU/ml where (p<0.001). The second test group (Neem) showed values of 7.32 ± 0.49, 6.31 ± 0.54, 5.07 ± 0.60 respectively in CFU/ml where (p<0.001) as shown in (Table 1)

Table 1:

Comparison of mean of total viable count (TVC) in each of the study groups.

Group	Procedure	Mean	SD	P-value
2% CHX	Pre-excavation	7.48	0.95	<0.001*
	Post-excavation	7.01	1.30	
	Post-disinfection	2.86	0.68	
Neem	Pre-excavation	7.32	0.49	<0.001*
	Post-excavation	6.31	0.54	
	Post-disinfection	5.07	0.60	

**; significant (p<0.05) SD: Standard deviation;*

Pair-wise comparison of viable bacterial colony was done for each study group at different phases; pre-excavation, post-excavation and post-disinfection which showed statistically significant difference in the number of bacterial colonies between each phase in each group (p<0.05) as shown in (Table 2).

Table 2:

Pairwise comparison of mean TVC count on blood agar of different phases in each of the study groups.

Group	Procedure		Mean difference	Percentage Reduction	P-value
2% CHX	Pre-excavation	Post-excavation	0.47	6.28	0.021*
	Pre-excavation	Post-disinfection	4.62	61.7	<0.001*
	Post-excavation	Post-disinfection	4.15	59.2	<0.001*
Neem	Pre-excavation	Post-excavation	1.01	13.7	0.001*
	Pre-excavation	Post-disinfection	2.25	30.7	<0.001*
	Post-excavation	Post-disinfection	1.24	19.6	0.003*

**; Significant (p<0.05)*

When pair wise comparisons were done between both groups, there was no statistically significant difference among the two study groups in the total viable count at two phases of experiment: pre-excavation and also post-excavation where (p=0.666) and (p=0.158) respectively. But when bacterial counts were compared post-disinfection there were statistically significant differences among the groups ($P < 0.01$) as shown in (Table 3). It showed higher reduction of TVC with CHX than neem extract.

Table 3:

Pairwise comparison of the number of bacterial colonies between the study groups before excavation, after excavation and after disinfection.

	Group		Mean difference	P-value
	CHX	Neem		
Pre-excavation	7.48	7.32	0.16	0.666ns
Post- excavation	7.01	6.31	0.7	0.158ns
Post- disinfection	2.86	5.07	-2.21	<0.001*

**; Significant (p<0.05) ns; non-significant (p>0.05)*

4. DISCUSSION

Modern dentistry is focusing on minimally invasive strategies and arresting caries instead of traditional restorative dentistry which is based on complete removal of demineralized and infected tissue [10].

ART was considered from the main techniques used as a minimally invasive modality[11]. However, the failure to completely remove the infected tooth structure and limitations such as accessibility and operator's fatigue for obtaining sterilized cavity can lead to micro leakage, increased pulp sensitivity and pulpal inflammation, secondary caries and failure of the restoration [12].

To overcome the problem of remnant bacteria, several antimicrobial agents have been widely used trying to disinfect the cavity. The CHX was considered the gold standard used to eliminate oral bacteria. Studies conducted by **(Taha et al.,2013)**[13] and **(Mahabala et al., 2016)**[14] showed powerful antibacterial effect of CHX. However, in some studies it has shown to have adverse effects on microtensile bond strength of composite resins [15], increases microleakage [16], constant increase in antibiotic-resistant strains and discoloration [17].

Therefore, alternate antibacterial agents are needed to overcome these drawbacks with preserving the privilege of cavity disinfection efficacy with low cost and safe properties. Natural products are considered good alternatives to synthetic chemicals [18]. Neem was used in our study to illustrate its efficacy as cavity disinfectants compared to CHX.

The present study showed significant reduction in total viable count after excavation of caries when compared with pre-excavation sample. This finding was confirmed by previous studies conducted by **(Ersin et al., 2006)**[19], **(Prabhakar et al., 2015)** [8] and **(Patri and Sahu, 2017)**[3] who showed that removal of carious dentine by ART significantly reduced TVC.

The results of the present study showed significant reduction of total viable count on the blood agar after application of 2% CHX. This is correspondence with **(Ersin et al., 2006)**[19] who found that CHX exhibited a greater significant reduction on TVC when compared to nondisinfected group, **(Borges et al., 2012)**[20] and **(Uday Mohan et al., 2016)**[7] showed that CHX was effective in reducing the bacteria in contaminated dentin.

These results may be attributed to the efficacy of CHX by binding to negatively-charged sites on the cell wall by its positively-charged molecule, therefore it destabilizes the cell wall and interferes with osmosis [21]

Results of the present study showed significant reduction of TVC after disinfection with neem leaves and twigs extract. This goes in accordance with **(Jodi et al., 2012)**[22] who showed antibacterial activity of leaf extract of neem against various oral bacterial species. In addition, **(Rajasekaran et al., 2008)**[23] and **(Das et al., 2014)**[24] stated that leaf extracts limited the growth of both Gram positive and Gram negative bacterial species tested.

The antimicrobial activity of neem could be attributed to its constituent compounds, the most important being the Azadirachtin. The significant

antimicrobial effect on bacteria is found to be due to presecnce of Azadirachtin by rupturing cell wall and inhibition of cell growth, also the rupture of cell wall disturb osmotic pressure and leads to cell death [25]. Moreover, the polyphenolic tannins in neem possess an antibacterial effect by binding to the surface associated bacterial proteins, leading to bacterial aggregation and glucosyltransferase activity loss [26].

In the current study, though neem extract showed significant reduction in TVC, it showed significant difference of antibacterial effect on total viable count between neem and 2% CHX which showed more count reduction.

5. LIMITATIONS

In this study some limitations were detected as the dentin samples were evaluated for microbial counts immediately after excavation and not after different follow-up periods. Also no follow-up was considered in the study to evaluate the success of the restoration after applying the disinfectant agents or identify occurrence of secondary caries.

6. CONCLUSIONS

By comparing the herbal antibacterial agent like Azadirachta indica (Neem) extract with the 2% Chlorhexidine; which is the gold standard of the disinfectants, the present study concluded that the Neem can be used as a potential cavity disinfectant agent for preventing secondary caries.

7. REFERENCES

1. Fisher-Owens SA, Gansky SA, Platt LJ, Weintraub JA, Soobader M-J, Bramlett MD, et al. Influences on children's oral health: a conceptual model. Pediatrics. 2007;120(3):e510–20.
2. Ntovas P, Loubrinis N, Maniatakos P, Rahiotis C. Evaluation of dental explorer and visual inspection for the detection of residual caries among Greek dentists. J Conserv Dent JCD. 2018;21(3):311.
3. Patri G, Sahu A. Role of herbal agents - Tea tree oil and aloe vera as cavity disinfectant adjuncts in minimally invasive dentistry-an in vivo comparative study. J Clin Diagnostic Res. 2017;11(7):DC05–9.
4. Guo L, Edlund A. Targeted antimicrobial peptides: a novel technology to eradicate harmful Streptococcus Mutans. J Calif Dent Assoc. 2017;45(10):557.
5. Packia Lekshmi NCJ, Sowmia N, Viveka S, Raja Brindha J, Jeeva S. The inhibiting effect of Azadirachta indica against dental pathogens. Asian J Plant Sci Res [Internet]. 2012;2(1):6–10. Available from: www.pelagiaresearchlibrary.com
6. Adyanthaya S, Pai V, S MJMD. Antimicrobial potential of the extracts of the twigs of Azadirachta indica (Neem): an in vitro study. J Med Plants Stud. 2014;2(6):53–7.
7. Uday Mohan PVM, Uloopi KS, Vinay C, Rao RC. In vivo comparison of cavity disinfection efficacy with APF gel, Propolis, Diode Laser, and 2% chlorhexidine in primary teeth. Contemp Clin Dent. 2016;7(1):45–50.
8. Prabhakar AR, Karuna YM, Yavagal C, Deepak BM. Cavity disinfection in minimally invasive dentistry-comparative evaluation of Aloe vera and propolis: A randomized clinical trial. Contemp Clin Dent. 2015;6(1):S24.
9. Ammari MM, Moliterno LFM, Hirata Júnior R, Séllos MC, Soviero VM, Coutinho Filho WP. Efficacy of chemomechanical caries removal in reducing cariogenic microbiota: a randomized clinical trial. Braz Oral Res. 2014;28(1):1–6.
10. Mount GJ. Minimal treatment of the carious lesion. Int Dent J. 1991;41(1):55–9.
11. Horowitz AM. Introduction to the symposium on minimal intervention techniques for caries. J Public Health Dent. 1996;56(3):133–4.
12. Sharma V, Rampal P, Kumar S. Shear bond strength of composite resin to dentin after application of cavity disinfectants–SEM study. Contemp Clin Dent. 2011;2(3):155.
13. Taha MY, Author S, Al-habeeb A, Nayif M. Antibacterial Effects of Diode Laser and Chlorhixidine gluconate on Streptococcus mutans in Coronal Cavity. WebmedCentral Dent [Internet]. 2013;4(8):1–10. Available from: http://www.webmedcentral.com/article_view/4179%5Cn
14. Mahabala KY, Shrikrishna SB, Natarajan S, Nayak AP. Ethanolic extracts of Aloe vera and propolis as cavity disinfectants: An in vitro study. Dent Hypotheses. 2016;7(2):61–6.
15. Hiraishi N, Yiu CKY, King NM, Tay FR. Effect of 2% chlorhexidine on dentin microtensile bond strengths and nanoleakage of luting cements. J Dent. 2009;37(6):440–8.
16. Singla M, Aggarwal V, Kumar N. Effect of chlorhexidine cavity disinfection on microleakage in cavities restored with composite using a self-etching single bottle adhesive. J Conserv Dent JCD. 2011;14(4):374.
17. Singla S, Mali S, Tyagi S, Godbole E, Kulkarni P, Helge S. Efficacy of Liquorice and Propolis Extract Used as Cavity Cleaning Agents against Streptococcus mutans in Deciduous Molars Using Confocal Microscopy: An In Vitro Study. Int J Clin Pediatr Dent. 2019;12(3):194–200.
18. Pai MR, Acharya LD, Udupa N. Evaluation of antiplaque activity of Azadirachta indica leaf extract gel—a 6-week clinical study. J Ethnopharmacol. 2004;90(1):99–103.
19. Ersin NK, Uzel A, Aykut A, Candan U, Eronat C. Inhibition of cultivable bacteria by chlorhexidine treatment of dentin lesions treated with the ART technique. Caries Res. 2006;40(2):172–7.
20. Borges FM, De Melo MA, Lima JP, Zanin IC, Rodrigues LK. Antimicrobial effect of chlorhexidine digluconate in dentin: In vitro and in situ study. J Conserv Dent. 2012;15(1):22–6.
21. Puig Silla M, Almerich Silla JM. Use of chlorhexidine varnishes in preventing and treating periodontal disease. A review of the literature. Med oral patol oral cir bucal. 2008;13(4):257–60.
22. Jodi SM, Oyinbo K, Maishanu HM, Farouq AA, Junaidu AU, Shinkafi AL. Effect of Neem Extracts (Azadirachta indica) on Bacteria Isolated from Adult Mouth. Niger J Basic Appl Sci. 2012;20(1):64–7.
23. Rajasekaran C, Meignanam E, Vijayakumar V, Kalaivani T, Ramya S, Premkumar N, et al. Investigations on Antibacterial Activity of Leaf Extracts of Azadirachta indica A. Juss (Meliaceae): A Traditional Medicinal Plant of India. Ethnobot Leafl. 2008;12(2005):1213–30.
24. Das S, Chatterjee S, Mandal NC. Original Research Article Enhanced antibacterial potential of ethanolic extracts of neem leaf (Azadiracta indica A . Juss .) upon combination with bacteriocin. Int J Curr Microbiol Appl Sci. 2014;3(9):617–21.
25. Chatterjee A, Saluja M, Singh N, Kandwal A. To evaluate the antigingivitis and antipalque effect of an Azadirachta indica (neem) mouthrinse on plaque induced gingivitis: A double-blind, randomized, controlled trial. Vol. 15, Journal of Indian Society of Periodontology. 2011. p. 398–401.
26. Anand PJ, Athira S, Chandramohan S, Ranjith K, Raj VV, Manjula VD. Comparison of efficacy of herbal disinfectants with chlorhexidine mouthwash on decontamination of toothbrushes: An experimental trial. J Int Soc Prev Community Dent. 2016;6(1):22–7.

https://digitalcommons.aaru.edu.jo/fdj/vol7/iss2/5
DOI: https://doi.org/10.54623/fdj.7025