Al-Azhar Journal of Dentistry

Volume 10 | Issue 1 Article 20

Pediatric dentistry and orthodontics Issue (Pediatric Dentistry, Orthodontics)

1-1-2023

Evaluation of the Effect of Tea Tree Oil Mouthwash on Streptococcus Mutans as Compared with Chlorohexidine in A Group of Egyptian Children

Alaa Khalil
Dentist at Ministry of Health, El-Behira, Egypt., dentistlolo7@gmail.com

Mohamad Mostafa
Assistant Professor and Head of Pedodontic and Oral Health Department, Faculty of Dental Medicine for Girls Al-Azhar University, Cairo, Egypt, dr.farouz00@gmail.com

Salam El-Araby
Lecturer of Pedodontic and Oral Health Department, Faculty of Dental Medicine for Girls Al-Azhar University, Cairo, Egypt, drpeace33@gmail.com

Maisra El-Bouseary
Lecturer of Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt., maisra_mohammed@outlook.com

How to Cite This Article

Khalil, Alaa; Mostafa, Mohamad; El-Araby, Salam; and El-Bouseary, Maisra (2023) "Evaluation of the Effect of Tea Tree Oil Mouthwash on Streptococcus Mutans as Compared with Chlorohexidine in A Group of Egyptian Children," *Al-Azhar Journal of Dentistry*: Vol. 10: Iss. 1, Article 20.
DOI: https://doi.org/10.58675/2974-4164.1476

AL-AZHAR
Dental Journal
For Girls

The Official Publication
of The Faculty of Dental
Medicine For Girls,
Al-Azhar University
Cairo, Egypt.

Print ISSN 2537-0308 • Online ISSN 2537-0316

ADJ-for Girls, Vol. 10, No. 1, January (2023) — PP. 267:272

Evaluation of the Effect of Tea Tree Oil Mouthwash on Streptococcus Mutans as Compared with Chlorohexidine in A Group of Egyptian Children

Alaa R. Khalil [1*], Mohammed H. Mostafa [2], Salam M. El-Araby [3], Maisra El-Bouseary [4]

Codex : 3-10/23.01

azhardentj@azhar.edu.eg

http://adjg.journals.ekb.eg

https://doi.org/10.58675/2974-4164.1476

Pediatric Dentistry & Orthodontics
(Pediatric Dentistry, Orthodontics)

ABSTRACT

Purpose: The objective of this study is to evaluate the effect of Tea tree oil mouthwash on salivary Streptococcus mutans count in a group of children compared to chlorhexidine. **Materials and methods:** 24 children from both sexes participated in the study. Their ages ranged from 8 to12 years. Participants were divided into 2 groups (1&2), each group included 12 children. Group 1**:** every child was given a bottle of prepared tea tree oil mouthwash (0.2%) for rinsing. Group 2: every child was given a bottle of chlorhexidine mouthwash (0.12 %) for rinsing. Unstimulated saliva samples were collected and microbiological analysis was done. **Results:** There was a significant reduction in the mean number of Streptococcus mutans colonies in both tea tree oil and chlorohexidine groups. **Conclusion:** Tea tree oil mouthwash has significant antimicrobial activity and could be utilized as a natural substitute to chlorhexidine.

KEYWORDS

Tea tree oil, Streptococcus mutans, Chlorhexidine

INTRODUCTION

Oral health has an impact on the overall health and well-being of children. Dental caries is the most prevalent oral disease and a serious health problem affecting humans all around the world[(1)]. It is caused by complex actions of microbial agents, nutrition factors, and host factors. Pain as a result of untreated carious lesions may have an effect on nutrition, speaking, school activities, and consequent growth of children[(2)].

- Paper extracted from Master thesis titled "Evaluation of the Effect of Tea Tree Oil Mouthwash on Streptococcus Mutans as Compared with Chlorohexidine in A Group of Egyptian Children"

1. Dentist at Ministry of Health, El-Behira, Egypt.
2. Assistant Professor and Head of Pedodontic and Oral Health Department, Faculty of Dental Medicine for Girls Al-Azhar University, Cairo, Egypt.
3. Lecturer of Pedodontic and Oral Health Department, Faculty of Dental Medicine for Girls Al-Azhar University, Cairo, Egypt.
4. Lecturer of Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt.

* Corresponding author email: dentistlolo7@gmail.com

Streptococcus mutans is a gram positive facultative anaerobic bacteria that belong to the normal flora of the mouth. S. mutans is that the primary etiologic agent of dental caries[1]. Therefore, finding substances that have the ability to remove or decrease these microbial species is of high importance in the prevention of dental caries. Because dental caries is a localized disease, the utilization of local anticaries agents such as mouthwash is more effective than systemic antimicrobials[3].

Chlorhexidine is the most popular antimicrobial mouthwash, out of the varied mouthwashes the foremost persistent decrease in S.mutans count has been accomplished by chlorhexidine[4]. But its side effects like discoloration of teeth, taste disturbance, and mucosal burning hinder it from getting utilized widely in dental caries prevention, particularly in children[5]. So, searching for alternatives continues, and the focus is inclined toward natural products. Recently, the use of herbal mouthwashes has become more acceptable in oral health due to their antibacterial, antifungal, anticancer, and fewer side effects[6]. The natural phytochemicals obtained from plants like essential oils offer a good alternative[7].

Melaleuca alternifolia essential oil is Australian in origin, popular as Tea tree oil (TTO). It is produced by hydrodistillation of leaves and terminal branches of M. alternifolia shrub called a paperbark tree. It has broad-spectrum antibacterial, antifungal, antiviral, and anti inflammatory properties. It has been utilized as a powerful antimicrobial substance for decades in the medical field, especially in skin conditions[7]. The main components responsible for the antimicrobial activity of TTO are terpinen-4 ol and 1,8-cineole[8].

Tea tree oil was recently brought into the oral health world. It has been consistently used as an amazing material in treating herpes lesions and mouth ulcers because of its antibacterial and antiviral activity[9]. Previous studies on Tea tree oil have shown promising results in the reduction of S.mutans count and in controlling plaque[9,10]. Thus, the present research aimed to determine the effectiveness of tea tree oil mouthwash in the reduction of S. mutans count in children after rinsing for a week. Also, comparing it with chlorhexidine mouthwash.

MATERIAL AND METHODS

This study was approved by the Research Ethics Committee, Faculty of Dental Medicine for Girls, Al-Azhar University (code is REC-PE-21-07).

Selection of patients: 24 children were selected from a Qur'an school (Dar Al-Etqan), Tanta, Egypt. A full detailed treatment plan was demonstrated to the Participants and guardians and informed written consent was assigned by guardians. Also, verbal consent from the children was obtained**.** Inclusion criteria[11]: Systemically healthy children, no orthodontic appliances, no history of oral prophylaxis for at least 3 months before the study, and children with low caries index (DMF&def: ≤ 4)**,** Exclusion criteria[11]: Children who had received any form of antibiotic 2 weeks prior to the study, history of using antimicrobial mouth rinse in the last 12 hs., or history of fluoride treatment in the past 2 weeks.

Methods:

Preparation of Tea tree oil mouthwash 0.2%[12]: Pure Tea tree oil was purchased from Nefertari company. The ingredients used to prepare 0.2% TTO mouthwash include: TTO (0.5 g), Glycerine (5 g), Propylene glycol (5 g), Tween-80 (2.5 g), and purified water. Glycerine and propylene glycol were weighed separately and mixed. Tween-80, used as a surfactant, was then weighed and added to that mix. Tea tree oil (Nefertari natural essential oil of M. alternifolia) was then weighed and mixed with it. Water was added to the above preparation to make the volume up to 100 ml and mixed well. As tea tree oil has its own flavor, no flavoring agent was added.

24 Children were randomly distributed into two equal groups 1&2, in which 12 children were in each group. The two groups followed the same oral hygiene instructions.

Group 1: each participant was given a bottle of prepared 0.2% Tea tree oil mouthwash to be used.

Group 2: each participant was given a bottle of 0.12% Chlorhexidine mouthwash to be used.

Children in both groups has been informed to: Use 10 ml from the given mouthwash for rinsing and retain it in the mouth for 30 seconds before expectorating it, rinse twice a day for 7 days under the supervision of their parents, stop eating or drinking for at least one hour after rinsing.

Collection of saliva samples:

Unstimulated saliva samples were collected from children by asking them to spit into sterilized, labeled containers till an appropriate amount of saliva was obtained. Possible fluctuations of saliva microbial counts were controlled by sampling at least 1 hour after breakfast and immediately transferred to the laboratory (Pharmaceutical Microbiology Department, Faculty of Pharmacy, Tanta University).

Baseline sample (S1): pre-rinse sample was taken before using the mouthwash.

Second sample (S2): The second sample was taken post-rinse after an interval of 10 minutes.

Third sample (S3): The third sample was taken after using the respective mouthwash for 7 days.

Preparation of the media:[13]

The selective medium Mitis Salivarius Bacitracin Agar (MSBA) was prepared as follows: 90 grams of dehydrated mitis salivarius agar were solved in one liter of distilled water. The medium was heated to dissolve the components and it was placed in the autoclave at 121° C for 15 minutes. Then it was left to cool to 55° C. then 1 ml of 1% sterilized potassium tellurite and 1ml of 200 units sterilized bacitracin was added. Each plate was filled with about 20 ml of MSBA and left 24 hours to dry at room temperature under 5-10% CO2 tension.

Microbiological analysis of streptococcus mutans:

One milliliter of each saliva sample was transferred to sterile tubes which contain 4 milliliters of Brain Heart Infusion Broth using a sterile disposable syringe. Then, saliva and BHI broth were mixed by a vortex-mixer. Using a micropipette, 0.1ml of the vortexed diluted samples were transferred and plated on Petri dishes containing Mitis Salivarius Bacitracin Agar in the laminar flow cabinet to prevent contamination. The plates have been incubated anaerobically for 48 hours at 37°C in 5% CO2. S.mutans colonies were examined, and the mean number of colony-forming units of original saliva was calculated.

Statistical analysis:[14]

Data analysis was done by the statistical program SPSS (version 25, IBM Co., USA). Colony forming units values were presented as mean and standard deviation values. Data were explored for normality using Kolmogorov-Smirnov test. The result revealed that data were parametric. So, paired t-test was used. P-value ≤ 0.05 is statistically significant.

RESULTS

The results revealed that in tea tree oil group (group1) there was a statistically significant decrease in the mean number of colony-forming units post 10 minutes and after 7 days of intervention. The percentage of reduction of (CFU) of S.mutans was 70.1% after 10 mins. and 86.8 % after 7 days. P value was < 0.001 which indicating a statistically significant difference by time within group1.

An intergroup comparison demonstrated that there was no statistically significant difference between the mean CFU of groups 1&2 at different time intervals (p-value = 0.51, 0.78, and 0.47 for Baseline, post 10 min, and Post 7days, respectively) which indicated that tea tree oil and chlorhexidine had a similar efficacy against S. mutans. (Table 1, Fig. 1).

Table (1) *CFU/ml of S. mutans in tea tree oil and chlorhexidine groups at baseline, post 10 mins., and post 7 days and significance of difference within each group.*

Colony forming unit of S. mutans	Tea Tree Oil Group			Chlorhexidine Group			Comparison of P-value between both groups at different time intervals
	Mean	SD	P-value compared to baseline within a group	Mean	SD	P-value compared to baseline within a group	
Baseline	54875	±8635.8	----	57041.7	±7022.7	----	0.51*
Post 10 mins	16408.3	±5389.6	< 0.001*	17133.3	±6936.3	< 0.001*	0.78*
Post 7 days	7245	±3583.5	< 0.001*	8619.7	±5376.2	< 0.001*	0.47*

**P significant at ≤ 0.05*

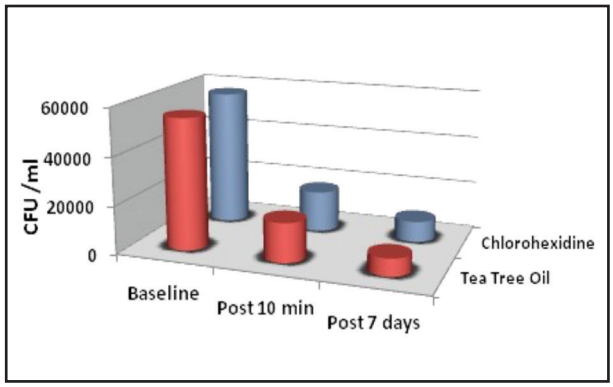

Figure (1): 3D cylindrical chart showing the mean of CFU/ml for both groups at different time intervals

In chlorhexidine group (group2) there was a statistically significant decrease in the mean number of (CFU/ml) post 10 minutes and after 7 days of intervention. The percentage of reduction of (CFU/ml) of S.mutans in group 2 was 69.9% after 10 mins. and 84.9% after 7 days. P value was < 0.001 which indicating statistical significant difference by time within group 2. (Table 2, Fig. 2)

Table (2) *The percentage of reduction % of CFU/ml of S.mutans in tea tree oil and chlorhexidine groups at baseline, post 10 min., and post 7 days.*

Time	Reduction %	
	Tea Tree Oil Group	Chlorhexidine Group
Baseline	0	0
Post 10 mins	70.10	69.96
Post 7 days	86.80	84.89

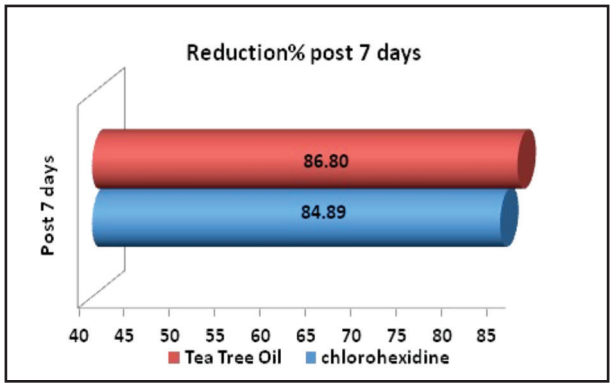

Figure (2): Bar chart represents the percentage of reduction of CFU/ml in both groups post 7 days

DISCUSSION

Dental caries is the most common oral disease and, despite the decrease in caries prevalence over the last decades, it remains a major challenge especially in developing countries. S. mutans is the major causative bacteria in dental caries. Even though many antimicrobial substances are used for caries prevention, searching for an effective substance which has a positive effect on oral health and fewer adverse effects remains important. From this point of view, natural plant products have attracted much attention from research teams within the last few years[15]. Herbal mouthwash was recently introduced in order to control plaque microorganisms due to their antibacterial, antifungal, anti inflammatory, antioxidant, and fewer side effects[16]. Thus, in the present study, a natural tea tree oil mouthwash was the one of choice.

In the current study, 0.2 % tea tree oil mouthwash was prepared, this was in accordance with a recent study[12]. Chlorhexidine was used in the present study in the control group because it has been considered as a gold standard among oral antiseptic mouthwashes that inhibit microbial plaque effectively, and the efficacy of other anti-plaque agents are compared to it[17].

The caries risk in an individual is directly proportional to the count of Salivary streptococcus mutans, Thus reduction of the count of these bacteria has been regarded as equivalent to a decrease in dental diseases[9]. Thus, S. mutans was the bacteria of interest in the present study. Mitis Salivarius Bacitracin Agar media was selected for detection of Streptococcus mutans as it is a commonly used selective medium for isolating and counting S. mutans in clinical and epidemiological cariology studies[18]. S.mutans are resistant to bacitracin. Hence, the addition of bacitracin to the media allows S. mutans to grow and form colonies and inhibit the growth of most other oral bacteria.

In our study, unstimulated saliva samples were selected as it is easier, and because this type of saliva predominates during most of the day, and previous research has documented that non-stimulated saliva represents the basal salivary flow rate[19]. This was agreed with a previous study[9]. In contrast, another study used paraffin wax stimulated saliva[12]. The population studied included children their ages (8-12) years who are adequately old to cooperate in the procedures and that they can rinse their mouth without swallowing the mouthwash to avoid swallowing reflex. This was in accordance with a previous study[20].

In this study, tea tree oil mouthwash has reduced the count of S.mutans significantly. The percentage of reduction of (CFU/ml) of S.mutans in tea tree oil group was 86.80% after 7 days. Thus, tea tree oil, if used as a mouthwash, has an excellent antimicrobial action on eradicating streptococcus mutans in vivo. Similar results have been reported in a previous study that compared the effectiveness of aloe vera and TTO mouthwashes with chlorhexidine, in children and found that they were as effective in decreasing streptococcus mutans count and in control of plaque and gingival inflammation as chlorohexidine[12].

Also, the present study is in accordance with another study that confirmed the antimicrobial effect of TTO on planktonic and biofilm forming S. mutans and revealed that it can inhibit S. mutans growth, acid generation, and adhesion[10]. Therefore, it can be suggested that using Tea tree oil as a natural mouthwash is effective as anticaries agent, and can be used as an alternative to chemical antimicrobial mouthwashes.

CONCLUSION

Tea tree oil 0.2% mouthwash showed antibacterial action against salivary streptococcus mutans when compared with the gold standard chlorhexidine, and being a natural product it could be suggested as a mouth rinse for oral hygiene maintenance and as a preventive measure for dental caries.

RECOMMENDATIONS

Further studies on tea tree oil mouthwash for a longer duration to explore the possible adverse effects of its long-term usage and to evaluate its substantivity.

ACKNOWLEDGEMENT

We are thankful to Dr. Amin Mohammed El-Kony, Assistant Lecturer at Chemistry Department, Faculty of Science, Al-Azhar University for performing statistical analysis.

Conflicts Of Interest

Authors declare no conflict of interest.

Funding

This research received no external funding.

REFERENCES

1. Anil S, Anand PS. Early childhood caries: prevalence, risk factors, and prevention. Front Pediatr. 2017; 5:157.
2. Sajadi F, Farrokhi S, Sharifi M, Saffari F, Sepehri Gh. Antibacterial Effect of Two Herbal Extracts on the Level of Salivary Streptococcus mutans in Children. Journal of Evolution of Medical and Dental Sciences. 2021; 10: 299-
3. Ghorbani F, Haghgoo R, Aramjoo H, Rakhshandeh H, Jamehdar SA, Zare-Bidaki M. The antibacterial effect of Magnolia mouthwash on the levels of salivary Streptococcus mutans in dental plaque: a randomized, single-blind, placebo-controlled trial. Iran J Microbiol. 2021; 13: 104-11.
4. Hegde RJ, Kamath S. Comparison of the Streptococcus mutans and Lactobacillus colony count changes in saliva following chlorhexidine (0.12%) mouth rinse, combination mouth rinse, and green tea extract (0.5%) mouth rinse in children. J Indian Soc Pedod Prev Dent. 2017; 35: 150-5
5. James P, Worthington HV, Parnell C, Harding M, Lamont T, Cheung A, et al. Chlorhexidine mouth rinse as an adjunctive treatment for gingival health. Cochrane Database Syst Rev. 2017; 3: CD008676.
6. Radvar M, Moeintaghavi A, Tafaghodi M, Ghanbari H, Fatemi K, Mokhtari MR, et al. Clinical efficacy of a herbal mouth wash composed of Salix alba, Malva sylvestrais and Althaea officinalis in chronic periodontitis patients. J Herb Med . 2016; 6: 24-7.
7. Jasmine V, Ravishankar PL, Saravanan AV, Padmaja V, Visithiriyan G, Preethikaa G . An in-vitro evaluation of the efficacy of the essential oils from Leptospermum Scoparium and Melaleuca Alternifolia as an antimicrobial agent against P.Gingivalis. Annals of R.S.C.B. .2021; 25:10291-6.
8. Ripari F, Cera A, Freda M, Zumbo G, Zara F, Vozza I. Tea Tree Oil versus Chlorhexidine Mouthwash in Treatment of Gingivitis: A Pilot Randomized, Double Blinded Clinical Trial. Eur J Dent. 2020; 14: 55-62.
9. França Leite KL, Martins ML, Medeiros MM, Iorio LP. Antibacterial activity of Melaleuca alternifolia (tea tree essential oil) on bacteria of the dental biofilm. Brazilian Research in Pediatric Dentistry and Integrated Clinic. 2017; 17 : 1-8.
10. Song YM, Zhou HY, Wu Y, Wang J, Liu Q, Mei YF. In vitro evaluation of the antibacterial properties of Tea Tree Oil on planktonic and biofilm-forming Streptococcus mutans. AAPS PharmSciTech. 2020; 21: 227.
11. Goyal AK, Bhat M, Sharma M, Garg M, Khairwa A, Garg R. Effect of Green tea mouth rinse on streptococcus mutans in plaque and saliva in Children: An in vivo study. J Indian Soc Pedod Prev Dent. 2017; 6: 35-41.
12. Kamath NP, Tandon S, Nayak R, Naidu S, Anand PS, Kamath YS. The effect of aloe vera and tea tree oil mouthwashes on the oral health of school children. Eur Arch Paediatr Dent. 2020; 21: 61-6.
13. El sharkawy MS , ELmalt M , Mostafa MH. Evaluation of the antimicrobial effect of pomegranate extract on Streptococcus mutans.ADJ for Girls. 2019; 6: 467-73.
14. Kim TK. T test as a parametric statistic. Korean J Anesthesiol. 2016; 68: 540.
15. Li J, Wu T, Peng W. Effects of resveratrol on cariogenic virulence properties of Streptococcus mutans. BMC Microbiol. 2020; 20: 99.
16. Raj D, Thangavelu L, Ganapathy D. Herbal mouthwash. Drug Invention Tody. 2019; 8: 2325–9.
17. Shetty V, Bhandary S, Pereira R. Evaluation of antiplaque and antimicrobial activity of Cocoa Bean Extract: An in vivo study.World J Dent. 2021; 12: 150-5.
18. 18. Zeng Y, Youssef M, Wang L, Alkhars N, Thomas M, Cacciato R, et al. Identification of non- streptococcus mutans bacteria from predente infant saliva grown on Mitis-Salivarius-Bacitracin Agar. J Clin Pediatr Dent. 2020; 44: 28-34.
19. Padiyar B, Marwah N, Gupta S, Padiyar N. Comparative Evaluation of Effects of Triphala,Garlic Extracts, and Chlorhexidine Mouthwashes on Salivary Streptococcus mutans Counts and Oral Hygiene Status. Int J Clin Pediatr Dent. 2018; 11: 299-306.
20. Bersy DA, Mostafa MH, El-Araby SM. Evaluation of the Antibacterial Effect of Cinnamon Extract on Streptococcus Mutans.ADJ for Girls. 2021; 8 : 123-8.