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. 7, No. 2, April (2020) — PP. 313:318

Evaluation of the Antimicrobial Effect of Thyme Extract on Streptococcus Mutans

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Codex : 39/2004

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http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2020.7484.1073

Pediatric Dentistry & Orthodontics
(Pediatric Dentistry, Orthodontics)

ABSTRACT

Purpose: This study was conducted to evaluate the effect of Thyme extract mouth rinse against Chlorhexidine mouthwash on salivary Streptococcus mutans count in a group of Egyptian children. **Materials and Methods:** A total of 70 Egyptian children were included in this study. Children ages ranged from 5 to 10 years old in a good physical condition. Children were randomly distributed into two groups A & B each of 35 children. In children of group A (using Thyme extract) each participant was instructed to rinse with 10ml of thyme extract for one minute. In group B (control group using Chlorhexidine mouthwash 0.2%) each participant was instructed to rinse with 10ml of CHX for one minute. **Results:** There was a significant reduction in Streptococcus mutans count in both groups. **Conclusion:** Thyme extract mouthwash was successful as an antimicrobial agent. It significantly reduced the total bacterial count in the saliva of children when compared to a potent antiseptic like Chlorhexidine.

KEYWORDS

Thyme extract,
Chlorhexidine mouthwash,
Oral microorganisms.

INTRODUCTION

Dental caries involves a dynamic process of demineralization and then remineralization (demineralization caused by acids of microbial origin and remineralization caused by salivary or therapeutic factors), but finally the oral environment directed toward demineralization [(1)].

Several factors affect the tooth structure [(1)]. The oral cavity contains several types of bacteria, but only streptococcus mutans and lactobacillus are thought to cause dental caries. After fermentation of dietary

- Paper extracted from Master thesis titled "Evaluation of the Antibacterial Effect of Thyme Extract on Streptococcus Mutans"

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sugar these organisms produce high amounts of lactic acid and resist the adverse effects of low PH [2].

Chlorhexidine (CHX) mouthwash is used to reduce count of oral bacteria and dental plaque. It has both bactericidal besides bacteriostatic activity as it has the ability to be adsorbed onto the pellicle-coated enamel surface [3]. Chlorhexidine activity continues in the mouth more than other mouthwashes (the reason that CHX is preferred over other mouth washes for gingivitis) [4]. Prolonged use of Chlorhexidine mouth wash has the ability to cause oral tissue stains and resin restorations stains, also it reduces taste sensations (the symptom which can be countered by stopping the use of chlorhexidine) [5].

Recently, there is a marked interest towards phenolic compounds found in plant food. This is because of their beneficial effects in humans.

Thyme is one of these phenolic compounds. The most important ingredient of thyme is thymol. Thymol can be used as an active component in mouthwash preparations such as Listerine [6]. Thymus Vulgaris has a strong antimicrobial action. Several studies suggested that thymol has a synergistic effect with carvacrol in reduction of bacterial resistance to antibiotics [7]. Also, thyme oil exhibited post antibacterial action against some micro-organisms which related to inhibiting the growth, inhibiting lactic acid production and decreasing cellular glucose uptake (CGU) [8].

MATERIAL AND METHODS

Materials used:

Thymus Vulgaris plant was obtained from dried herb market, Cairo, Egypt. Thyme extract was prepared as (5% infusion), Chlorhexidine mouthwash (0.2%): (oraxine, medical cosmetic products Ltd, KSA) and Mitis Salivarius Bacitracin (MSB): Base enriched with sucrose to selectively isolate oral streptococci and inhibit other micro-organisms.

Case Selection:

A total number of 70 children were selected from the outpatient clinic of pediatric Dentistry Department, faculty of Dental medicine for girls, Al-Azhar University. Inclusion criteria*:* Cooperative children of ages ranging from (5-10) years, and in a good physical condition. No history of recent antibiotic administration or using antimicrobial mouth rinse (within the previous 2 weeks). Exclusion criteria: Children with any systemic diseases, Children with any untreated active carious lesions, children with topical fluoride treatment, Children with history of allergy to any mouth rinse or drug.

Methods: Children were randomly distributed into two groups A and B; each of 35. **(Group A**) consisted of 35 children using thyme extract (5%). **(Group B**) consisted of 35 children using Chlorhexidine mouthwash (0.2%). Each subject was directed to rinse with 10 ml of the mouthwash; for one minute; three times a day for 5 days, Saliva sampling from each subject was carried out before intervention and after 5 days of intervention at least 1 hr after a meal.

Collection of saliva sample

Non stimulated saliva samples were taken from each child by asking him/her to spit in a sterile plastic container till a suitable amount collected, at least 1 hr after meal.

Baseline sample (S1): The initial sample was taken before using the mouthwash.

Second sample (S2): The second sample was taken after using the mouthwash.

Statistical Analysis

Statistical analysis was done using a commercially available software program (SPSS 19; SPSS, Chicago, IL, USA) to compare the mean bacterial count within and between groups as data was parametric, significance of the difference between groups was evaluated using unpaired t test, whereas

pre-treatment and post treatment mean values were compared using paired t test.

The percentage of change was calculated by the following formula:

$$\frac{\text{Value after-value before}}{\text{Value before}} \text{ X10}$$

The level of significance was set at $P < 0.05$

RESULTS

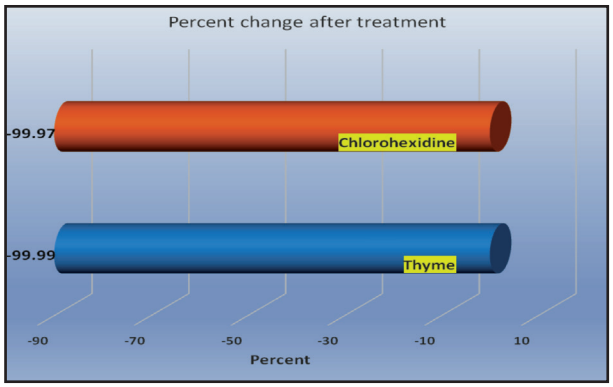
The results of our study showed that after treatment, a greater mean percent decrease in colony forming unit of Streptococcus mutans (%) was recorded in thyme group (99.99±0.02), in comparison to chlorohexidine group (99.97±0.05). However, unpaired t test revealed that the difference in percent decrease of CFU of Streptococcus mutans in both groups was not statistically significant (table1, figure1).

Table (1): *Comparison of mean percent change in colony forming unit of Streptococcus mutans (%) in thyme and chlorhexidine groups after treatment*

Groups	Mean	Std. Dev	Std. Error Mean	Mean Difference	Std. Error Difference	95% CI of Difference		t value	P value
						Lower	upper		
Thyme	99.99	.02	.00	.01	.01	.03	.01	1.16	.25ns
Chlorhexidine	99.97	.05	.01						

95% CI= 95% Confidence Interval of difference

Significance level p<0.05, ns=non-significant



Figure(1) Bar chart showing mean percent change in colony forming unit of Streptococcus mutans (%) in Thyme and chlorhexidine groups after treatment.

DISCUSSION

Dental caries involves demineralization of hard tooth structure by acids of microbial origin and other several factors affect the tooth structure [1]. The oral cavity contains several types of bacteria, but only streptococcus mutans and lactobacillus are thought to cause dental caries.

The main initiator of enamel caries is mutans Streptococci (S.mutans) [9]. Streptococcus mutans make the first step in tooth decay metabolizing sucrose to lactic acid [1] creating acidic environment that increase the chance for tooth decay. Application of antimicrobial agent (such as dentifrices and mouth rinses) reduces and controls microorganisms in plaque biofilm [10]. Mouth rinses are used to rinse the mouth for removing or destroying bacteria, act as an astringent and relieving infection or preventing dental caries [11].

The aim of our study was to use a natural product which is safe, available and easy to prepare. Prolonged CHX rinsing causes stains of oral tissues and restorations [5]. Recently, there has been a great interest in phenolic compounds found in plant food. This is because of their beneficial effects in humans. Thyme is one of these phenolic compounds.

The most important ingredient of thyme is thymol. Thymol can be used as an active component in mouthwash preparations such as Listerine [6].

Thyme is locally known as Zaatar widely used in folk medicine.

Thyme was used in this study because it has a strong antimicrobial attributes due to inhibiting the growth decreasing lactic acid production and decreasing cellular glucose uptake (CGU)[12]. The actual mode of action is unknown, but in some evidences the biocidal properties of thymol is due to membrane disruption[13] .Thyme has Anti-microbial activity reported in vitro[14] against several bacteria (Salmonella typhimurium, Staphylococcus aureus and Helicobacter pylori)[15] . Activity against caries causing bacteria and periodontopathogenic bacteria such as P. Gingivalis, S. Sorbinus, Selenomonas artemidis and S. mutans is due to perforation of cell membrane leading to intracellular components efflux [16].

In addition, thymol (which is the main constituent of thyme plant) reduces resistance of bacteria to common drugs such as penicillin through a synergistic effect with carvacrol [17]. Thymol is primarily responsible for anti-oxidative activity of thyme plant [18]. Thyme plant is rich in vitamins and minerals which protect from lung and oral cavity cancers[19].

It exhibits antispasmodic, bactericides, antiseptics, antioxidants, anthelmintic properties and has lately been suggested to be a substitute as cancer prevention agent [20].

Additionally, thymol has antifungal action due to its lipophilic property enabling it from interaction with the cell membrane, changing its permeability permitting the loss of macromolecules [21].

In vitro clinical trials discussed the antiviral activity of an aqueous extracts of thymus vulgaris against Herpes simplex viruses (type1, type2 and an acyclovir-resistant strain of HSV-1) [22]. A dry extract of thyme and evening primrose showed better healing from acute bronchitis [23].

Another trial found that thyme extract and ivy leaves resulted in 50% of patients healing from cough fits 2-days sooner than in those taking the placebo [24] and it has been found to be safe for children aged 2-17 [25].

In the present study, thyme extract was prepared by steeping 5 grams of dried leaves per 100 ml boiling water for 10 minutes and straining (5%) infusion [26]. Five percent is the lowest concentration that has an acceptable taste for the child. Saliva samples were collected from each group before rinsing and processed for bacterial count then the first group was instructed to rinse with 10ml thyme extract and the second group to rinse with 10ml CHX. Then saliva samples were collected after 5 days rinsing and processed.

Results showed that rinsing with 5% thyme extract gave nearly the same inhibitory effect on salivary S.mutans as Chlorhexidine. Another study found that thyme extract (aqueous) suppressed S.mutans adherence to the epithelial cells of the buccal mucosa [26]. Another experiment studied a rinsing with (5%, 10%, 20%) aqueous thyme extract[27], and the results showed that aqueous thyme extract inhibit the adherence of S. mutans to epithelial cells of buccal mucosa and showed that oral rinsing with chlorhexidine have almost an equal activity as that of rinsing with 10% aqueous thyme extract on preventing S. mutans adherence to BEC.

Thyme has great nutritional value as its leaves are considered a rich source for important minerals and vitamins [28], this, in addition to the safety [2], make it a promising effective antimicrobial mouthwash for children.

CONCLUSION

The daily use of thyme extract (5% infusion) mouthwash can reduce the salivary level of Streptococcus mutans which are the main etiologic factor in dental caries. Thyme extract is safe and prepared easily so, can be used as home care product.

From these trials we can conclude that thyme is a natural, safe and available plant can be prepared easily at home which make it a promising product in health care and inhibition of caries causing bacteria specially streptococcus mutans.

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