Antimicrobial Efficacy of Chlorhexidine and Licorice Mouthwashes In Children

Çocuklarda Meyan Kökü ve Klorheksidin İçerikli Gargaraların Antimikrobiyal Etkinliğinin Değerlendirilmesi

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Abstract

Objective: The aim of this in vivo study is to evaluate the effectiveness of a simple herbal caries-prevention protocol for reducing salivary *Streptococcus mutans* (SM) levels in children.

Materials and Methods: A total of 90 individuals were recruited randomly divided into three groups (n=30). Mouthwashes including chlorhexidine (CHX), licorice and saline were used as tested antimicrobial agents, and saliva samples were collected before rinsing, at the end of 5 min (T1) and 60 min (T2) following rinsing, and the differences were calculated within 5-60 min (T3). Plaques were evaluated following incubation, and counts of the growing colonies were performed in Colony Forming Units (CFU)/ml. Decreases in CFU were calculated in ratio for statistical analysis. Data were analyzed with Kruskal Wallis, Mann-Whitney U, Friedman and Wilcoxon signed rank tests by Bonferroni correction, using a 0.05 of significance level.

Results: CHX caused significantly different decreases for T1 and T2 (p<0.05), but, there were no significant differences between CHX and licorice for T3 (p>0.05).

Conclusion: Licorice might be a useful agent for dental procedures and further studies are needed to learn more about the dose of licorice, the ratio of glycyrrhizin and the duration of dental therapy.

Keywords

Licorice, mouthwash, chlorhexidine, children, antibacterial

Anahtar Kelimeler

Meyan Kökü, gargara, klorheksidin, çocuk, antibakteriyel

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Öz

Amaç: Bu in vivo çalışmanın amacı, çocuklarda tükürük streptokok mutans (SM) düzeylerini azaltmaya yönelik basit bir bitkisel çürük önleme protokolünün etkinliğini değerlendirmektir.

Gereç ve Yöntemler: Yapılan bu çalışmada Klorheksidin (CHX), meyan kökü ve serum fizyolojik kullanılmıştır. Toplam 90 hasta, her bir grup için 30 kişi olacak şekilde rastgele 3 gruba ayrıldı. Gargara öncesi, gargaradan 5 dk sonra ve gargaradan 1 saat sonrasında tükürük örnekleri toplanmıştır. Değerlendirmeler gargara sonrası 5.dk (T1), 1 saat sonra (T2) ve aradaki farklılıkları tespit amacıyla 5-60 dk (T3) olarak hesaplanmıştır. Ekimi yapılan örneklerin enkübasyon süresi sonunda plaklar değerlendirmeye alınarak üreyen kolonilerin Colony Forming Unit/ml (CFU ml⁻¹) olarak sayımları yapılmıştır. CFU değerlerindeki azalmalar istatistiksel olarak analiz edildi. Elde edilen verilerin değerlendirilmesinde Kruskal-Wallis, Mann-Whitney U and Bonferroni düzeltilmiş Friedman, Wilcoxon işaret ve testleri ile yapıldı ve yanılma düzeyi =.05 olarak alındı. Bulgular: CHX, T1 ve T2 zamanları içerisinde istatistiksel olarak anlamlı düşüşler gösterirken (p<.05), T3 grubunda CHX ve meyan kökü grubunda istatistiksel olarak anlamlı bir farklılık görülmedi (p>.05).

Sonuç: Meyan kökü dental işlemlerde kullanılabilecek yararlı bir ajan olabilir ve meyan kökü oranının, içerisindeki glisirik asit yüzdesinin ve tedavinin süresi ile ilgili daha ileri çalışmaların yapılması gerekmektedir.

Introduction

Dental caries is the most common, multifactorial, infectious disease, caused by a specific group of cariogenic bacteria (1, 2). As estimated by the World Health Organization (WHO), 5 billion people of world population are affected by dental caries (3). Dental caries has been a highly prevalent and costly disease in Turkey as well, particularly during childhood. In 2004, caries prevalence was 69.8% (4) and in 2011, it was 46.9% in Turkey (5).

Clinical studies indicated that oral colonization of *Streptococcus mutans* (SM) promotes dental caries development in humans (6, 7). SM is the major contributor to acid production and plays an important role in the dental caries. SM show adherence in retentive areas of the tooth structure, especially pit and fissures (7). Several molecular mechanisms by which SM efficiently enhance biofilm biomass in the presence of sucrose and promote ecological shifts that lead to emergence of acidogenic and aciduric organisms have been described (8).

Dental caries is preventable by adopting healthy behaviors, such as regular tooth brushing, favorable dietary habits and regular dental check-up (9). However, in some instances mechanical methods need to be combined with chemoprophylactic agents to prevent tooth caries (10). Various antibacterial agents have been tested for decreasing the number of cariogenic bacteria, reducing acid production and preventing biofilm formation (2). One such successful chemotherapeutic agent is chlorhexidine (CHX). CHX has antimicrobial activity against SM and also demonstrates antiplaque and antigingivitis properties (11, 12). On the other hand, the use of CHX has some side effects such as staining, altered taste sensation, vomiting, increased risk of caries due to fermentation and alcohol content, development of resistant bacterial strains or oral cancers (13, 14).

Innovations and recent researches in dentistry presented a new herbal material named licorice. *Glycyrrhiza glabra*, commonly known as licorice root, is a medicinal plant has been used since ancient times to relieve coughs, sore throats, and gastric inflammation as a traditional herbal remedy (15). *Glycyrrhiza glabra* has been used as a sweetener in food and drug industry, and it is listed generally regarded as safe (GRAS) by Food and Drug Administration (FDA) (1).

Licorice root compounds with their anti-adherence, antimicrobial and anti-inflammatory effects may help treating oral diseases including dental caries (16, 17). Glycyrrhizin, a sweettasting compound (50 times sweeter than sucrose) and it can increase salivary flow due to acting as a gustatory stimulus, and then can provide a beneficial effect against dental caries (18).

In recent years, a few studies regarding anti-cariogenic properties of licorice root has been published. Chaiya et al. (19) have demonstrated that *Glycyrrhiza glabra* inhibited the growth of SM as well as the adherence ability of the bacteria. Jain et al. (15) have showed antimicrobial and cariostatic efficacy of liquorice extracts and recommend that liquorice can be used as a preventive regimen in pediatric dental practice. Hu et al. (20) and Peters et al. (1) have demonstrated that licorice containing lollipop led to reduction in salivary SM in their studies.

The aim of this *in vivo* study was to evaluate the effectiveness of a simple herbal caries-prevention protocol for reducing salivary SM levels in children.

Materials and Methods

Ethical approval for this study was obtained from the XXXXX University Clinical Research Ethic Committee (2014-02/15) and the study was conducted at the Department of Pediatric Dentistry, Faculty of Dentistry, XXXXX University, XXX, XXX.

Sample Distribution

In this study, with an $\alpha=0.05$, $\beta=0.20$ and $(1-\beta)=0.90$, a total of 90 individuals [48 girls (53.3%), 42 boys (46.7%)] were included in this study, and the power of the study was $p=0.81$.

The subjects were selected using simple lottery method from patients who came to routine dental treatments, aged 10-13 years (mean age: 11.33), have a clinical picture of basic features of gingivitis (with/without gingivitis). The same procedures were followed for all patients. The patients who have unknown allergies or idiopathic allergies, who had received antibiotic therapy within past 1 month, using any mouthwash for maintaining oral hygiene, having any painful, debilitating oral condition, and patients who failed to comprehend the task were excluded from the study.

Three groups were formed as;

CHX Group

Unstimulated saliva samples were taken from all patients (baseline) and then the patients rinsed their mouth with CHX for 1 min (0.2% of chlorhexidine solution, Klorhex, Drogosan, Ankara, Turkey). Two more saliva samples were taken at the end of 5 min and 60 min.

LICORICE Group

Saliva samples were taken from all patients (baseline) and then the patients rinsed their mouth with freshly prepared licorice for 1 min. Two more saliva samples were taken at the end of 5 min and 60 min.

SALINE Group

Saliva samples were taken from all patients (baseline) and then the patients rinsed their mouth with saline for 1 min. Two more saliva samples were taken at the end of 5 min and 60 min.

Licorice Root Extract Solution Preparation

A modified protocol of Sedignnia et al (21) were used for this study. Two hundred mg of licorice was weighed and mixed with a 20 ml of a solution composed of a mixture of distilled water and ethyl alcohol at a rate of 70:30 at 60°C for 25 minutes. The mixed solution was centrifuged at 3000 rpm for 10 min and the extract was separated. The extracts were then concentrated through ethanol evaporation. Finally, the supernatant was filtered from a membrane filter and was prepared for use. Seventy-five ml rinse solution was freshly prepared to be used in patients in 15 ml cups for single use and prepared again when it finished.

Measurements

Saliva samples were collected before gargling, at the end of 5 min (T1) and 60 min (T2) following rinsing and was transferred to laboratory as soon as possible. The differences were calculated within 5-60 min (T3). After collecting saliva samples, the samples were sent to microbiology laboratory immediately.

SM Count

A saliva sample of 0.01ml was obtained and added to a physiologic serum solution of 9.99 ml. A 0.01 ml of the obtained suspension was taken using a sterile plastic loop and was cultured in Difco™ Mitis Salivarius Agar (Becton, Dickinson and Company, Sparks, MD, USA). The agars were incubated for 24-48 h in an environment with 5% CO₂ at 36.5°C. Plaques were evaluated following incubation and counts of the growing colonies were performed in Colony Forming Units/ml (CFU ml⁻¹). Elevated colonies that grew in the plaques that were faded blue in color and had a granular ground-glass appearance were accepted as SM. Colonies evaluated to be SM in the agars were confirmed using Microflex LT MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and BD Phoenix 100 system (Becton Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) and Streptococcus definition panels (BD Phoenix SMIC/ID-11 Becton Dickinson, Sparks, MD, USA).

Statistical Analysis

The data was enrolled with Statistical Package for Social Sciences (SPSS) 17.0 for Windows (SPSS Inc., Chicago, IL, USA). The compliance of normal distribution of data was performed by Shapiro Wilk test. Kruskal Wallis and Mann Whitney U test was performed with Bonferroni correction. Variations by time were performed with Friedman Wilcoxon signed rank test by Bonferroni correction. *p* value of 0.05 was referred statistically different.

Results

After counting the SM colonies, the percentages were calculated by the ratio of the decreases in SM count. Table 1 shows the minimum, maximum and mean CFU of the groups. CHX caused 100% decrease in some samples for the first 5 min (T1) and showed significantly different decreases in SM count when compared with the other groups. CHX and licorice showed 100% decrease in some samples for T2.CHX

Table 2: Binary comparisons of the groups (*: there were no significant differences for T3 between CHX and licorice)

	Saline			Licorice			CHX		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Saline	-	-	-	,001	,000	,000	,000	,000	,003
Licorice	,001	,000	,000	-	-	-	,000	,000	,167*
CHX	,000	,000	,003	,000	,000	,167*	-	-	-

and licorice showed significantly higher decreases in binary comparisons of the groups (Table 2).

CHX showed significantly different decreases for T1 and T2 but there were no significant differences for T3 between CHX and licorice ($p>0.05$). Decreases were seen in all samples of CHX and licorice groups (Figure 1).

The rankings of reducing of the number of bacteria colonies were CHX>Licorice>Saline for T1 and T2 but there were no significant differences for T3 between CHX and licorice ($p>0.05$).

Licorice and CHX decreased SM count 100% in some samples (Figure 2).

Discussion

The main purpose of dentistry is to help patients to maintain their oral health (2). The most common

Table 1: The minimum, maximum, mean and standard deviations of the groups

TIMES			
	T1	T2	T3
GROUPS			
Saline	23,14+11,27	20,08+10,71	11,03+10,27
Licorice	39,70+16,18	69,18+14,88	29,47+17,01
Chlorhexidine	69,26+20,86	89,33+12,88	20,87+12,92

CFU ml-1 values are shown as mean± standart deviation

Figure 1: CHX and licorice showed decreases in all samples (M_1 : Licorice T1, M_2 : Licorice T2, M_3 : Licorice T3, K_1 : CHX T1, K_2 : CHX T2, K_3 : CHX T3).

Figure 2: 100% decreased amount of SM (M_1 : Licorice T1, M_2 : Licorice T2, M_3 : Licorice T3, K_1 : CHX T1, K_2 : CHX T2, K_3 : CHX T3)

dental problems were caused by microorganisms and they play a key role in pulpitis, infection mediated necrosis and their extension to the periapical tissues. Current anti-microbial strategies to treat dental caries have consisted primarily of mechanical dental plaque removal or generalized destroying of oral bacteria with antibacterial compounds (22, 23).

One of these compounds used for chemical plaque control and inhibition of bacterial colonization to the tooth surfaces is CHX. CHX is an effective antibacterial agent and has effects on a wide range of microorganisms in the oral cavity. CHX has been used to prevent and reduce carious lesions, because SM species are particularly sensitive to CHX. CHX reacts directly with the microbial cell surface, destroys the integrity of the cell membrane, penetrates the cell, and precipitates the cytoplasm leading to cell death (15, 24). CHX or CHX containing oral health products has the strongly suppresses SM with bacteriostatic effect which makes CHX gold standard (11, 25).

CHX gluconate is a cationic bisguanide with the broad-spectrum antimicrobial action, whose effectiveness in decreasing the formation of dental biofilm and gingivitis have been demonstrated in several clinical studies (26, 27). However, CHX has some side effects; including bitter and metallic taste and altered taste perception and the formation of extrinsic stains on the teeth and tongue, increased risk of caries due to fermentation and alcohol content, and cytotoxic effects on cells (15, 24). All these disadvantages have led current research to more natural and biocompatible agents like herbal medicines (25).

History of herbal medicines originated from ancient civilizations and they have been used for many years as the main source of medicines. Herbal

medicines are still the mainstay of about 75-80% of the whole population, mainly in developing countries for primary health care because of their better cultural acceptability and compatibility with human body; and fewer side effects. Hence, studies are being carried out to discover new alternative plant-based bioactive compounds which will be safer, easily available, and might substitute standard pharmaceutical remedies (21-24).

Antimicrobial activity of licorice was shown in many studies (28, 29). It is used for many purposes such as antibacterial agent, mouthwash, therapeutic agent for ulcerous lesions, endodontic infections and periodontitis. It has anti-inflammatory, antimicrobial, anticarcinogenic and hepatoprotective effects (21, 24, 28, 30-32). One of the aims of using licorice is to reduce the bacteria colonies which cause dental caries and licorice was added into lollipop, rinsing solutions and gels for this aim (20, 28).

In this study, licorice was used as a rinsing solution. There are two forms of licorice rinsing solution. One is aqueous licorice form and the other is alcoholic licorice form. Ajagannanavar et al. (22) declared that alcoholic root extract of licorice was superior to aqueous form and CHX. Sedighinia et al. (21) declared that the ethanolic extract of licorice had promising minimum inhibitory concentration (MIC) value against all oral bacteria, especially *S. mutans*, *A. viscosus*, and *E. faecalis*. Ahn et al. (24) found that three purified compounds that were isolated from ethanol-extracted licorice root, were less cytotoxic to normal human gingival fibroblast (NHGF) cells than CHX. Jain et al. (15) concluded that alcohol was a better solvent than water and that the reduction in colony counts of ethanolic licorice group was more significant than the control groups. In line with the results of these studies, alcoholic form of licorice was preferred in this study.

CHX has been considered to be the gold standard of oral therapies due to its prolonged broad spectrum antimicrobial property, but it is accompanied by altered taste perception, metallic taste, and staining of teeth. (15, 26, 33). CHX was one of the mouthwashes used in this study. CHX showed more decrease in SM count than the other mouthwashes for T1 and T2 and the decreases were statistically different. However for T3, licorice showed 30% and CHX showed 19.37% decrease, though there were no significant differences

between these groups. This has led to a conclusion that licorice showed its effect in a long-time period. The authors declared that one of the limitations of this study was that the study was completed in one hour and long-term follow up studies are needed to support this conclusion.

In some samples, both licorice and CHX decreased SM count 100% and this result indicated that the licorice mouthwash is a dose dependent material which shows its effectiveness by glycyrrhizin. Glycyrrhizin is the most important ingredient of licorice which might be in a high concentration and provide the antimicrobial effect of licorice (15, 28). The high concentration of glycyrrhizin (5%, 10%) slightly inhibited bacterial growth, but completely abolished plaque formation (34). Soderling et al. (28) declared that the liquorice extract used in their study inhibited acid production effectively *in vivo* with concentrations ranging from 2.5 to 10%. Glycyrrhiza glabra extract containing 7-7.5% glycyrrhizin was found advantageous in terms of cytotoxicity because 87% of periodontal ligament fibroblasts were alive after 48 h (30). Another limitation of this study is that the ratio of glycyrrhizin could have been calculated initially, but no other study were done about the ratios of glycyrrhizin. When side effects of chlorhexidine, the need of herbal products were taken into account, these kinds of herbal mouthwashes will be good alternatives and the authors hope this *in vivo* study could give more opinions for further studies about glycyrrhizin.

Conclusion

The results of this study indicated that licorice might be a useful tool for dental procedures and further studies are needed to learn more about the dose of licorice, the ratio of glycyrrhizin and the duration of dental therapy.

Ethics

Ethics Committee Approval:

Informed Consent:

Peer-review:

Authorship Contributions

Conflict of interest: The authors declare that they have no conflict of interest.

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