AL-AZHAR
Journal of Dentistry

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

Print ISSN 2974-4156 • Online ISSN 2974-4164

AZJD, Vol. 10, No. 2, April (2023) — PP. 503:509

Evaluation of the Antibacterial Effect of Licorice Extract on Oral Microflora and its Effect on Salivary PH

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Codex : 3-02/23.04

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http://adjg.journals.ekb.eg

DOI: 10.21608/adjg.2023.103433.1433

Pediatric Dentistry & Orthodontics
(Pediatric Dentistry, Orthodontics)

ABSTRACT

Purpose: To evaluate the antibacterial effect of Licorice (as a mouth wash) on total salivary bacterial count in relation to chlorohexidine mouthwash on a group of Egyptian children and assess the effectiveness of Licorice extract rinse on salivary PH. **Materials and methods :**A total of 60 normal apparently healthy children aged between 6-12 years from both genders were involved in this study and equally divided into four groups(n=15) regarding to the type and concentration of the received mouthwash **:** group I : included children used licorice extract mouth-rinse (75%), group II: included children used licorice extract mouth rinse (50%), group III: included children used licorice extract mouth-rinse (25%) and group IV: as a control group in which children used chlorohexidine 0.12% mouth wash for one minute. They were told to gargle with 10 ml of mouthwash for one minute three times a day for five days. **Results:** Significant reduction in the total bacterial count with the four studied groups after mouth rinsing for 5 days. However, chlorohexidine showed a significant reduction in total bacterial count when compared to Licorice extract .Regarding the pH value and the percent change in pH, the highest mean value was recorded in group I, and the difference between the 4 groups was highly statistically significant. **Conclusion:** Licorice aqueous root extract has antimicrobial effect against total bacterial count and it is more efficient in comparison with Chlorohexidine. Licorice aqueous root extract rapidly rise salivary PH.

KEYWORDS

Cariogenic Microflora,
Egyptian Children,
Licorice extract

INTRODUCTION

Tooth caries is a complicated sugar-driven disease caused by biofilms that causes phasic demineralization and remineralization of tooth hard tissues. Later in adulthood caries can affect the crowns of

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teeth as well as exposed root surfaces and it can affect both primary and permanent dentitions [1].

Dental caries is caused through interactions between tooth structure, microbial biofilm on the tooth surface and carbohydrates as well as salivary and genetic factors. The dynamic caries process consists of rapidly alternating phases of tooth demineralization and remineralization which culminates in the development of specific caries lesions at anatomical predilection points on the teeth if net demineralization continues for a long enough length of time [2].

Preventing dental caries entails preserving healthy tooth structure, reducing enamel demineralization and promoting natural healing processes. Health policy, legislation, regulation and public health strategies can all be used at the population level to encourage healthy behaviors and change wider social determinants of health. To ensure equality prevention interventions can either target the entire population (for example water fluoridation and sugar levies) or higher-risk populations to maximize cost effectiveness [3,4].

Oral rinses are a typical way to get therapeutic substances to all of the mouth's accessible surfaces including the interproximal hard surfaces. Mouth rinses eliminate the need for manual dexterity and allow the duration of the treatment to be controlled. However, mouthwashes now on the market have been linked to various negative consequences including an unpleasant taste, an increased risk of cavities due to fermentation and alcohol content and tooth discoloration. Because of its long-lasting broad-spectrum antibacterial properties, chlorhexidine is regarded the "gold standard" of oral preventative medicine . However, continuous use causes changed taste perception, metallic taste and tooth discoloration [5].

Many chemical compounds that have been reported to affect bacterial metabolism and adhesion such as chlorhexidine, delmopinol and triclosan have shown substantial inhibitory activity against biofilm growth and maturation. The breakdown of the serosa permeability barrier against bacterial cells is thought to be the mechanism of chlorhexidine's bactericidal effect. This chemical can cause partial cytoplasmic leakage at low concentrations, whereas high doses cause cytoplasm condensation, denaturation and sterilization [6].

Licorice is a Glycyrrhiza family substance that is utilized in the form of dentifrice, chewing gums, lollipops and gels. The efficacy of licorice root extract on the biofilm lowering Streptococcus mutans (SM) count and preventing dental caries in children has been reported in the literature [7]. Despite the fact that liquorices' anticariogenic properties have been disputed for many years, there have been few studies published on its role as an anticariogenic agent. Liquorices' anti-inflammatory properties have recently received a lot of attention [8].

MATERIALS AND METHODS

This study was conducted on total of 60 Egyptian children from both sexes' 30 males and 30 females in which their age ranged from 6-12 years old. All the procedures and instructions were explained to all children parent or guardians and to sign a formed consent. Ethical approval was obtained from the research and ethical committee of the Faculty of Dental Medicine of Al-Azhar University for Girls Cairo-Egypt (REC-21-13) .

Using a sterile diagnostic set that included a plane mirror, sterile explorer, and tweezers, all children were clinically evaluated for dental caries.

Sample size calculation

It was done according to previous study[9] that compare of total bacterial count using 3 different aqueous concentrations of licorice in distilled water and chlorohexidine initially and after 5 days with values ranged from 5.48±0.27 using chlorohexidine to 5.08±0.36 using aqueous extract of licorice (1.5g/10ml) and 4.83±0.64 using ethanolic extract (375mg/ml). A total sample size of 48 (24 in control group and 24 in study group (further sub divided into 8 patients in each concentration each group) .

Subjects grouping

Children were equally divided into four groups (n=15) according to type of the received mouth rinse: group I: included children who rinsed with 75% licorice extract containing mouth wash, group II: included children who rinsed with 50% licorice extract containing mouth wash, group III: included children who rinsed with 25%licorice extract containing mouthwash and group IV: included children who rinsed with 10 ml of chlorohexidine mouth wash for one minute for 5 days three times a day (control group) .

Preparation of Licorice mouth-rinse:

Liquorice roots was dried shade and coarse ground in an electric blender. A total of 30 g of licorice were soaked in 100 ml distilled water for 24 h with intermittent shaking. The extract was considered as 100% in concentration. The crude extracts were filtered. The concentrations of 75% , 50% and 25% were made respectively by diluting the concentrated extract with the required volume of distilled water [8] figure (1).

Patients Instructions:

The enrolled children were instructed to rinse their mouth with 10 ml of the solution for 1 min three times per day for 5 days after tooth brushing followed by expectoration of the residual mouth rinse. Tooth brushing and mouth rinsing techniques were demonstrated for every child. After mouth rinsing the subjects were adjusted not to eat or rinse for the next 30 minutes and frequent reminders were given to supervisors (parent's/caregivers) to insure compliance and not to take any antibiotics without reference to operator.

Collection of saliva Sample

Prior to the start of the experiment the subject's salivary concentration of microorganisms calculated from a sample of saliva in order to establish the baseline level (S1).The salivary samples (S2) collected after 5 days of treatment with mouth wash therefor two saliva Samples (S1andS2) were taken for each individual [10].

Samples were delivered as soon as possible to the microbiological lab at Microbiology and Immunology Department, Faculty of Medicine for Girl's, Al-Azhar University. Plate Count Agar (PCA), also called Standard Methods Agar (SMA), is a microbiological growth medium commonly used to assess total bacterial growth of samples that cultured using spread technique to produce single colonies [11] .

Determination of PH [10]:

A chair side kit was used to determine the pH of entire saliva taken at each interval (MQuant, Universal indicator). After dipping the pH test paper in the sample for at least 10 seconds the color changes were compared to the manufacturer's chart as represented in figure (2).

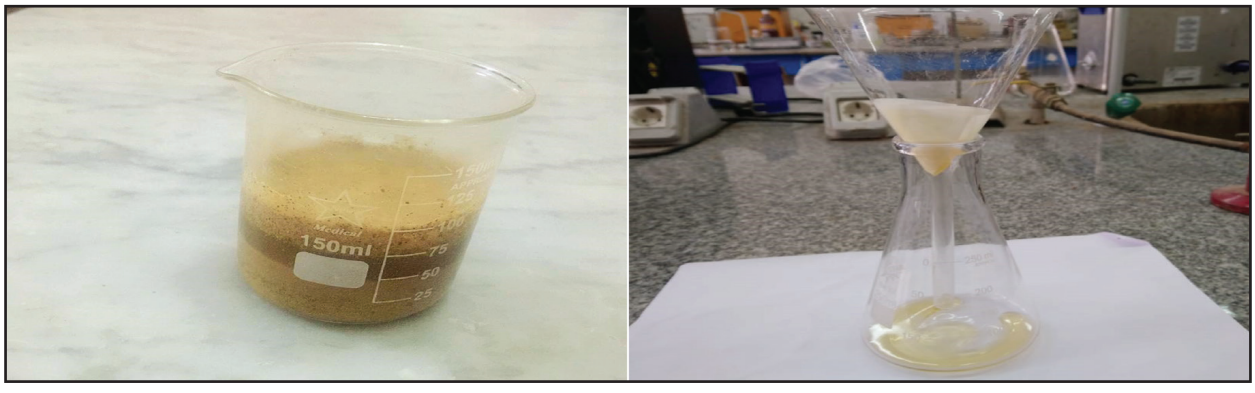

Figure (1) Licorice extraction.

Figure (2) pH indicators color coding strips

Statistical analysis

Statistical analysis was performed using a commercially available software program (SPSS Chicago, IL, USA). Numerical data described as mean and standard deviation or median and range. Data compared using ANOVA test or Kruskall Wallis test according to normality. The level of significance was set at P .0.05. All tests will be two tailed.

RESULTS

Demographic data

The mean age for group I was 9±2.12, while group II was 9.4±2.07, the mean age in group III was 8.2±2.68 and the mean age was 9.2±2.39 for group IV. There was no significant difference between groups regarding age (p=0.518). Regarding gender distribution group I consisted of 20% males and 80% females, group II consisted of 60% males and 40% females, group III consisted of 80% males and 20% females and group IV consisted of 40% males and 60% females. There was a significant difference between groups regarding gender distribution (p=0.007).

Colony forming unit (CFU)

Pre-treatment there was no discernible difference between groups (p=0.495). Post-treatment the highest mean value was recorded in Group III (6.26±1.01) followed by group II (4.25±0.9) then group I (2.86±0.66) while the least value was recorded in-group IV (2.34±0.71). The difference between the four groups was statistically significant (p=0.000). Regarding the percentage change, the highest mean percent decrease was recorded in Group IV (-68.88±1.01) followed by group I (-59.77±0.88) then group II (-41.73±6.83) while the least value was recorded in group III (-17.74±7.06). There was a statistically significant difference between the four groups (p=0.000) as represented in figure (3)

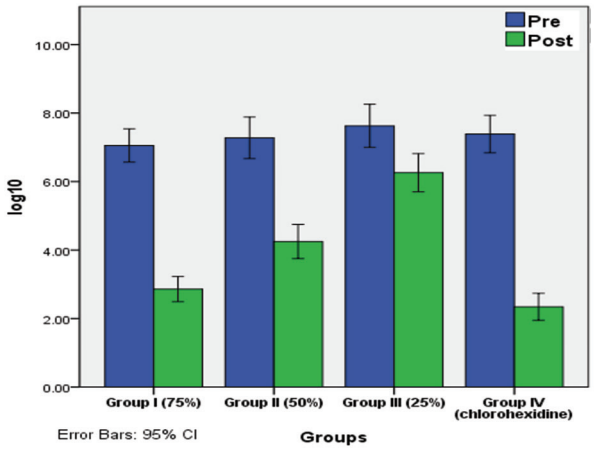

Figure (3) Bar chart illustrating mean log10 of Colony forming unit of total bacterial count in different groups.

PH Evaluation

Pre-treatment there was no significant difference between groups (p=0.468). Post-treatment the highest mean value was recorded in Group I (7.8±0.45), followed by group II & III (7.4±0.55), while the least value was recorded in group IV (5.2±0.45). The difference between the four groups was statistically significant (p=0.000). Tukey's post hoc test revealed no significant difference between Groups I, II and III. These three groups recorded a significantly higher value compared to group IV.

Regarding the percentage change, the highest mean percent increase was recorded in Group I (40.67±18.92), followed by group II & III (32.67±9.55), while group IV recorded a percent decrease (-13.33±7.45). The difference between the

four groups was statistically significant (p=0.000). Tukey's post hoc test revealed no significant difference between Groups I, II and III. These three groups recorded a significantly higher value compared to group IV as represented in figure (4).

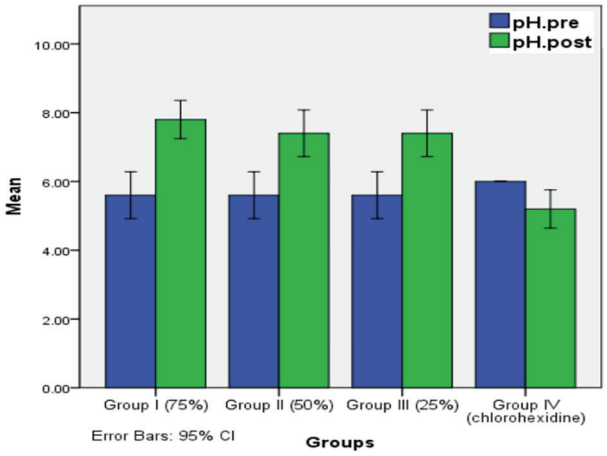

Figure (4) Bar chart illustrating mean value of pH in different groups

DISCUSSION

Caries is a chronic infectious illness caused by bacterial colonization of hard tooth structures and despite global efforts to reduce its prevalence, its prevalence remains high. Along with the traditional mechanical treatments, chemical antibacterial agents such as mouth rinses are regarded a significant tool for controlling and/or reducing bacterial colonization [11].

This research was carried out on youngsters of both sexes ranging in age from 6 to 12 years old, because they may easily use mouthwash without swallowing the rinse liquids, avoiding deglutition reflexes [9].

In this study the antibacterial activity of Licorice extract on oral microbiota and its impact on salivary PH are compared to those of chlorohexidine mouthwash. Salivary pH is crucial because it provides an acidogenic environment for aciduric bacteria to proliferate which leads to tooth decay, which lowers salivary pH even further, producing a vicious cycle. Plate Count Agar (PCA), also known as Standard Methods Agar (SMA) is a microbiological growth medium used to determine or monitor the amount of "total" or viable bacterial growth in a sample [13].

Mouthwashes aid in the reduction of microbial burden in the oral cavity. In this investigation, chlorhexidine mouth rinse was utilized as an active control. Because CHX is the most widely used and most effective antibacterial agent, it is advertised as the gold standard among all mouthwashes. However, because of its known flaws there is a constant need to develop new, safer mouthwashes for youngsters [14,15] .

Herbal extracts with their active phytochemical constituents are thus the least hazardous and most genuine solution for dental health rehabilitation. Licorice (Glycyrrhiza glabra Linn) is a less expensive and generally safe medicinal herb [12] .

Licorice is an alkaline food that has been shown to help with gastroesophageal reflux disease (GERD). Phytochemicals such tannins, triterpenoid saponins and flavonoids are thought to be responsible for Licorice's antibacterial properties. Glycyrrhizin the active ingredient is known to suppress bacterial growth and acid production [17].

This study utilized three strengths of Licorice extract as a mouth wash in this investigation, rinsing three times each day for five days. The extracts' antimicrobial activity was determined by calculating their inhibitory and cidal activity. The in vitro investigation demonstrated that Licorice extract has superior antibacterial action [16] .

The results revealed that Licorice has a substantial antibacterial effect when compared to the control group. Several studies have shown that licorice has antibacterial properties. According to our findings a study revealed the new chemical glycyrrhizol from Licorice root extract which has potent antibacterial activity against cariogenic bacteria. One of its key ingredients glycyrrhizin has been shown

to block the glucosyltransferase activity of mutans streptococci which is involved in the manufacture of insoluble glucans required for biofilm formation in a dose-dependent way [11,19] .

Glycyrrhizin increases fluoride uptake and reduces enamel solubility when added to an acidulated phosphate-fluoride solution according to some authors most likely due to a surface-coating action and its deposition in the porous structure of demineralized enamel. Some research showed that licorice-containing gel inhibited acid generation in an in vivo acid production test which is consistent with our findings [19-21] .

Due to possible fluctuations in saliva microbial counts which occur throughout the day saliva was collected in the morning before meal (S1) and children were asked to rinse their mouth with 10 ml of specific mouth wash for about 1 minute then rinse three times per day (after breakfast, after lunch and before sleeping) for five days and saliva samples were collected after five days from each subject (S2). Because it is a quantitative test and delivers a more accurate score using a chair-side kit (GC Saliva Check) produced correct results [22] .

When the four groups in this study were compared the salivary pH value increased significantly, peaking 30 minutes after the rinse. As a result alkalinity increased suggesting that it could be used as a preventative approach to reduce tooth caries. The elevation in pH caused by Licorice extract mouthwash was in line with findings from recent research indicating Licorice extracts limit acid formation [23].

There is a significant difference between groups (p=0.495) as a result of this investigation. The highest mean value was found in Group III, followed by Group II, Group I, and Group IV with the lowest value found in Group IV. Licorice mouthwash was found to be effective in the prevention of dental caries, with a significant reduction in total bacterial count when compared to chlorohexidine mouthwash.

CONCLUSIONS

According to the findings of this study, Licorice aqueous root extract has an antibacterial action against total bacterial growth, as well as a quick rise in salivary PH that is more efficient than Chlorhexidine.

RECOMMENDATIONS

Herbal mouthwashes should be researched and regarded as a viable alternative to commercial mouthwashes. Licorice extracts, at various concentrations, should be examined as an alternative mouthwash. Different Licorice extract procedures should be examined in future investigations.

CONFLICT OF INTEREST

No conflict of interest.

FUNDING

No funding was received for this study

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