

Comparison of Oral Disease Prevention Effects between Saline and *Glycyrrhiza Uralensis* Mouthwash

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ABSTRACT

Chemical mouthwashes are widely used in oral health care. However, they are unpleasant to use due to their strong smell and taste, and they have many side effects after long-term use. This study was conducted to confirm the preventive effect on oral diseases of using oral gargles with *Glycyrrhiza uralensis*, a natural medicine that has broad antibacterial activity and has been proven to be harmless to the human body. Fifteen patients who had no systemic disease and had complete data from among the patients who visited M Dental Clinic in Busan, South Korea were divided into three groups: five in the control group with no gargle; five in the saline gargle group, and five in the *Glycyrrhiza uralensis* gargle group. They were all instructed to gargle with 15 ml of their assigned mouthwash for 1 min and then to spit for 1 min to remove the remaining gargle solution. Then their O'Leary index, dental caries activity, bacterial species, and bacterial motility were measured. Significant decreases in all four indices were noted in the *Glycyrrhiza uralensis* gargle group, unlike in the saline gargle group ($p < 0.05$). Thus, safe and effective oral care can be achieved by gargling with *Glycyrrhiza uralensis*.

Keywords

Bacteria; Dental caries; *Glycyrrhiza uralensis*; Periodontitis

Introduction

Oral health refers to a state in which the oral cavity has teeth and oral tissues and structures capable of performing three functions: mastication, aesthetics, and pronunciation [1]. If one of these functions is abnormal, a normal social life is difficult to lead. Therefore, prevention of oral diseases is of utmost importance because such diseases cause oral dysfunction. The most common oral diseases are dental caries and periodontal disease, occurring in more than 75% of adults [2]. Dental caries is a typical irreversible chronic disease that occurs frequently in all age groups. In it, the tooth enamel interacts with food residues in the oral cavity and with acid produced by plaque bacteria, causing demineralization [3]. Among the bacteria involved in dental caries, *Streptococcus mutans* (*S. mutans*) is the most important because it causes the early formation of a biofilm that attaches to the tooth surface, proliferates, and produces acid, which causes dental caries [2].

Prevention of periodontal disease is most important because it causes inflammation of the supporting tissues of the teeth, formation of periodontal pockets, and loss of alveolar bone, which is the main cause of tooth loss [4]. When the tooth structure is affected by dental caries and periodontal disease, it can no longer be restored [5]. The main causative bacteria of periodontal disease are anaerobic bacteria such as *Filamentous* and *Spirochetes*. To prevent and treat periodontal disease, these bacteria must be reduced and eliminated by preventing their colonization [6]. Since mechanical plaque control methods such as toothbrushing are more difficult to do than chemical plaque control methods such as gargling with a mouthwash, the use of simple and portable mouthwash products has been increasing in recent years [7]. Common mouthwash products are chlorhexidine (CHX) [8], Listerine [9], and fluoride [10]. However, side effects have been noted such as toxicity and changes in normal oral flora [11], so research on new mouthwash products that are safe and can be used continuously without side effects is being conducted. Therefore, there is growing interest in natural substances that are effective in preventing oral diseases, and in research on natural antibacterial agents as

materials for next-generation antibiotics [12].

More and more studies are seeking to confirm the anti-caries properties of natural extracts. Herbal extracts that have exhibited an inhibitory effect on the proliferation of *S. mutans* include *Coptidis rhizoma*, *Magnoliae cortex*, citric acid-mixed preparations [13], *Grapefruit seed*, *Cassiae torae semen*, and *Angelicae gigantis Radix* [14], and *Kochia scoparia* Schrader and *Scutellaria baicalensis* Georgi [15] have also shown anti-caries effects. *Glycyrrhiza uralensis*, the root of which is widely used as a sweetener in mainland China and many other countries, is widely used as a natural antibacterial agent because it not only inhibits the growth of *S. mutans* but is also seen to have potential as an anti-caries agent [16]. Hu et al. [17] reported that when 26 adults were fed sugar-free lollipops mixed with *Glycyrrhiza uralensis* extract twice a day for 10 days, the number of *S. mutans* in the oral cavity of most of them significantly decreased. This effect is attributed to the substance Glycyrrhizol A in the *Glycyrrhiza uralensis* extract; but Villinski et al. [18] reported that *Glycyrrhiza uralensis* extract that contained small amounts not only of Glycyrrhizol A but also of licoricidin, licorisoflavan A, licorisoflavan C, licorisoflavan D, and licorisoflavan E effectively inhibited *S. mutans*. However, *Glycyrrhiza uralensis* has been reported to exhibit excellent antibacterial effects not only on bacteria that cause dental caries but also on bacteria that cause periodontal disease [19], such as the gram-negative oral anaerobic bacterium *Porphyromonas gingivalis* (*P. gingivalis*) [18]. Glycyrrhizin, the main component of *Glycyrrhiza uralensis*, has been found to inhibit cytokine production induced by lipopolysaccharide (LPS), another causative factor of periodontitis [20]. In terms of cytotoxicity, *Glycyrrhiza uralensis* has met the international guidelines for use of herbal substances in products for human consumption [21].

Therefore, this study investigated the possibility of use and the oral improvement effects of a mouthwash containing *Glycyrrhiza uralensis* in order to evaluate its clinical applicability for the prevention and the suppression of the progression of oral diseases.

Materials and Methods

Study subjects

This study was conducted on 15 patients who did not have a systemic disease and had complete data from among the patients who visited M Dental Clinic in Busan, South Korea within the period of March to May 2021. A dental hygienist with more than 10 years' experience directly explained to them the purpose of the study. They were divided into three groups: five in the control group that did not use a gargle (Group A), five in the saline gargle group (Group B), and five in the *Glycyrrhiza uralensis* gargle group (Group C).

Study method

Measurement of oral environmental parameters

For all the groups, the O'Leary index measurement and the Snyder test were performed in the same manner as dental caries activity tests, and the bacterial amount and motility of the subjects were measured with a phase-contrast microscope. Group A was asked not to gargle; Group B, to gargle with 15 ml of saline for 1 min; and Group C, to gargle with 15 ml of *Glycyrrhiza uralensis* for 1 min. The changes in the oral environment of all the subjects were measured after they were asked to spit for 1 min to remove the remaining gargle solution from their mouths.

O'Leary index

After all the teeth of each subject were colored with a disclosing agent, their plaque control score (O'Leary index) was calculated to determine the percentage of dental plaque attachment

to their teeth. In the index, 1 point is given if dental plaque is found attached to four tooth surfaces (i.e., the mesial, distal, buccal, and lingual surfaces), and 0 point is given if it is not [22].

Snyder test

The Snyder test was performed to colorimetrically measure the acid production rate of the oral bacteria. Saliva (0.2 ml) was injected into a test tube that contained a 5ml medium (Difco™ Snyder Test Agar, BD, Franklin Lakes, NJ, USA) and was incubated for 72 h, during which the teeth color was observed for changes every 24 h. A change in the teeth color to yellow after 24h incubation was deemed indicative of high activity and given 3 points; after 48h incubation, moderate activity with 2 points; and after 72h incubation, low activity with 1 point. No color change during the 72h incubation was considered inactivity and given 0 point.

Phase-contrast microscope

To identify the microorganisms, a sterile probe was used to collect the supragingival and subgingival dental plaque from the lingual surfaces of the mandibular left and right posterior teeth. Then the plaques were placed on a slide, and a drop of normal saline was released on them. After covering them with another slide to prevent the formation of air bubbles, the movements of the live microorganisms in the oral cavity were observed on a monitor using a phase-contrast microscope (DCS6002, Doctor Prevent Co., Seoul, South Korea) at 4300X magnification.

Data analysis

The resulting O'Leary index, Snyder test, and amount of quantified bacteria that were determined via a program during the phase-contrast microscopy were statistically analyzed according to the parameters of the oral environmental change in each group, using IBM SPSS ver.21.0 (IBM Co., Armonk, NY, USA). To test the significance of the differences among the groups, one-way ANOVA and Tukey's test as a post-hoc test were performed.

Results

Comparison of O'Leary index

The O'Leary index was high in the control group and lower in the *Glycyrrhiza uralensis* gargle group than in the saline gargle group. Significant differences were seen among the three groups (Table 1), but the most significant reduction was seen in the *Glycyrrhiza uralensis* gargle group ($p < 0.05$).

Comparison of the dental caries activity with the Snyder test

In the comparison of the dental caries activity, significant differences were found among the three groups ($p < 0.05$). In the Snyder test for the control group, the subjects' teeth color changed to yellow after 24h incubation, which indicates that it was a high-risk group; in the saline gargle group, the teeth color changed to yellow after 48h incubation, which showed moderate to low activity; and in the *Glycyrrhiza uralensis* gargle group, the teeth color did not change during the 72h incubation, which showed inactivity, the safest condition in terms of dental caries activity (Fig. 1, Table 1).

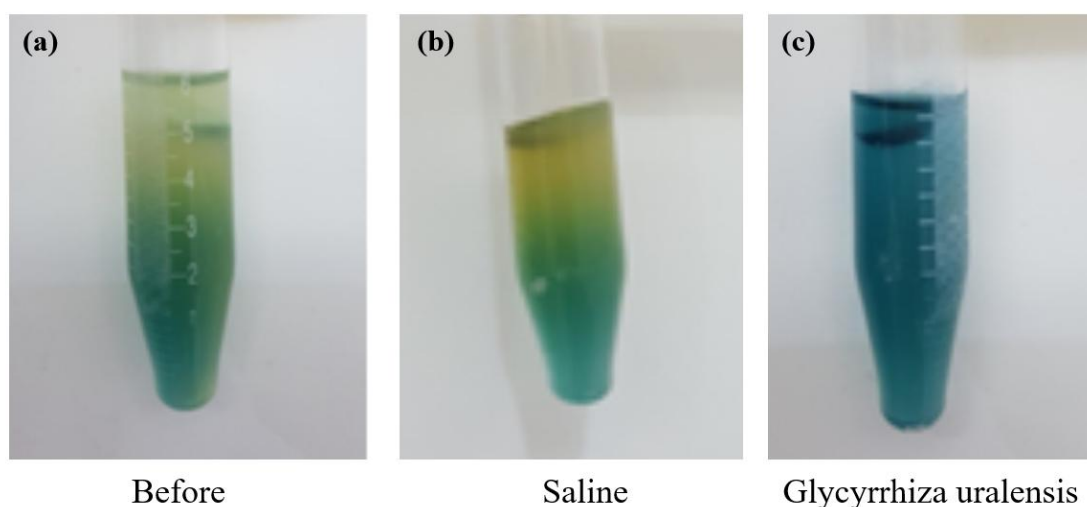


Figure 1. Comparison of the dental caries activity with the Snyder test

a: Control

b: Oral care with 0.9% Saline solution (1 min/15 ml)

c: Oral care with *Glycyrrhiza uralensis* (1 min/15 ml)

Observation of oral bacteria via phase-contrast microscopy

The observation of the bacterial activities of *Cocci*, *Bacillus*, *Filamentous*, and *Spirochetes* in the three groups using phase-contrast microscopy showed significant differences among the three groups ($p < 0.05$). All the bacteria in the control group showed active movement; reduced movement in the saline gargle group; and significantly reduced movement in the *Glycyrrhiza uralensis* gargle group. In particular, there was a definite decrease in the *Cocci* and distinct decreases in the *Filamentous* and *Spirochetes*, the bacteria that cause periodontal diseases. There were more significant decreases in the *Filamentous* activity in the *Glycyrrhiza uralensis* gargle group than in the saline gargle group (Fig. 2, Table 1).

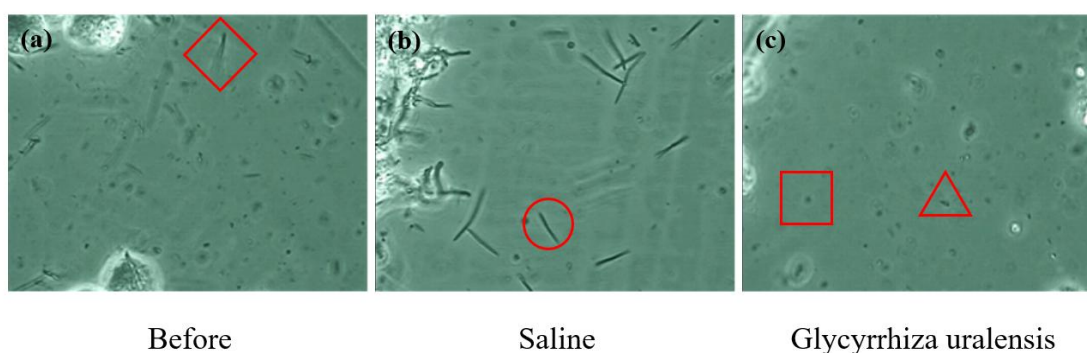


Figure 2. Comparison of the observation results of the Saline group and the *Glycyrrhiza uralensis* group using a phase-contrast microscope.

a: Control

b: Oral care with 0.9% Saline solution (1 min/15 ml)

c: Oral care with *Glycyrrhiza uralensis* (1 min/15 ml)

□ *Cocci* △ *Bacillus* ○ *Filamentous* ◇ *Spirochetes*

Comparison of the oral disease parameters

There were significant differences among the three groups. However, the O'Leary index, Snyder test, bacterial motility, and *Filamentous* amount most significantly decreased in the *Glycyrrhiza uralensis* gargle group (Table 1, $p < 0.05$).

Table 1. Comparison of the oral disease parameters

Test group	Mean $\pm$ SD			p-values
	Before	Saline	<i>Glycyrrhiza uralensis</i>	
O'Leary index	93.80 $\pm$ 1.48 ^a	84.00 $\pm$ 1.00 ^b	51.60 $\pm$ 1.14 ^c	0.000 [*]
Snyder test	2.40 $\pm$ 0.55 ^a	1.80 $\pm$ 0.84 ^a	0.20 $\pm$ 0.45 ^b	0.000 [*]
Motility	98.20 $\pm$ 2.49 ^a	71.00 $\pm$ 1.87 ^b	58.60 $\pm$ 1.67 ^c	0.000 [*]
<i>Coccus</i>	57.80 $\pm$ 5.63 ^a	58.80 $\pm$ 5.72 ^a	34.80 $\pm$ 4.27 ^b	0.000 [*]
Bacterial <i>Bacillus</i>	17.80 $\pm$ 4.38 ^a	15.60 $\pm$ 5.13 ^a	15.00 $\pm$ 4.69 ^a	0.000 [*]
<i>Filamentous</i>	34.20 $\pm$ 4.27 ^a	45.60 $\pm$ 4.39 ^b	0.00 $\pm$ 0.00 ^c	0.000 [*]
<i>Spirochetes</i>	6.60 $\pm$ 1.67 ^a	5.80 $\pm$ 0.84 ^a	0.00 $\pm$ 0.00 ^b	0.000 [*]

*The p-values were determined via one-way ANOVA and Tukey's tests ($p < 0.05$).

Discussion

When plaque accumulates around teeth and prostheses, it can develop into dental caries or periodontal disease. Dental caries is caused by the destructive power of acid produced by oral bacteria such as *S. mutans*, and periodontal disease accompanies gum infection, leading to inflammation and bone loss [23]. The purpose of the treatment of these oral diseases is to stop their progression, as it is still unknown if a complete cure is possible [24]. Antibiotics are used as representative ancillary methods of preventing infection after treatment of oral diseases, and mouthwash products are used to reduce the number of pathogenic bacteria in the mouth. However, antibiotics, antibacterial agents, and anti-inflammatory drugs, which are pharmacological approaches, are being used on a limited scale due to their side effects of developing resistant bacteria and causing hypersensitivity reactions, gastrointestinal disorders, and teeth discoloration [25]. To overcome the side effects of existing drugs, studies on the use of natural products to prevent and treat oral diseases are being actively conducted [15,26]. Natural agents that have been reported as effective against the bacteria that cause dental caries include *pine needle and twig* [27], *Akebia quinata* [28], *Juniperus rigida* S. et Z. [29], and *Sophora flavescens* [30]. *Corn* extract [31] has been reported as an effective natural agent against bacteria that cause periodontal disease, and as particularly effective in reducing the plaque and gingival index [32]. The mixture of *Corn unsaponifiable* extract and *Magnoliae cortex* extract has been reported to be more effective against periodontal disease-causative bacteria than when each drug is used alone [33]. *Safflower* extract has also been reported to be effective in periodontal ligament cell regeneration [34]; *Curcuma xanthorrhiza*, to have an inhibitory effect on plaque and gingivitis [35]; and *Scutellariae Radix*, to improve the inflammation and enhance the cell biological function of gingival fibroblasts [36]. *Eclipta prostrata* L. extract was applied to an animal model on which periodontitis was induced, and

its effects of periodontal tissue regeneration and improvement of alveolar bone loss were confirmed [37].

This study was conducted to evaluate the applicability of *Glycyrrhiza uralensis* extract as an oral disease prevention agent. *Glycyrrhiza uralensis* is an herbaceous perennial plant that belongs to the legume family. Its dried root, which is called “licorice” because it tastes sweet, is used as a medicinal material. As it is easy to grow in suitable climates and soils due to its strong growth potential and resistance to blight and harmful insects, it has been cultivated as a medicinal resource for a long time in China and North Korea [38]. It is known to be effective in reducing the causative bacteria of periodontal disease and dental caries [39].

This study confirmed that the O’Leary index decreased in the saline gargle group but more significantly decreased in the *Glycyrrhiza uralensis* gargle group. The results of the Snyder test, which colorimetrically evaluated the oral acid production ability to confirm the association with dental caries induction, showed inactivity in the *Glycyrrhiza uralensis* gargle group and were significantly lower in the *Glycyrrhiza uralensis* gargle group than in the saline gargle group ($p < 0.05$). These indicate that *Glycyrrhiza uralensis* gargling has no effect on acid-producing bacteria and no dental caries activity. In terms of the oral bacteria observed through the phase-contrast microscopy, it was confirmed that the motile bacteria significantly decreased in the *Glycyrrhiza uralensis* gargle group compared to the saline gargle group ($p < 0.05$). In the case of Coccus, a bacterial species related to dental caries, the *Glycyrrhiza uralensis* gargle group showed a statistically significant decrease, which indicates that gargling with *Glycyrrhiza uralensis* is effective in preventing and suppressing dental caries. *Filamentous* and *Spirochetes*, which cause periodontal disease, were found in the saline gargle group but not in the *Glycyrrhiza uralensis* gargle group, which indicate that gargling with *Glycyrrhiza uralensis* has an excellent effect on periodontal disease and may thus prevent and inhibit the progression of two major oral diseases, periodontal disease and dental caries.

These results show that effective oral care is possible through dental plaque management, reduction of dental caries activity, reduction of bacterial acid production ability, and reduction of oral bacteria. For these purposes, *Glycyrrhiza uralensis* extract was confirmed to be an excellent natural substance. Therefore, a mouthwash containing *Glycyrrhiza uralensis* extract should be able to act as a natural mouthwash with excellent antibacterial effect for the prevention of dental caries and periodontal disease, two major diseases of the oral cavity.

Conclusion

Gargling with a mouthwash that contained *Glycyrrhiza uralensis* extract decreased the O’Leary index, Snyder test result, and bacterial motility of the subjects. There was also a significant decrease in Coccus, the causative bacteria of dental caries in the oral cavity. *Filamentous* and *Spirochetes*, the bacteria that causes periodontal disease, were not observed in the oral cavity. Mouthwash products that contain *Glycyrrhiza uralensis* extract reduce dental plaque, suppress dental caries activity, and have antibacterial effects against bacteria that cause periodontal disease as well as dental caries. Thus, the use of an oral mouthwash containing *Glycyrrhiza uralensis* extract will greatly contribute to the inhibition of dental plaque adhesion and to the prevention of oral disease.

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