The effects of *Scutellariae radix* extract gargling solution on the prevention of periodontal disease.

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Abstract

This study was to evaluate the effect of *Scutellariae radix* extract on periodontal disease. A 500 g of *Scutellariae radix* was added to 70% methanol and extracted for 3 h, followed by filtration and freeze drying. The freeze dried specimen was added to 10% Dimethyl Sulfoxide (DMSO) solution, and the diluted solution was used in this study, which was conducted after obtaining the approval of the IRB of Inje University (No. 2-1041024-AB-N-01-20140514-HR-055-04; approval date: 2014-08-27). The study enrolled patients without systemic disease or drawbacks with data collection. They visited the M Dentist Office located in Busan from September 2015 to January 2016. They were equally divided into three groups. The 10 subjects were placed in 0.9% saline (JW Pharmaceutical Corporation) gargle group, 10 subjects were placed in 0.005% chlorhexidine (Bukwang Pharmaceutical Co., Ltd.) gargle group, and 10 subjects were placed in 10% *Scutellaria* root gargle group. The periodontal disease variables for the groups prior to the gargle treatment were the same. Each group gargled a 15 ml solution for 1 min and expectorated for 1 min until there was no gargle solution left. The bacterial count was calculated by using a phase contrast microscope and the O'Leary index. As seen with the naked eye, the O'Leary index was reduced in the order of saline gargle group, chlorhexidine gargle group, and *Scutellaria* root gargle group, and the gap among the groups was distinct (p<0.005). However, for the active bacteria, *Spirochaeta*, the chlorhexidine gargle group and *Scutellaria* root gargle group showed a similar effect, and their gap was not statistically significant (p>0.005). Since many side effects are reported on the chemical solution, chlorhexidine, substituting it with natural *Scutellaria* root gargle solution will enable a safe and efficient oral care.

Keywords: Chlorhexidine, O'Leary index, Periodontitis, *Scutellaria radix*, *Spirochaeta*.

Accepted on May 17, 2017

Introduction

Periodontal disease occurs to 90% of adults and it is a threat to oral health because it can cause loss of function. Its morbidity rate and severity are increasing steadily and mostly high during and after late-middle-aged adults. Periodontal disease refers to an inflammation in the dental supporting apparatus due to bacteria. In addition, the bacterial composition in the dental plaque of a healthy gingiva and periodontal disease gingiva are different [1].

Studies on periodontal disease were mainly focused on controlling its progress by efficiently removing the dental plaque [2]. Dental plaque is formed when microorganisms adhere to the dental surface and constitute either a homotypic bond or a heterotypic bond between the cells, along with the production of an extracellular matrix. Dental plaque can inhibit external materials from entering, as its extracellular matrix forms a thick layer, and its resistance to antibiotics and antibacterial substances are high, as compared to bioaerosol due to genetic morphism through cell-to-cell interaction, thereby making it difficult to remove [3]. The deposition and maturity of the dental plaque determine the severity of the periodontal diseases. Therefore, maintaining a good oral hygiene and managing the dental plaque regularly are necessary for the prevention of periodontal diseases [4]. In particular, chlorhexidine was discovered in the late 1940s, and it was used diversely in gynecology, urology, and ophthalmology. It has been used for various purposes (e.g., treatment for skin infection or burn, and sterilization of surgical instruments before surgery) [5], since it has antimicrobial activity against gram-positive bacteria, gram-negative bacteria, yeast, fungi, and anaerobic species [6]. It has been recommended for the chemical dental plaque control of handicapped patients, who are having difficulties with tooth brushing, and for circumstances where the jaw fracture is fixed, including after periodontic surgery and after fixation of the orthodontic appliances [7]. In particular, it selectively
retrains *Streptococcus mutans*, which can be used for periodontal disease treatment and dental caries prevention purposes. However, its bitter taste makes it difficult to be used as a gargle solution for children, and it cannot be used long term, since it can proliferate on the dental surface and cause tongue discoloration [8]. The discoloration can take place in both hard tissue and soft tissue, and it mainly occurs on the dental surface with its severity, while the intensity varies according to the eating habit and tooth brushing pattern of the patients. While the mechanism of discoloration has not yet manifested, Rolla et al. [9] mentioned that the denaturation of chlorhexidine, formaldehyde, and tannic acid is due to the dark brown discoloration caused by the deposition of ferrous and sulfur ions, as well as the denaturation of protein inside the dental plaque and acquired films. Jansen [10] stated that the dental surface is pigmented by the ionic bond between pigment anion and chlorhexidine cation. Low-concentration chlorhexidine, zinc, lanthanum, fluorid, and oxidant were suggested as an attempt to minimize the discoloration [11-13]; however, a gargle solution made with natural Korean medicine extracts are actively being experimented due to the side effects caused by chemical agents. *Psoralea corylifolia* [14] and *Erythrina variegata* [15] have excellent antimicrobial effects. Meanwhile, *Curcuma aromatica* [16] and *Asarum sieboldii* Miquel [17] have been reported to have an antibiotic effect against *S. mutans*. For this reason, a gargle solution composed of natural extracts has an excellent antibacterial activity. It is also considered as an effective oral care method. In particular, the *Scutellaria* root (*Scutellaria radix*) is a perennial herbaceous plant that is used as a medicine and grown all over the country. Its main constituents are flavonoids, such as baicalein, wogonin, and skullcap flavone I and II [18]. Furthermore, it is known for its ability to inhibit contact hypersensitivity, as well as its antioxidant, antimicrobial, antiviral liver function protection, anti-inflammatory, anticonvulsant, antihyperlipidemic, and anti-anxiety effects [19]. Although many studies were conducted on *Scutellaria* root extract due to its excellent antibacterial activity [20-22], there are only a few reports regarding its use in dentistry. There are only a few studies conducted on periodontal disease, which is closely related to dental plaque adhesion, as well as oral bacteria count and activity, whereas a gargle solution composed of natural extracts is under active investigation. In addition, the clinical data obtained by the clinical application is deemed insufficient.

Therefore, this clinical study aims to assess the potential of *Scutellaria* root extract in order to prevent periodontal disease with its antimicrobial activity, and investigate its effect on the progress of periodontal disease.

**Materials and Methods**

**Scutellaria radix extract**

A 500 g of *Scutellaria radix* was purchased from Busan Hyundai herb merchandise. A 100 g of crushed lotus leaves was diluted 1:10 in 70% methanol and extracted for 3 h under 65°C heating mantle. The extract was filtered 3 times with a filter paper (Advantec No. 2, Toyo, Japan). An aspirator (A-3S, EYELA Co., Japan) and a freeze dryer (Ishin Lab. Co., Korea) were used to concentrate and freeze dry the *Scutellaria* root extract. The freeze dried specimen was dissolved and diluted with 10% dimethyl sulfoxide (DMSO) and stored at -20°C.

**Study subjects**

This study was conducted after obtaining the approval of the IRB of Inje University (No. 2-1041024-AB-N-01 – 20140514-HR-055-04; approval date: 2014-08-27). The study enrolled patients who visited the M Dentist Office located in Busan from September 2015 to January 2016. A dental hygienist with more than 10 y’ experience informed the patients about the objectives of the study, and among the 36 patients who filled out the questionnaire, only 30 of them who had no limitations in the data collection were selected. Each of the 30 patients was randomly assigned to three groups. The 10 patients in the 0.9% saline (JW Pharmaceutical) gargle group, 10 patients in the 0.005% chlorhexidine (Bukwang Pharmaceutical Co., Ltd.) gargle group, and the remaining 10 patients in the 10% *Scutellaria* root gargle group were checked for periodontal disease variables before and after the gargle process that included a one-minute gargle with 15 ml of designated solution, and expectorated until there was no gargle solution left. The overall design of the experiment proceeds is the same as Figure 1.

**Clinical application**

The O’Leary index was measured in the saline gargle group, chlorhexidine gargle group, and *Scutellaria* root gargle group. Meanwhile, the *Spirochaeta* (spiro) and active bacteria count were assessed by the phase microscope. The X1 group gargled for 1 min by using 15 ml of 0.9% saline, the X2 group gargled for 1 min by using 15 ml of 0.005% chlorhexidine, and the X3 group gargled for 1 min by using 15 ml of 10% *Scutellaria* root. The microbe count was carried out again after the subjects expectorated until there was no gargle solution left.

**Figure 1. Illustration of the experimental design for the in vivo study.**

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Biomed Res- India 2017 Special Issue

Special Section: Health Science and Bio Convergence Technology

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**O’Leary index**

O’Leary et al.’s dental plaque test (O’Leary index) was carried out. We discolored all teeth in the oral cavity with a dental surface discoloration agent and calculated the level of adherence (%) by using the plaque control score (O’Leary index), which gives 1 point if the dental plaque adheres to the dental surfaces of 4 teeth (mesial, efferent, facies, and lingual) and 0 if not [23].

**Phase microscope**

A sterilized probe was used to confirm the type of microbes. The subgingival and supragingival dental plaque of the left and right post-lingual surface of the mandible was collected and smeared on a slide. After applying a drop of saline, the slide was mounted with a slide cover without making any bubbles, and then it was observed by using a phase microscope (DCS6002, Doctor prevent Co., Seoul, Korea) by a factor of 4,300. The movement of the microbes were monitored without staining, and they were differentiated to active bacteria and inactive bacteria. The relative increase of *Spirochaeta* may influence the progression of periodontal disease.

**Statistical analysis**

The statistical analysis of the saline gargle group, chlorhexidine gargle group, and *Scutellaria* root gargle group was conducted by using the IBM SPSS ver. 21.0 (IBM Co., Armonk, NY, USA). The O’Leary index and *Spirochaeta* count, which was quantified by a program that studies microbes observed with a phase microscope, proceeded to one-way ANOVA test and Tukey’s post hoc test under p=0.05 significance level in order to test the significance among the groups.

**Results**

**Comparison of the O’Leary index**

The result of the O’Leary index before using a gargle solution was high in all groups, while it was reduced in the order of saline gargle group, chlorhexidine gargle group, and *Scutellaria* root gargle group after using a gargle solution (Figure 2). As shown in Table 1, there was a distinct difference between the chlorhexidine gargle group and the *Scutellaria* root gargle group (p<0.05).

**Change in phase microscope image**

The active bacteria count before using a gargle solution was high in all groups, and it was reduced in the order of saline gargle group, chlorhexidine gargle group, and *Scutellaria* root gargle group after using a gargle solution (Figure 3). As shown in Table 2, the chlorhexidine gargle group and *Scutellaria* root gargle group showed similar antimicrobial effects against active bacteria and *Spirochaeta* (p<0.05).
Table 2. Bacterial count before and after the use of a gargle solution of the saline gargle group, chlorhexidine gargle group, and Scutellaria root gargle group.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Before M ± SD</th>
<th>p-values</th>
<th>After M ± SD</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Micro_a</td>
<td>X1</td>
<td>10 80.00 ± 7.89</td>
<td>0.202</td>
<td>68.00 ± 32.93</td>
<td>0.004*</td>
</tr>
<tr>
<td>BIG</td>
<td>X2</td>
<td>10 86.00 ± 14.29</td>
<td>0.000</td>
<td>37.00 ± 23.94</td>
<td>0.000</td>
</tr>
<tr>
<td>Chl_a</td>
<td>X3</td>
<td>10 75.00 ± 24.49</td>
<td>0.100</td>
<td>26.40 ± 21.18</td>
<td>0.000*</td>
</tr>
<tr>
<td>Spiro</td>
<td>X1</td>
<td>10 6.00 ± 5.16</td>
<td>1</td>
<td>4.00 ± 5.16</td>
<td>0.007*</td>
</tr>
<tr>
<td>BIG</td>
<td>X2</td>
<td>10 6.00 ± 8.43</td>
<td>0.000</td>
<td>0.00 ± 0.00</td>
<td>0.000*</td>
</tr>
<tr>
<td>Chl_a</td>
<td>X3</td>
<td>10 6.00 ± 6.22</td>
<td>0.000</td>
<td>0.00 ± 0.00</td>
<td>0.000*</td>
</tr>
</tbody>
</table>

The p-values are determined by ANOVA (p<0.05). a, b, c Different letters indicate statistically significant differences. X1: Oral care with 0.9% saline solution (1 min/15 ml); X2: Oral care with 0.005% chlorhexidine (1 min/15 ml); X3: Oral care with 10% Scutellaria root (1 min/15 ml).

**Discussion**

Pathogenic bacterium produces many kinds of diseases, and it causes pain and loss of teeth. In particular, periodontitis is a mixed infection of multiple bacteria strains that is characterized by gingivitis due to the disposition of the dental plaques. It has progressed into damaging the dental supporting apparatus and loss of teeth when inflammation is spread by the reactions in the periodontal tissues [24]. Although various physical methods are used to manage dental plaque, the measures conducted by the experts are short term and limited. Tooth brushing is a self-care method that has been proven in many studies; however, its efficacy depends on a person’s motivation [25], and it will be laborious to a person with limited hand movement. Chemical dental plaque care is used in order to make up for these limitations, and the gargle solution, in particular, is easy and convenient to use. Various gargle solutions in the overseas market were assessed for their antibacterial activity on dental plaques, and their efficacy has been proven in both laboratory and clinical studies. Upon the assessment of the marketed products with the main ingredients of chlorhexidine, essential oil, fluoric amine, and fluoric tin, each product had different antibacterial activity on dental plaque [26,27]. This study compared the periodontal disease variables among the saline gargle group, chlorhexidine gargle group, and Scutellaria root gargle group. As a result of this study, the O’Leary index after the use of a gargle solution was reduced in the order of saline gargle group, chlorhexidine gargle group, and Scutellaria root gargle group. The gap among the three groups was statistically significant and distinct between the chlorhexidine gargle group and the Scutellaria root gargle group. The number of active bacteria after using a gargle solution, as seen in the phase microscope, was reduced in the order of saline gargle group, chlorhexidine gargle group, and Scutellaria root gargle group. The objectives of periodontal disease treatment are to cease the progress of inflammation. There is still an ongoing debate as to whether it can be cured completely [28]. Gargle solution and antibiotics are generally used to decrease pathogenic bacteria and help prevent secondary infection after surgical intervention. However, antibiotics, antimicrobials, and anti-inflammatory agents are applied in a limited manner, since they account for side effects, such as manifestation of resistant bacteria, anaphylaxis, gastrointestinal disturbance, and dental discoloration [29]. The limitations of this study are that Scutellaria root gargle group did not have a significant effect on active bacteria and *Spirochaeta*, but they can be used to prevent and treat periodontal disease more effectively by using natural ingredients without the side effects.

In order to overcome the side effects of the conventional products, investigations of the natural ingredients for their application in the prevention and treatment of dental disease are actively being made [30,31]. Liquorice [32] is known to effectively diminish bacteria that can cause periodontal disease and dental caries. Moreover, Their et al. has reported the efficacy of corn extract in periodontitis [33], dental plaque index decreasing effect and gingiva index decreasing effect [34].

*Zea mays* L. and *Magnolia* extract complex have shown more effective antimicrobial effects against germs that can cause periodontal disease, as compared to using its ingredient separately [35]. This study used *Scutellariae radix*, the root of *Scutellariae baicalensis* with its periderm removed [36], of which the extract was reported to have an anti-inflammatory effect and the ability to enhance the cytological function of gingiva fibroblasts [37]. For the antibacterial activity, it was reported that *Scutellaria* root extract has an antimicrobial effect against *Streptococcus mutans*, which usually causes dental caries [20], and an antibiotic effect against *Pseudomonas aeruginosa* [21]. The antibacterial activity of *Scutellaria* root extract was also reported [22]. This study was conducted in order to obtain fundamental data regarding the clinical application of *Scutellariae radix* extract and its efficacy.

This study clinically applied and comparatively analysed the saline gargle group (X1), chlorhexidine gargle group (X2), and *Scutellaria* root gargle group (X3). As a result, the O’Leary index was reduced in the saline gargle group, chlorhexidine gargle group, *Scutellaria* root gargle group, and the gap among the groups was distinct (p<0.005). The gap between the chlorhexidine gargle group and *Scutellaria* root gargle group was not statistically significant, which means that their effects are similar against the active bacteria and *Spirochaeta* (p>0.005), although, the three groups showed a significant difference in the phase microscope image.

Therefore, *Scutellaria* root extract can help prevent the progression of periodontal disease by inhibiting the growth of pathogenic bacteria, and it is capable of dental plaque generation care. Although the chlorhexidine gargle group and *Scutellaria* root gargle group did not show any significant effect on active bacteria and *Spirochaeta*, periodontal disease will be more thoroughly prevented and treated by using nature-
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Conclusion
In conclusion, all periodontal disease variables have decreased after using the gargle solution in the order of saline gargle group, chlorhexidine gargle group, and Scutellariae root gargle group. The three groups did not show any significant difference in the O’Leary index, active bacteria, and Spirochaeta prior to the use of the gargle solution; however, after using the gargle solution, the three groups showed a significantly different efficacy in the O’Leary index. In addition, the chlorhexidine gargle group and Scutellariae root gargle group showed a similar efficacy on the bacteria. Dental plaque care and oral care that reduce bacterial activity are critical to preventing periodontal diseases. Therefore, it is better to use the natural Scutellariae root extract that shows a similar effect but with less side effects than the commonly used chlorhexidine in dental practice.

Conflict of Interest
The authors report no conflicts of interest related to this study. The author does not have any financial interest in the companies whose materials are included in the article.

References


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