

Effectiveness of Two Kinds of Traditional Chinese Mouthwash on the Oral Anaerobic Bacteria and Volatile Sulfur Compounds

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Abstract: Objective To evaluate two kinds of traditional Chinese mouthwash on the anaerobic bacteria of oral cavity and levels of the volatile sulfur compounds. Methods A dual-center, randomized, double-blind, controlled clinical trials lasted for one month was adopted. A total of 60 subjects was selected and randomly allocated in three groups—Xipayi group, Jinzhi Jieyin group and placebo group. Recordings were assessed at the baseline, two weeks and one month. They included the changes of the volatile sulfur compounds and bacteria of the oral cavity. Results: At the two weeks and one month, the Xipayi group and Jinzhi Jieyin group resulted in a significant reduction in the VSCs scores. And the two groups got a greater change in comparison to the placebo group ($P < 0.05$). At the same time, the Xipayi group and Jinzhi Jieyin group resulted in a significant reduction in the total anaerobic bacteria of oral cavity. The changes of the two groups were more than the placebo group ($P < 0.05$), whereas no significant reduction was observed between the two groups. Conclusion The Xipayi group and Jinzhi Jieyin group are effective in the treatment of the VSCs and the anaerobic bacteria of oral cavity.

Keywords: Traditional Chinese Mouthwash; Anaerobic Bacteria; VSCs.

1. Introduction

Halitosis is a bad smell in the mouth and is a common symptom of the mouth. It not only affects people's social communication, but also causes burden to patients and affects patients' oral health related quality of life (OHRQoL) [1]. Studies [2] have shown that 90% of bad breath is caused by the production of volatile sulfur compounds (VSCs), which are produced by some gram-negative anaerobic bacteria in the mouth that break down proteins in the mouth.

A large number of studies have confirmed that mouthwashes containing certain antibacterial ingredients, which can reduce the degree of bad breath [3]. However, at present, most commercially available mouthwashes contain only a single chemical ingredient, such as mouthwashes containing cetylpyridinium chloride, which can cause side effects such as oral microecological imbalance, tongue coating and mucous membrane staining [4-5]. Other studies have shown that many Chinese medicinal materials not only have good antibacterial effects, but also have few side effects and rarely produce drug resistance [6]. Therefore, this study selected two common Chinese herbal gargles on the market, which include Xipayi Guying liquid and Jinzhi Jieying gargling liquid. In order to compare and evaluate the anti-halitosis clinical efficacy of the two gargles by detecting the changes in the number of oral anaerobes and the level of oral volatile sulfide in patients with bad breath, so as to provide data for the clinical application of Chinese herbal gargling liquid.

2. Materials and Methods

2.1. Experimental Equipment and Materials

Xipayi solid gingival liquid (Xinjiang Qikang Habowei Pharmaceutical Co.LTD.), Jinzhijie Gingival Gargle (Sichuan Enwei Pharmaceutical Co,LTD.), Halimeter halitosis measuring instrument (Interscan Company of the United

States), colony counting instrument (interscience Company of France), Bovine Heart and Brain Infusion Medium (BHI Medium) (Beijing Luqiao Technology Co, LTD.)

2.2. Inclusion of Exclusion Criteria

The subjects were all patients with bad breath in our hospital, and all subjects signed informed consent.

Inclusion criteria Firstly, the age of 18-70 years old, which were male and female. Secondly, Sensory measurement score (OM) ≥ 2 . Thirdly, halimeter detected VSCs concentration levels greater than 150. Fourthly, the patient had good compliance and could cooperate with the completion of this study.

Exclusion criteria Firstly, pregnant and lactating women with bad oral prostheses and other oral diseases, such as severe periodontitis, pericoronitis, mucous membrane disease. secondly, antibiotics have been used within 1 month. Thirdly, Major systemic diseases.

2.3. The Method of Application

The patient should use the mouthwash provided at home in the morning and evening, and use it once after brushing in the morning and evening. The dosage is about 20ML each time, and the time of each gargle is about half a minute. In addition, the patient was asked not to drink, gargle or eat for half an hour.

2.4. The Method of Research

The period of this study was one month. The bacterial quantity and VSCs concentration, which were detected at baseline, two weeks and one month respectively. A total of sixty participants were randomly divided into three groups, which contain twenty participants in each group according to the random number table method. Divided into Xipai solid gingival liquid group, Jinzhi Jie gingival gargle group, distilled water group, each group is 250ml, by the researchers unified packaging. To avoid bias among investigators, a

researcher with a senior title, who was assigned to measure the total amount of oral anaerobes and their oral volatile sulfide levels at baseline, two weeks, and one month. Do not eat, brush your teeth, rinse your mouth, chew gum or wear perfume two hours before the test.

2.5. Evaluation Indicators for Clinical Efficacy

2.5.1. Detection of Oral Anaerobic Bacteria

During the examination, the subject first gargled with purified water, then chewed paraffin to stimulate saliva secretion, and then collected two to three ml of saliva using a sterile tube, and then quickly placed into a solution of ten ml TD. Next, after oscillating with a vortex oscillator for one minute, the bacterial solution was taken and diluted in double ratio, and then inoculated in bovine cardio-brain infusion medium and cultured for three days at 80% N₂, 10% CO₂, and 10% H₂. Finally, the colony counter was used to count the bacteria

2.5.2. The Level Determination of VSCs

VSCs levels in patients with bad breath, which were detected by Halimeter. The test was carried out at around 10 o'clock in the morning. Before the test, the subjects were asked to breathe through the nose and keep their mouth shut for one minute. Then, a disposable straw with a length of about ten cm, which was extended into the patient's mouth for about two cm. The patient was asked to breathe normally. The maximum value measured by each instrument is the VSCs result measured at that time, and the steps are repeated more than three times in a row, and the average value is taken.

2.6. Statistical Treatment

SPSS22.0 software package was used to analyze the test results. Results were expressed as $\pm s$. The analysis of variance and SNK method were used for ptwo comparison, and the test level was set as 0.05.

3. Result

3.1. Basic Characteristics of the Research Object

All subjects ranged in age from 20 to 56 years, with a mean age of 35.63 ± 6.78 years, which including 28 male patients and 32 female patients. All subjects completed the entire study.

3.2. Total Number of Oral Anaerobic Bacteria in Each Group

Table 1 shows the results of comparison of the total number of oral anaerobic bacteria in the three groups at baseline, two weeks, and one month. The results showed that at baseline, there was no significant difference in the measurement results of the total number of oral anaerobes among the three groups ($P > 0.05$), indicating that the results were comparable. At two weeks and one month, the total number of anaerobic bacteria in Xipaiyi Guggingival liquid group and Jinzhijiegingival gargle group decreased, and the difference was statistically significant compared with that in distilled water group ($P < 0.05$). There was no significant difference in the total number of anaerobic bacteria between Xipaiyi Guggingival liquid group and Jinzhi Jiegingival gargle group ($P > 0.05$).

Table 1. Comparison of measurement results of total number of anaerobic bacteria in each group ($\bar{x} \pm s$, log CFU/ml)

	baseline	Two week	One month
Xipaiyi Guggingival liquid group	8.86 ± 0.84	$7.62 \pm 0.83^{a, b}$	$7.47 \pm 1.02^{a, b}$
Jinzhijiegingival gargle group	9.01 ± 1.05	7.89 ± 0.91^c	7.29 ± 0.93^c
distilled water group	8.97 ± 0.96	8.84 ± 0.92	8.64 ± 1.13

Note: a: Compared with distilled water group, $t_{two\ week} = 23.8000$, $P = 0.0000$, $t_{one\ month} = 24.5737$, $P = 0.0000$; b: Compared with Jinzhi Jiegingival gargle group, $t_{two\ week} = 3.1331$, $P = 0.1524$, $t_{one\ month} = 3.0532$, $P = 0.1434$; c: Compared with distilled water group:, $t_{two\ week} = 22.3426$, $P = 0.0000$, $t_{one\ month} = 24.1012$, $P = 0.0000$

3.3. Oral VSCs Levels of Each Study Group

Table 2 compares the oral VSCs levels of each group at baseline, two weeks, and one month. The results showed that there was no significant difference in VSCs results among the three groups at baseline ($P > 0.05$). At two weeks and one

month, the VSCs results of Xipaiyi Guggingival liquid group and Jinzhijiegingival gargle group decreased, and the difference was statistically significant compared with that of distilled water group ($P < 0.05$). However, there was no significant difference in VSCs between Xipaiyi Guggingival liquid group and Jinzhijiegingival gargle group ($P > 0.05$).

Table 2. Comparison of measurement results of oral VSCs in each group ($\bar{x} \pm s$,ppb)

	Base line	Two week	One month
Xipaiyi Guggingival liquid group	291.5 ± 81.4	$112.6 \pm 21.4^{a, b}$	$118.9 \pm 23.3^{a, b}$
Jinzhijiegingival gargle group	278.3 ± 92.4	105.2 ± 24.7^c	110.4 ± 21.6^c
distilled water group	286.5 ± 84.5	257.3 ± 78.1	267.5 ± 80.5

Note: a: Compared with distilled water group, $t_{two\ week} = 103.4523$, $P = 0.0000$, $t_{one\ month} = 106.0695$, $P = 0.0000$; b: Compared with Jinzhijiegingival gargle group, $t_{two\ week} = 6.772$, $P = 0.0963$, $t_{one\ month} = 7.1663$, $P = 0.0892$; c: Compared with distilled water group, $t_{two\ week} = 134.3924$, $P = 0.0000$, $t_{one\ month} = 138.3786$, $P = 0.0000$.

4. Discussions

4.1. The Analysis of Results

The effective component of Sipayi gingival solid solution is galli, which contains tannin components. Tannin

components can bind with protein to precipitate it, and reduce enzyme activity, thus significantly inhibiting the growth of odorous bacteria [7]. Jinzhi Jieying mouthwash contains 12 kinds of prescription, such as honeysuckle, scutellaria, gardenia, matrine, etc., which has good antibacterial, anti-inflammatory and antiviral effects [8]. In this study, both

Xipayi Solid gingival solution and Jinzhijie Gingival gargle can effectively reduce the number of anaerobic bacteria in the mouth. Oral anaerobic bacteria can produce putrefying effect, through the process of decomposing methionine and cysteine in the mouth to produce volatile sulfides (VSCs), which is the main cause of bad breath. The results of this study showed that Xipaii Gugingival solution and Jinzhijiegingival gargle, which could effectively reduce the total number of oral anaerobes at two weeks and one month, when compared with the distilled water group. There was no statistical significance in the reduction of the total number of oral anaerobes between the two groups ($P > 0.05$), which indicating that the two effects were similar.

The measurement of VSCs in oral cavity is an important index to evaluate the anti-halitosis effect. It is mainly produced by anaerobic bacteria such as *Prevotella intermedia* (Pi), *Porphyromonas gingivalis* (Pg), and *Treponema denticola* (Td). The results of this study showed that both Xipaii Gugingival solution and Jinzhijiegingival gargle could reduce the concentration of VSCs in the mouth to a certain extent at two weeks, Then they kept the concentration of VSCs at a low level at one month, which indicating that the two gargles could produce anti-halitosis effect more quickly and sustainably.

4.2. The Significance of Research

Halitosis is a common symptom in the mouth. Studies have shown that volatile sulfides produced by odorous bacteria in the mouth not only affect patients' social and oral health-related quality of life, but also produce cytotoxicity to oral gingival epithelial cells, and even play an important role in the occurrence and development of periodontal disease [9]. In addition to mechanical washing to reduce bacteria and nutrient sources that can be used by bacteria, mouthwash also has sterilization, cover halitosis gas, and reduce the number of anaerobic bacteria in the mouth.

In recent years, more and more researchers have paid attention to natural Chinese herbs because of their good antibacterial effect and less toxic side effects. In this study, two commonly used Chinese herbal gargles were selected to compare the anti-halitopathy clinical efficacy of the two kinds of gargles, which detecting the changes of oral anaerobes and the levels of oral volatile sulfide in patients with bad breath, so as to provide data for their further clinical application.

4.3. Research Deficiencies and Prospects

In summary, this study conducted a randomized double-blind clinical study to detect the total number of anaerobic bacteria in the mouth and the concentration of volatile sulfide

in the mouth of the subjects before and after the experiment, which indicate that the two traditional Chinese medicine garrinse, Xipayi Gugingiwai liquid and Jinzhijiegingiwai garrinse, can alleviate halitosis to a certain extent. The mouthwash prepared from the active ingredients of natural Chinese medicinal materials should have broad application prospects, because of its safety and small side effects. It should be emphasized that for patients with bad breath, the use of combined tongue scrapers [10] can also be used to achieve better halitosis treatment. In addition, in future studies, further extension of the study time is needed to verify the long-term effect of mouthwash against bad breath.

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