The Antiplaque Effects of *Salvadora persica* and Padina Essential Oil Solution in Comparison to Chlorhexidine in Human Gingival Disease; a Randomized Placebo-controlled Clinical Trial

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**Abstract:** In this study the effects of Persica and Padina mouthrinses on plaque accumulation and gingivitis were compared with chlorhexidine gluconate 0.2%. This study was a single blind randomized clinical trial. Forty patients with chronic gingivitis participated in this study and were divided into four groups. Gingival index, gingival bleeding index and plaque index were recorded at baseline and after 1, 2, 3 and 4 weeks. Subjects of each group were asked to rinse one of the coded mouth rinses (Chlorhexidine, Persica, Padina and Placebo) twice a day for 4 weeks. During the first two weeks the patients had their habitual oral hygiene but after 2 weeks, oral hygiene was instructed and teeth were scaled and planed. Adjunctive use of Persica and chlorhexidine had only a modest advantage over placebo or Padina. This held true for both plaque (p=0.296) and gingival inflammation indices (p=0.007). However, when these mouthwashes were used as the only oral hygiene procedures and in the presence of calculus, none of them proved to be clinically effective regardless of statistical difference observed between groups (p=0.176 and p=0.001 for plaque and gingival bleeding index, respectively). Use of mouthwashes of any kind alone could not be regarded as substitute for mechanical tooth brushing. In addition adjunctive use of chlorhexidine or Persica proved to have some benefits over placebo and Padina mouthwashes.

**Key words:** Chlorhexidine, Persica, Padina, mouthwash, antimicrobial therapy, chemical plaque control, periodontal disease, therapy

**INTRODUCTION**

Microbial dental plaque is the main cause for both dental and periodontal diseases. Therefore, reduction or elimination of plaque is essential in preventing, reducing or treating these diseases.

Current therapies are directed toward preventing or reducing oral disease through a combination of mechanical and chemical plaque controls. Removal of supra-gingival plaque is usually sufficient to prevent inflammation, however effective treatment of all forms of periodontitis also requires the control of subgingival plaque. Retention of plaque in inaccessible sites can provide a nidus for re-infection, which may allow growth of pretreatment microflora and subsequent disease. The most widely used aid for oral hygiene is the tooth brush with a dentifrice. Systemic and topical chemotherapeutic agents have been used as adjunctive methods for preventing gingivitis and periodontal disease. Today, many oral rinses have been used as daily irrigation by patients as part of a home-based oral hygiene program.

The use of wood sticks for cleaning the teeth is traditionally and widely used in several countries in the Middle East, Asia and Africa. In the Middle East it is known as Miswak (or Siwak) which in Arabic means ‘sticks for rubbing the teeth’ and there is a strong belief that its use reduces plaque and gingivitis. The widespread use of Miswak among moslems was attributed to the Prophet Mohammad (PBUH), who recommended its use for tooth cleaning before each of the five daily prayer rituals. The plant most commonly used for Miswak is *Salvadora persica* (Salvadora ceae), a small tree, growing wild with a very wide geographical distribution. It has been used for many centuries by different communities as an oral hygiene aid. The stem of this plant is presented in the form of a short stick which, when softened with water and chewed gently at its tip will become frayed like the bristles of a tooth brush.

Chewing sticks have been shown to have a therapeutic effect on the gingiva and surrounding structures in addition to their mechanical effect. The therapeutic effect could be due to certain chemical factors, such as volatile oils, tannins, and other components. The use of such natural products may be a viable alternative to chemical agents in the management of oral health.
constituents such as fluorides, silicones, alkaloids, essentials, tannins, resins, gum and anthraquinones. 

In Indo-Pakistan, the sticks are obtained from a plant called Azadirachta indica which is known by the natives as “Neem”. In some part of Africa these sticks are obtained from a plant known as Fagara zanthoxyl. The value of Miswak extract like that of Persica has not been fully substantiated clinically, while chlorhexidine gluconate is well accepted as both an efficient plaque reducer and a valuable agent for prevention of gingivitis. Chlorhexidine gluconate (CHX) has been identified as an appropriate chemotherapeutic agent at a concentration of 0.2% CHX. CHX has been used effectively for many years as an antiseptic. In 1970, Loe and Schiott presented their observations of complete inhibition of formation of soft deposition on teeth when using two daily mouth rinses with CHX. Since then, their results have been confirmed in numerous publications. In the early 1970s CHX was incorporated at 0.2% concentration into mouth rinses in Europe and in 1986 it was incorporated at 0.12% in a mouth rinse in the United States. These mouth rinses were effective in reducing the supra-gingival flora, had a high safety margin and had no reported bacterial resistance. A substantial reduction in actinomyces counts after six months of daily application of a 0.12% solution of CHX has been reported. CHX, a bactericidal agent, kills microorganism by shorter exposure time than a bacteriostatic agents such as tetracycline, which inhibits protein synthesis. CHX is a long molecule with a positive charge that is attracted to the negatively charged surface of the biologic membranes of bacterial cells, it attaches to the cell wall of bacteria, disrupting the cell and entering the cell. As CHX enters the cells, it disrupts the cytoplasm. When the cytoplasm flows out of the ruptured cell wall, bacterial death occurs.

Padina (Essential oil oral rinse) is composed of three plant: Clove, Thyme, Spearmint which contains: Eugenol, Thymol, Menthol, Caraceral, Pinen, Phellandren.

To date, there has been no report of comparing CHX, Persica and Padina mouthwashes. Today these mouthwashes are being marketed and used. The manufacturers believe that they are effective in preventing gingivitis, therefore there is a need to study the effectiveness of these oral rinses. Furthermore, viewing tooth discoloration as an unwanted effect seen following the use of CHX, this comparison sounds warranted.

The aim of this study is to evaluate the effects of a mouthwash containing Salvadora persica extract (Persica) and an essential oil oral rinse (Padina) in comparison to CHX 0.2% rinse on plaque accumulation and gingivitis.

MATERIALS AND METHODS

This study was a single blind, randomized, placebo-controlled clinical trial. Forty patients (18 male and 22 female), aged between 20 and 30, with generalized chronic gingivitis participated in this study. Patients were selected from those attending for treatment in Periodontal clinic of Mashhad Dental School during 1999 and 2000. The following criteria were used in patient selection: No relevant medical history, good medical health, no pregnancy, not receiving any medication or antibiotic within the past six month, no periodontal treatment during past 3 month, at least 10 sites with chronic mild gingivitis with bleeding on probing, no pockets more than 3 mm.

The nature of the clinical trial was explained and informed consent was obtained from all patients.

Experimental design: Subjects were randomly divided into 4 groups of 10, as follow:

Group A: The subjects were asked to rinse with 15 mL of placebo (distilled water) for one minute, twice a day until two weeks as control group.

Group B: The patients were asked to rinse with 15 mL of CHX 0.2% (Sharedar Pharmaceutical Co., Tehran, Iran) twice a day until two weeks.

Group C: The subjects were asked to rinse in the same protocol with 15 mL of Salvadora Persica oral rinse (Persica, Poursina Pharmaceutical Laboratories, Tehran, Iran). Prior to use, the mouthwash was prepared as a 7% dilution as this was the recommendation of the manufacturer.

Group D: The patients rinsed in the same manner with 15 mL of essential oil oral rinse (Padina, Padina Co., Mashhad, Iran). Prior to use, the mouthwash was prepared as a 30% dilution as this was the recommendation of the manufacturer. Forty uniform bottles with secret code as the content for each group were prepared. The trial was designed as a single blind study, the patients and the operator who recorded the indices during the study was not aware of the randomization and code of each group.

Baseline measurement: The gingival index, gingival bleeding index and plaque index as clinical indices of improvement were recorded at baseline and after 1, 2, 3 and 4 weeks.

Then the subjects in each group were asked to rinse for one minute of coded mouth rinse, twice a day for
During the first 2 weeks the patients had their habitual oral hygiene. After 2 weeks, instruction were given to maintain a high standard of oral hygiene, using Bass technique for the rest of trial and the teeth were scaled and planed if needed.

Statistical analysis: All data were presented as mean±SE. Parametric data were analyzed by ANOVA. The comparison between treating groups was carried out by post hoc Tukey’s test. The p value of 0.05 was considered the threshold value to declare a statistically significant difference. The power of study was 80% to prevent a type II statistical error (β=0.20).

RESULTS

Both the CHX and the Persica groups showed greater reductions in the Gingival Bleeding Index (GBI) than the Padina and Placebo groups at the 1st week. However, the difference between the GBI reductions of the CHX and Persica groups were not statistically significant (p>0.05).

At the 2nd week follow up visit, the only significantly different groups were CHX versus Padina and CHX versus placebo groups (p<0.001).

At the 3rd and 4th weeks of the study, the reductions of the GBI (%) in the Persica group were significantly greater than those of the Placebo groups (p<0.01 and p=0.007, respectively). There were no other significant differences among other groups at these stages of the study (Table 1).

Plaque Index (PI) showed no significant difference among groups at the baseline (p=0.277) (Table 2). At the 1st week visit, the reduction in the PI were minimal across the groups with no significant differences among them. However, at the 2nd week visit, the CHX group showed the greatest reduction of PI (0.2±0.12) which was significantly greater than the PI reduction observed in the Padina group (p=0.02). When all patients were subjected to oral hygiene instruction and scaling, an improvement in the PI was noted at the 3rd week and 4th week visits in all groups, however, no statistical difference existed among the groups (p=0.329 and p=0.296, respectively).

Gingival Index (GI) were above 1 in all groups at the baseline with no significant difference among them (p=0.638) (Table 3). At the 1st and 2nd week follow up visits, the improvements in the GI were small. At the 1st week, the GI reduction in the CHX group turned out to be significantly greater than the placebo and Padina groups (p<0.001). At the 2nd week there was no significant difference between the groups (p=0.09). Once the mechanical tooth cleaning was re-instituted in addition to mouth rinsing, considerable improvement took place in all groups. However, no significant difference existed between groups neither at the 3rd nor at the 4th week visits.

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**Table 1: Gingival Bleeding Index (GBI) at baseline and the reduction at 1st, 2nd, 3rd and 4th weeks among the study groups**

<table>
<thead>
<tr>
<th>GBI</th>
<th>Group 1 Placebo</th>
<th>Group 2 CHX</th>
<th>Group 3 Padina</th>
<th>Group 4 Persica</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline (%)</td>
<td>94.59±5.69</td>
<td>98.29±5.29</td>
<td>99.36±4.59</td>
<td>99.90±5.16</td>
<td>-</td>
</tr>
<tr>
<td>1st week reduction</td>
<td>0.21±0.66</td>
<td>1.98±1.50</td>
<td>0.22±0.63</td>
<td>1.54±1.20</td>
<td>0.001*</td>
</tr>
<tr>
<td>2nd week reduction</td>
<td>0.52±1.33</td>
<td>6.40±0.75</td>
<td>0.59±1.27</td>
<td>3.83±3.89</td>
<td>0.09**</td>
</tr>
<tr>
<td>3rd week reduction</td>
<td>77.15±7.29</td>
<td>83.40±6.2</td>
<td>82.37±5.50</td>
<td>85.33±3.72</td>
<td>0.04**</td>
</tr>
<tr>
<td>4th week reduction</td>
<td>88.56±6.24</td>
<td>93.10±8.34</td>
<td>93.17±3.06</td>
<td>94.85±4.19</td>
<td>0.007##</td>
</tr>
</tbody>
</table>

Data are mean±SE. *Significantly different groups (Tukey test): [Placebo and CHX],[Placebo and Persica],[Placebo and CHX],[Persica and Padina]. #Significantly different groups: [Placebo and CHX],[CHX and Padina]. **Significantly different groups: [Placebo and Persica]. ## Significantly different groups: [Placebo and Persica].

**Table 2: Plaque Index (PI) at baseline and the reduction at 1st, 2nd, 3rd and 4th weeks among the study groups**

<table>
<thead>
<tr>
<th>PI</th>
<th>Group 1 Placebo</th>
<th>Group 2 CHX</th>
<th>Group 3 Padina</th>
<th>Group 4 Persica</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.19±0.1</td>
<td>1.28±0.06</td>
<td>1.12±0.37</td>
<td>1.31±0.24</td>
<td>0.277</td>
</tr>
<tr>
<td>1st week reduction</td>
<td>0.008±0.01</td>
<td>0.09±0.07</td>
<td>0.002±0.06</td>
<td>0.11±0.26</td>
<td>0.176</td>
</tr>
<tr>
<td>2nd week reduction</td>
<td>0.02±0.03</td>
<td>0.20±0.12</td>
<td>0.02±0.04</td>
<td>0.16±0.28</td>
<td>0.02*</td>
</tr>
<tr>
<td>3rd week reduction</td>
<td>1.08±0.13</td>
<td>1.14±0.07</td>
<td>1.00±0.36</td>
<td>1.19±0.25</td>
<td>0.329</td>
</tr>
<tr>
<td>4th week reduction</td>
<td>1.14±0.11</td>
<td>1.21±0.07</td>
<td>1.08±0.37</td>
<td>1.26±0.25</td>
<td>0.296</td>
</tr>
</tbody>
</table>

Data are mean±SE. *Significantly different groups: [Padina and CHX].

**Table 3: Gingival Index (GI) at baseline and the reduction at 1st, 2nd, 3rd and 4th weeks among the study groups**

<table>
<thead>
<tr>
<th>GI</th>
<th>Group 1 Placebo</th>
<th>Group 2 CHX</th>
<th>Group 3 Padina</th>
<th>Group 4 Persica</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>1.24±0.07</td>
<td>1.22±0.11</td>
<td>1.14±0.38</td>
<td>1.24±0.06</td>
<td>0.638</td>
</tr>
<tr>
<td>1st week reduction</td>
<td>0.002±0.06</td>
<td>0.02±0.02</td>
<td>0.002±0.06</td>
<td>0.01±0.01</td>
<td>0.09</td>
</tr>
<tr>
<td>2nd week reduction</td>
<td>0.06±0.01</td>
<td>0.07±0.04</td>
<td>0.05±0.20</td>
<td>0.05±0.07</td>
<td>0.09</td>
</tr>
<tr>
<td>3rd week reduction</td>
<td>1.06±0.05</td>
<td>1.06±0.13</td>
<td>0.95±0.41</td>
<td>1.04±0.04</td>
<td>0.521</td>
</tr>
<tr>
<td>4th week reduction</td>
<td>1.18±0.07</td>
<td>1.16±0.10</td>
<td>1.08±0.38</td>
<td>1.19±0.05</td>
<td>0.694</td>
</tr>
</tbody>
</table>

Data are mean±SE. *Significantly different groups: [Placebo and CHX],[CHX and Padina]
DISCUSSION

The present investigation studied the effect of Persica and Padina in comparison to CHX as the most effective mouthwash and a gold standard positive control. CHX could not be recommended as a permanent routine daily mouthwash because of its unsightly tooth discoloration.

Present results indicated that the adjunctive use of Persica and CHX had only a modest advantage over Placebo or Padina groups. This held true for both plaque and gingival inflammation indices (Table 1-3). However, when these mouthwashes were used as the only oral hygiene procedures and in the presence of calculus, none of them proved to be clinically effective regardless of statistical difference observed between groups. The studies done by other investigators supported our study, for example, Gazi et al. compared Salvadora Persica slurry and CHX 0.2% rinse, as adjuncts to mechanical plaque control. Both mouth rinses produced a significant reduction in the GI, bleeding point index and PI. However, they found superiority of CHX over Salvadora Persica tooth paste rinse as an anti-plaque agent.

Darout et al. concluded that periodontal status of Miswak users in Sudanese population is better than tooth brush users. They found significant health gains from Miswak as an oral hygiene tool. Al-Khatib et al. found that, the frequent use of the Miswak is associated with a lower need for treatment. Moghaddas and Mahdavi found Persica as a mouth wash helpful in reducing the bleeding index and plaque accumulation in patients with gingivitis. Almasi compared anti-microbial activity of Neem and Salvadora Persica (Arak) chewing sticks aqueous extracts at various concentrations. He suggested both chewing stick extracts effective at 50% concentration on S. mutans and S. sanguinis. He recommended chewing sticks as oral hygiene tool for health promotion in developing countries.

Padina mouth wash proved not superior to placebo in our study. This agent needs further investigation before it could be recommended as an effective mouthwash.

In GBI score of first two weeks of the study, there was a significant difference between CHX and Persica in one hand and Placebo and Padina groups in the other hand. Although these differences were statistically significant, the improvements of 6.40 and 3.83%, respectively observed in GBI of CHX and Persica groups do not seem to be clinically important during the first 2 weeks of study. This lack of clinical improvement in the gingivitis and plaque levels of all groups might be attributed to the fact that during the first 2 weeks of the study the patients had their habitual oral hygiene and their plaque retentive factors such as supra- and subgingival calculus remained intact. CHX or any other chemotherapeutic agents when applied using a mouthwash may fail to reach interproximal areas. Little improvement in plaque and gingivitis when CHX rinse alone was compared to other methods of tooth cleaning has been reported. This is, however, in contrast to the studies conducted by other investigators who demonstrated that CHX rinse might reduce plaque up to 30 to 40% in gingivitis subjects. When scaling and root planning were performed together with oral hygiene instruction, all groups showed improvements in plaque and gingivitis levels. CHX and Persica groups showed a small benefit over the other two groups at this stage of the study, with Persica group being statistically superior to the placebo in terms of GBI scores. This highlights the fact that advantage resulted from mechanical debridement and tooth brushing is far greater than that could be achieved by a mouthwash alone.

Present data indicates that the use of mouthwashes of any kind alone could not be regarded as substitute for mechanical tooth brushing. In addition, although adjunctive use of CHX or Persica proved to have some benefits over placebo and Padina mouthwashes, this benefit should not be overemphasized.

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