Evaluation of anti-aphthous activity of decoction of Nicotiana tabacum leaves as a mouthwash: a placebo-controlled clinical study

Siavash Vaziri, Mahdi Mojarrab, Mohammad Hosein Farzaei, Farid Najafi, Ali Ghobadi

OBJECTIVE: To determine the effects of decoction derived from the leaves of Nicotiana tabacum (L.) as a mouthwash on minor recurrent aphthous.

METHODS: A randomized double-blinded placebo-controlled clinical trial was conducted on 60 patients with minor recurrent aphthous. Treatment comprised of application of tobacco or placebo mouthwash (10 mL 3 times a day) for 5 days. Clinical evaluation included pain level using a visual analog scale and ulcer size on days 1, 3, and 5 were measured. Adverse effects after mouthwash application were recorded, and the oral mucosa was examined by the investigator at each visit.

RESULTS: A total of 54 subjects with the mean age (38 ± 10) years fulfilled the study. No minor and major adverse effects were observed. In the treatment group, ulcer pain score was decreased by 79.2% and 93.8% and ulcer size was reduced by 69.1% and 92.2% (days 3 and 5, respectively), which was significantly greater than the control group (P < 0.01).

CONCLUSION: The decoction prepared with of Nicotiana tabacum leaves, used as mouthwash are well-tolerated and safe, and can be used for the management of recurrent aphthous.

Key words: Stomatitis, aphthous; Tobacco; Pain; Ulcer; Medicine, Traditional; Complementary therapies; Mouthwashes

INTRODUCTION

Recurrent aphthous ulcers (also termed canker sores) are currently one of the most common inflammatory ulcerative disorders of the oral mucosa. This condition is characterized by painful, recurrent and single or multiple ulceration of the oral cavity that affect nearly 10%-20% of the general population. The ethiopathogenesis of aphthae is unclear but some factors such as heredity, immune dysregulation, hematine deficiency (like iron, folic acid, vitamin B6 and B12), stress, local trauma, infections and systemic diseases (Behcet’s syndrome) have been proposed as causative factors. Despite the multi-factorial etiology of the disease, most of the treatments for aphthae are designed to reduce pain and inflammation. However, in order to eliminate or reduce the symptoms, we need to search safer and more effective agents with multi-bioactivities. Nowadays, there is an increasing tendency to use herbal med-
icines for the treatment of aphthae and several herb’s extracts have been evaluated for this propose. The genus Nicotiana (L.) (Family: Solanaceae) comprises about 100 species and natively distributed in tropical America. Three species of this genus, Nicotiana glauca, Nicotiana rustica and Nicotiana tabacum are cultivated in Iran with the common Persian name of "Tanbakoo." Nicotiana tabacum, of the most commercially valued agricultural herbs in the world, has half hardy annual subshrubs with rose-coloured flowers and elliptic-ovate leaves. In folk medicine, the leaves of tobacco have been used in the treatment of backache, toothache, lumbago, gout, ulcers and wounds. As a medicinal plant, leaves have been used due to their sedative, narcotic, emetic and antispasmodic activities and applied for the rheumatic swelling and skin disorders. According to the literature, different biological compounds, such as, isoflavones, phenolic acids, sesquiterpenes, diterpenoids, and alkaloids were isolated from tobacco but alkaloid constituents, chiefly nicotine, are the most important part of these components due to their main pharmacological and biological activities. Moreover, several reports revealed that smoking has potential inhibitory effects on the occurrence of aphthous ulcers. In the light of above findings, it is reasonable to assess the anti-aphthous activity of tobacco leaves extract against aphthous ulcers. In the present study, anti-aphthous activity of the leaf decoction from Nicotiana tabacum as a mouthwash was evaluated on patients with recurrent aphthous ulcers.

MATERIALS AND METHODS

Preparation of decoction and formulation (Sample preparation)
Dried leaves of Nicotiana tabacum were purchased from local bazaar market, Kermanshah, Iran. 500 grams of Nicotiana tabacum leaves was ground and decocted with 10-folded mass of water (5000 mL) for 30 min, filtered, and then let the decoction be cooled down to room temperature. For preparation of a mouthwash, 3000 mL of filtrated decoction was mixed with 3 grams of a mixture of methylparaben and propylparaben (9:1) as preservatives. In addition, placebo was prepared in similar container including distilled water with approved color additives which looked the same as the tobacco mouthwash. The placebo mouthwash also had similar label as the tobacco mouthwash preparation.

Subjects and study design
In this randomized double-blinded placebo-controlled clinical study, 60 patients were assigned into group of placebo (n = 30) and group of tobacco treatment (n = 30). Patients were seen at the Infectious Diseases Clinic, Kermanshah University of Medical Sciences, Kermanshah. Randomization of equal number of subjects to placebo or treated group was achieved using a simple random allocation strategy, using block randomization method according to CONSORT Statement (2010). The participants were instructed to apply 10 mL of mouthwash, 3 times a day for 5 days. Likewise, the patients in placebo group were instructed to use the placebo mouthwash the same as treatment group (10 mL of mouthwash, 3 times a day for 5 days). The baseline factors were taken and recorded on the day of the first visit. All of the subjects were free to withdraw at any time during the course of study. All participants signed a written informed consent before recruiting in the study. The Ethics Committee of Kermanshah University of Medical Sciences approved this clinical study (approval number: KUMS. REC.1394.14).

To exclude potentially confounding systemic diseases, all patients underwent careful examination by dermatologists, gastroenterologists, and ophthalmologists before enrollment. The clinical diagnosis was made by infectious medicine specialists based on clinical appearance, location, and patient history. All patients were selected according to specific inclusion and exclusion criteria. The inclusion criteria were as follows: (a) men and women (males and females) aged 15-65 years old who can follow the doctor’s advice; (b) Willingness to participate and sign the informed agreement forms; (c) patients with 1 to 5 aphthous ulcers (less than 48 hours’ duration); (d) an anticipation that their ulcers normally take 5 or more days to resolve without treatment; (e) ulcers must be in positions simply accessible for Evaluation and treatment, such as the labial mucosa, buccal mucosa, or the tongue; and (f) patients with normal sense of pain. Exclusion criteria were the following: (a) a known history of severe drug hypersensitivities, particularly allergies to tobacco or nicotine; (b) pregnancy or lactation; (c) ulcers as a manifestation of a systemic disease process, including Behçet disease, serious anemia Crohn’s disease, ulcerative colitis, or acquired immune deficiency syndrome; (d) simultaneous clinical conditions which represent a health risk to the subjects, such as severe heart, liver, or kidney disorder; (e) treatment with systemic non-steroidal anti-inflammatory drugs, systemic steroids or other immunomodulatory agents, oral antihistamines, or systemic antibiotics within 2 weeks study entry; (f) treatment of the ulcer with any medication within 72 h before study entry; and (g) attendance of any clinical studies within 2 months before trial entry.

Measurement
In all studies, the size and number of ulcers were calculated by the investigator, and the pain was assessed by the subjects before the first mouthwash application and at each subsequent evaluation. For patients who had more than 1 ulcer, only the ulcer that occurred lately and was easy for evaluation was selected. Periodic telephone interviews were performed to supervise the
administration on schedule. Adverse effects after mouthwash application were recorded, and the oral mucosa was examined by the investigator at each visit. Evaluation of pain was performed using a visual analog scale (VAS) containing a 10-cm horizontal line between the poles of "no pain" and "unbearable pain". Patients were instructed to mark the line with a vertical line at the point that best characterized the present pain level of the aphthous. In order to calculate the size of the ulcers, the investigators measured the distance between 2 opposite outside edges of the white border in term of millimeter. Two measurements approximately 90 degrees from each other were determined; the largest distance was used as one of the measurements. The 2 measurements were then multiplied to represent the cross-sectional areas of the aphthous.

In order to compare the efficacy of treatment statistically at different time points the data were calculated by means of the following formula \[\frac{(visit 1-visit 3 or 5)}{visit 1}\times 100\%\] (visit a referring to the therapeutic indices measured at day a), as described previously.  

Statistical analysis
Comparison of baseline characteristics between placebo-control and treatment group were performed by t-test and c² test. Differences between tobacco treatment group and placebo group were evaluated in each visit using Chi-square and the Mann-Whitney U test. Statistical significance was set at \(P < 0.05\). All data were analyzed with SPSS software (Released 2009, SPSS Statistics for Windows, Version 18.0. SPSS Inc. Chicago, IL, USA).

RESULTS
A total of 60 subjects were recruited for this study. Only 3 subjects in treatment group and 3 patients in placebo-control group dropped out because of conflicting schedule and not following the treatment. Thus, 27 subjects in treatment group and 27 subjects in placebo-control group fulfilled the study.

Baseline characteristics
There were no statistically significant differences in baseline characteristics between the 2 groups. Hence, the groups were homogenous with respect to age, sex, as well as known allergies. Likewise, the baseline levels of ulcer size, numbers, as well as pain score were not significantly difference between treatment and control groups (\(P > 0.05\)). Age of the patients ranged from 21 to 58 years with the mean age (38 ± 10) years. Among the patients, 48.2% were women and 51.8% were men (Table 1).

Improving pain
As shown in Table 2, the mean pain score of the treatment group and the placebo-control group were not significantly different (\(P > 0.05\)) at the baseline (day 1, before administration of the treatment). The ulcer pain score in all of the study days after treatment with tobacco mouthwash was significantly lower than that of placebo-control values (\(P < 0.01\)). At day 3, ulcer pain score in the tobacco group was decreased by 79.2%, which was significantly higher than that of the control group (22.3%, \(P < 0.01\)). Likewise, at the day 5 of study, ulcer pain score in the treatment group (tobacco mouthwash) was lessened by 93.8%, which was statistically different in comparison with the level of placebo group (44.6%, \(P < 0.01\)).

Ulcer measurement
Table 3 shows the ulcer size in term of millimeter at study entry day and after treatment with tobacco mouthwash and placebo. At day 1, mean ulcer size of the treatment and control groups were not significantly different (\(P > 0.05\)). The ulcer size in all of the days after treatment was significantly different in comparison with placebo-control group (\(P < 0.01\)). At the day 3 of study, the ulcer size in the treatment group (tobacco mouthwash) was decreased by 69.1%, which was higher than the 22.9% observed in the placebo group (\(P < 0.01\)). On day 5 of the study, the ulcer size decreased by 92.2% which was significantly greater than the level of placebo group (50.1%, \(P < 0.01\)).

### Table 1 Clinical characteristics of patients with aphthous (\(\bar{x} \pm s\))

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients (n)</th>
<th>Gender (n)</th>
<th>No. of ulcers</th>
<th>Year of age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Female</td>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Tobacco</td>
<td>27</td>
<td>14</td>
<td>13</td>
<td>3.1±0.7</td>
</tr>
<tr>
<td>Placebo</td>
<td>27</td>
<td>12</td>
<td>15</td>
<td>3.2±0.8</td>
</tr>
</tbody>
</table>

Notes: patients with aphthous in tobacco group were treated with mouthwash of Nicotiana tabacum leaves decoction, and patients with aphthous in placebo group were treated with blank mouthwash which looked the same as the tobacco mouthwash.

### Table 2 Ulcer pain score of treatment and placebo-control groups at different time points (\(\bar{x} \pm s\))

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco</td>
<td>4.1±2.3</td>
<td>2.4±2.2</td>
<td>1.1±1.4</td>
<td>0.7±1.0</td>
<td>0.4±0.6</td>
</tr>
<tr>
<td>Placebo</td>
<td>4.0±2.2</td>
<td>3.6±1.8</td>
<td>3.1±1.8</td>
<td>2.7±1.5</td>
<td>2.1±1.3</td>
</tr>
</tbody>
</table>

Notes: patients with aphthous in tobacco group were treated with mouthwash of Nicotiana tabacum leaves decoction, and patients with aphthous in placebo group were treated with blank mouthwash which looked the same as the tobacco mouthwash. Compared with the placebo-control group, \(P < 0.01\).

Notes: patients with aphthous in tobacco group were treated with mouthwash of Nicotiana tabacum leaves decoction, and patients with aphthous in placebo group were treated with blank mouthwash which looked the same as the tobacco mouthwash.
Safety evaluation
No clinical adverse effect including hypersensitivity, taste sense malfunction, and infection were reported. All subjects tolerated the mouthwash well with a good score.

DISCUSSION
Recurrent aphthous ulcers is an episodic and self-limit ed disease and period of the ulcers is usually 7-10 days but it causes remarkable pain, suffering ulceration and inconvenience for patients as well as adversely affecting the quality of life. For these reasons, reducing the pain, inflammation and ulcer size, decrease in the frequency of recurrence and also healing promotion are the main aims of therapy.26,27
Natural medicine is considered as a safe and efficacious complementary and alternative therapy for various diseases.26,27 In traditional and folklore medicine of different nations all over the world including Persian medicine several plant-derived natural remedies have been used for the management of dermal as well as mucosal ulcers for thousands of years.28,29
Results obtained from current study indicated that decoction of leaves of Nicotiana tabacum was administrated as a mouthwash and showed remarkable effect to treat aphthous ulcers. This is in line with the observations by Subramanyam,30 Koybasi et al.,31 Grady et al.32 and Shapiro et al.33 and supports their hypothesis that cigarette smoking has a protective activity on occurrence of aphthous ulcers.34-37 Combustible products of smoking are known to stimulate an increased keratinization of the oral mucosa and therefore resist formation of aphthous ulcers and reduce trauma or bacterial penetration of the mucous membrane in smokers when compared to non-smokers.38-40 This property may be attributed to the presence of the main alkaloid of Nicotiana tabacum, Nicotine, which showed remarkable protective effect in aphthous ulcers and Behcet syndrome by using oral nicotine replacement therapy.41 Nicotine and its metabolites exhibited immunosuppressive activity and lead to reduction in inflammatory condition by different known mechanisms including antibody-forming cell response, suppression of neutrophil mediated inflammatory action, inhibition of endothelial cell release of interleukin (IL)-8, inhibition of IL-1β, IL-2, IL-10, tumor necrosis factor (TNF)-α and interferon (IFN)-γ release, reduction in circulation levels of immune globulins, impairment of antigen-mediated signaling in T-cells and inducing T-cell allergy as well as attenuation of IFN signaling.42-45 Moreover, previous investigations revealed that different extracts of Nicotiana tabacum leaves have a significant range of inhibitory effect on different bacterial species that result in improvement of disease process.25,27
In conclusion, the present clinical study exhibits that the mouthwash of decoction of Nicotiana tabacum leaves possess a remarkable potential to reduce pain and promote ulcer healing with minimal safety concerns. Thus, current clinical practice confirmed the efficacy of Nicotiana tabacum leaves mouthwash as a well-tolerated and safe adjunctive therapy for the management of recurrent aphthous. More well-designed randomized controlled clinical trial is suggested in order to recognize the mechanisms of action and therapeutic aspects of this natural mouthwash.

ACKNOWLEDGEMENTS
We gratefully acknowledge Dr. Anita Yaghootti Poor (Faculty of Agriculture, Razi University, Kermanshah, Iran) for her kindly statistical advice.

REFERENCES
7 Moghadamnia AA, Motallebnejad M, Khanian M. The efficacy of the bioadhesive patches containing licorice ex-