

COMPARATIVE STUDY OF *ZATARIA MULTIFLORA* AND *ANTHEMIS NOBILIS* EXTRACTS WITH *MYRTHUS COMMUNIS* PREPARATION IN THE TREATMENT OF RECURRENT APHTHOUS STOMATITIS

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ABSTRACT

Recurrent aphthous stomatitis (RAS) is a prevalent and complicated disorder and its management is directed toward treatment of symptoms. The purpose of this study was to compare the efficacy of three herbal preparations in the management of RAS. One hundred and one out of 115 patients with minor aphthae were selected and randomly divided into four groups. Groups A, B and C received topical preparations of *Zataria multiflora*, *Anthemis nobilis* and a 50 % (v/v) mixture of *Zataria multiflora* and *Anthemis nobilis* respectively. Groups D (positive control) received *Myrthus communis* (Myrtle, mouth rinse), which reported to be efficient in the management of RAS. The time of pain elimination and the duration of the thorough healing were recorded. Mean time of pain elimination showed significant differences ($p < 0.01$) between groups A (3.00 ± 1.14 day), C (3.08 ± 1.84 days) and D (4.30 ± 2.12 days) with group B (5.20 ± 3.11 days). The mean duration of healing also showed significant differences ($p < 0.03$) between groups A (6.00 ± 2.80 days), C (6.70 ± 2.70 days) and D (7.60 ± 3.10 days) with B (8.70 ± 3.90 days). No significant differences were observed between groups A and C with group D (positive control). The result obtained for treatment with *Z. multiflora* (group A) was similar to that of group C, but better than *M. communis* (group D). Findings of this study revealed that *Z. multiflora* extract showed better effects than *M. communis* which is reported to be effective in the treatment of RAS. It is concluded that the *Z. Multiflora* extract is an effective product for the management of minor aphthae.

Keywords: *Zataria multiflora*, *Anthemis nobilis*, *Myrthus communis*, Aphthous stomatitis

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is among the most prevalent and complicated disorders of oral cavity. Despite much clinical attention, the causes of this disease is poorly understood and its management is directed largely toward treatment of symptoms (1,2). Several initiating factors have been implicated in the pathogenesis of RAS (3). Immunopathologic basis (4), nutrient deficiency (5, 6), psychologic stress (7), hormonal imbalance (3), microbial contamination (2,8), hereditary influence (9) and gluten sensitivity (10,11) are among the most important factors. Antibacterial, anti-inflammatory and anti-histaminic agents, analgesics, local anesthetics, and glucocorticoids have been used topically to manage the RAS (1-3, 9). Most of these therapies are associated

with side effects or unwanted reactions (2).

Few medicinal herbs are listed as anti-aphthous agents without any experimental or clinical references (12). Quercetin, a plant flavonoid, may have some effects on the management of RAS due to its structural similarity and functionalities with disodium cromoglycate and has been shown to be effective in the treatment of RAS (11). Azulene compounds found in several plant species, such as *A. nobilis* and *Matricaria recutita* are reported to possess anti-allergic, anti-inflammatory and mild anti-bacterial activities. *A. nobilis* is used for washing the mouth wounds and external use of *M. recutita* is approved for mucous membrane inflammations including those of the oral cavity and gums (13,14). Deglycyrrinized liquorice (DGL) is reported to be effective in promoting

healing of RAS (11).

Zataria multiflora Boiss (Avishan-e Shirazi in Persian) is a plant native to Iran. In the Iranian botanical sources (15,16) the Avishan term has been used as a Persian name for three different herbs: **a**; *Thymus kotschanus* Boiss & Hohen, **b**; *Ziziphora clinopodioides* Lam and **c**; *Zataria multiflora* Boiss. But, *Zataria multiflora* (Avishan-e Shirazi) is considered to be what recorded as Sa'atar or Zaatar in the old medical treatises and books of Iran (17-19). According to these classical references the Sa'atar (Avishan Shirazi) has been discussed to be useful in reliving the toothache, strengthening the gum, healing the wound and is used for other purposes (17,19).

Literature survey showed that only two clinical studies have been carried out to support indication of medicinal plants in the management of RAS lesions (20,21). Results of study on the use of essential oil of *M. communis* in a double-blind study showed that the severity of pain is decreased and duration of ulcers is shortened (20). In another clinical study, which is published recently, a *Z. multiflora* essential oil mouthwash has shown to be more efficacious than placebo (21). Antibacterial and anti-fungal effects of *Z. multiflora* are also reported (22).

Pertinent to the ethnobotanical use, as well as other data noted in the introduction, a randomized double blind clinical trial was designed to compare the efficacy of *Z. multiflora* (group A), *A. nobilis* (group B), and a combination of *Z. multiflora* and *A. nobilis* (group C) with *M. communis* (group D, positive control) in the management of the aphthous lesions. The overall results of this study revealed that the hydroalcoholic extract of *Z. Multiflora* could be a safe and effective agent for the management of minor aphthae.

MATERIALS AND METHODS

Plant material

Medicinal plants were selected on the basis of their bioactive constituents, supportive reports and traditional uses. Hydroalcoholic extracts of *Z. multiflora* and *A. nobilis* were prepared in the Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences. Dried and powdered leaves of *Z. multiflora* and powdered flowers of *A. nobilis* (500 g each) were extracted 3 times with 2 L of

ethanol (55% V/V) by percolation at room temperature. The extracts were kept to 2 L by addition of alcohol and labeled **A** for *Z. multiflora*; **B** for *A. nobilis*; **C** for a mixture of (50/50) (V/V) of **A** and **B**, respectively. *M. communis* (Myrtle) mouth rinse on the basis of previous clinical report (20) was purchased from the local market (Barije Essence Corporation, Iran) labeled **D** and used as a positive control.

Patients and methods

A total of 101 patients of which 43 (42.2%) were women and 58 (57.4%) were men, with mean age 30.49 years (9-64 years), with a history of RAS and currently suffering from ulceration located on the oral mucosa were recruited from the patients attending at the Oral Medicine Clinic, Faculty of Dentistry, Tehran University of Medical Sciences.

All patients were interviewed and the followings were excluded from the study. Patients with any systemic diseases or special syndrome that aphthous ulcer is one of its symptoms (Behcet's syndrome); those with aphthous lesions older than 4 days, patients subjected to any other treatment for at least 4 weeks before the start of the study and those who declined entering the study.

Diagnosis of minor aphthae was made on the basis of the patient's health history, clinical examination and the presence of a well-demarcated painful ulcer on the smooth unattached oral mucosa, which is surrounded by a light red areola (1,2).

All patients were asked to grant the informed consent as required by the local Ethics Committee. At the initial appointment, patients were asked to read and sign a letter of informed consent and complete a medication history questionnaire.

Study design

Patients under the study were assigned randomly to one of the four-treatment groups namely A, B, C, and D. Patients of each groups received 20 ml of anonymous herbal preparations and were instructed to apply medications five times daily by putting a small sterile cotton pad impregnated with 10 drops of each preparation (unknown to clinicians and patients) on the lesions for one minute and do not eat at least for 30 min after administration

of preparations. They were also instructed to record the date of pain elimination and duration of healing. The investigators kept in contact with patients to insure the correct use of drug, recorded results and any adverse reactions in questionnaire. Patients were re-examined after a week for elimination of the pain and complete healing.

RESULTS

One hundred and one out of 115 patients (58 males and 43 females) with RAS participated in the study. The mean age of patients was 30.49 years (9-64 years). Twenty five patients (24.8 %) received *Z. Multiflora* extract (group A); 27 patients (26.7 %) received *A. nobilis* extract (group B); 25 patients (24.8 %) received 50/50 mixture of *Z. multiflora* and *A. nobilis* extracts (group C) and 24 patients (23.8 %) received "Myrtle" preparation (group D, positive control). Figure 1 shows the mean duration of thorough healing of lesions in four groups. Figure 2 reveals average time of pain elimination in the same four groups. The mean duration of healing showed significant differences ($p < 0.03$) between groups A, C and D with group B (*A. nobilis*). No significant differences ($p > 0.1$) were observed among groups A and C with group D (positive control) as shown in figure 1. Results represented in Figure 2 reveal significance differences ($p < 0.01$) between groups A, C, and D with group B ($F = 5.71$), but again no significant differences ($p > 0.1$) were seen among groups A and C with group D.

DISCUSSION

Since no study has so far been carried out on the efficacy of *Z. multiflora* and *A. nobilis* extracts on the management of aphthous ulcers except what recently reported on the efficacy of *Z. multiflora* essential oil mouthwash (21), the discussion is originally focused on the results of this study. The main problems related to the use of herbal medicines in treatment of RAS are: the low documentation of the use of these medicines, lack of standardized preparation, low acceptance by the medical communities and low patient's compliance. In the present study a complete literature search was carried out and medicinal plants were selected according to their existing scientific data. Patients were instructed to use the drug locally,

but the length of time that the drug remained over a lesion might have been variable. The selected 30 min. time to remain the drug over the lesions seemed reasonable for the release of the agents and the results of the study were in support of the applied localized manner.

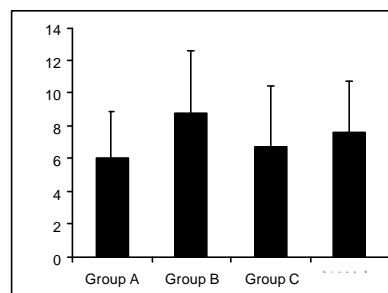


Fig. 1. Average time for complete healing of lesion after receiving *Z. multiflora* extract (group A); *A. nobilis* extract (group B); 50/50 mixture of *Z. multiflora* and *A. nobilis* extracts (group C) and *M. communis* (Myrtle) mouth rinse (group D). Values represent the mean \pm SD for each group. Unpaired student's *t*-test analysis shows that all test groups are significantly different from group B ($p < 0.03$), but there is no significant differences between groups A and C with D (positive control).

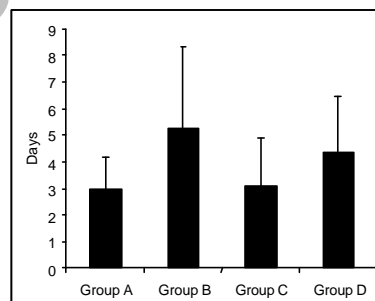


Fig. 2. Average time for elimination of pain among four groups after receiving *Z. multiflora* extract (group A); *A. nobilis* extract (group B); 50/50 mixture of *Z. multiflora* and *A. nobilis* extracts (group C) and *M. communis* (Myrtle) mouth rinse (group D). Values represent the mean \pm SD for each group. Unpaired student *t*-test analysis shows that all test groups are significantly different from group B ($p < 0.01$), but there is no significant differences between groups A and C with D (positive control).

Since the history of previous healing or pain elimination may affect the healing and pain elimination of in-used drugs, the analysis of covariance (ANOVA) was performed. The results showed significant altering effect of patient's history on drug effect. Thus, multiple regression analysis was used for estimation of the duration of thorough healing or pain elimination and it was found that the effect of *Z. multiflora* extract on RAS was even better

than that of Myrtle mouth rinse (figs 1 and 2). This effect may be either attributed to antimicrobial effect reported for *Z. multiflora* (21) or alteration of microbial flora of the mouth, which resulted in less immunologic damage as reported for the effect of some antibacterial mouth rinses against RAS (2,8). The *A. nobilis* extract which had been reported to have some antiphlogestic and antibacterial effects (13) did not present good results as a single preparation (group B) in this study. Furthermore, the effect of the combination of *A. nobilis* with *Z. multiflora* was not better than of *Z. multiflora* alone. Therefore, it seems that *A. nobilis* might have only a smooth effect on the management of RAS and combination of this plant with *Z. multiflora* did not present any probable synergism reported for some other

combinations (23). It may be concluded that azulene compounds may have little effect in the treatment of RAS in the applied doses. The effect of the *M. communis* (Myrtle) on RAS reported previously (20) was confirmed in this study. In conclusion, the results of this study suggest that topical application of hydroalcoholic extract of *Z. multiflora* may present an effective treatment for minor aphthae. Further studies are required to elucidate the probable mode of action of this plant and standardization of the product.

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