Original Article

Effectiveness of Garlic (Allium Sativum) Tablets on Minor Recurrent Aphthous Stomatitis: Unblinded Randomized Controlled Trial

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ABSTRACT

Background: Oral aphthous lesion is a painful and prevalent ulcerative condition of oral mucosa, causing inflammation and pain. Allicin, a bioactive compound derived from garlic (*Allium sativum*), has potential therapeutic applications.

Purpose: The present study aimed to evaluate the efficacy of allicin in the management of minor recurrent aphthous stomatitis.

Materials and Method: In this unblinded randomized clinical trial study, 60 patients divided in two groups were enrolled in the study based on the inclusion criteria, receiving oral garlic tablets (n=30), and placebo (n=30). Patients took garlic or placebo tablets (containing 1.1 mg of allicin) four times per day. Patients reported their pain intensity using a visual numerical scale during follow-up sessions. An oral medicine specialist examined all lesions before any intervention to confirm minor aphthous lesions that were recurrent and limited to the oral mucosa in patients who did not have any other symptoms of disease. Lesion diameter was measured by the examiner. Independent t-tests, Mann-Whitney, and chi-square tests were used for data analysis. Analysis was performed using SPSS 22 software, and the significance level was considered less than 0.05.

Results: The mean pain intensity on the third day was greater in the control group (*p* Value= 0.045), and no significant difference was observed between the two groups, on other days. The mean lesion diameter was significantly different on days 3 and 5 which was greater in the control group (p= 0.033 and p= 0.046, respectively). However, the results of the generalized estimating equations (GEE) analysis did not show a statistically significant difference in lesion diameter and pain intensity.

Conclusion: Allicin, at the dosage and formulation evaluated, did not significantly improve pain intensity or reduce lesion diameter compared with placebo in patients with minor recurrent aphthous stomatitis.

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Introduction

Recurrent aphthous stomatitis (RAS) is the most prevalent ulcerative condition of the human oral mucosa [1]. This condition commonly occurs in healthy subjects and causes unusual clinical manifestations in immunocompromised subjects [2]. Based on their manifestations,

aphthous lesions are clinically categorized into three groups including minor, major, and herpetic form. The most common category, involving more than 80% of lesions, is minor RAS. These lesions are superficial, smaller than 5mm, round or oval in shape with necrotic base and erythematous border, the lesions may be single or multiple. The other two lesions involve more severe manifestations, including larger size and more extended healing period [3-4].

The exact etiology of these lesions is unspecified, however due to their inflammatory nature, factors influencing immune system such as stress, genetics, food allergy, vitamin deficiency, mechanical traumas, and immunological dysfunctions are responsible for their occurrence [5-6]. The etiological factors impair oxidants and antioxidants balance in the body and result in production of free radicals [5]. Produced free radicals lead to cellular damage [7]. A previous systematic review by Estornut *et al.* [8] demonstrated the significant role of oxidative stress in developing aphthous lesions. They observed higher levels of oxidative stress in patients suffering from RAS.

Since the etiology of RAS is indefinite, only symptomatic therapies are available [9]. Furthermore, various topical and systemic treatments, including analgesics, anesthetics, anti-inflammatory agents, and steroids are administered [10]. Recently, the focus of dental researches on medicinal properties and applications of herbal extracts and their components has considerably increased. Allicin has been investigated for its potential role in promoting the healing of aphthous ulcers and its antibacterial activity against cariogenic bacteria. [5, 11-13]. Garlic (*Allium sativum*) has antibacterial, antioxidant, and anti-inflammatory properties [14].

The beneficial effect of garlic on health could be associated with phytochemicals generated during the extraction process, including aliin, methiin, and S-allyl-L-cysteine. Enzymes in garlic convert aliin into allicin, which has antimicrobial action against oral pathogens and confines promise to cure periodontal diseases, dental caries, and oral cancers [15]. Garlic extract exerts antioxidant properties by scavenging reactive oxygen species and enhancing cellular antioxidant enzymes, including superoxide dismutase, catalase, and glutathione peroxidase. Garlic is an essential source of antioxidants due to phytochemicals such as diallyl sulfide and Sallylmercaptocysteine [16].

Garlic components decrease the expression of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL) 1β, IL6, monocyte chemoattractant protein-1 (MCP-1), and IL-12; Thus, garlic is known to have anti-inflammatory properties [17].

In a systematic review, Shooriabi *et al.* [18] demonstrated a significant therapeutic effect of garlic on oral diseases. Lauritano *et al.* [19] conducted a study investigating the effectiveness of allicin mucoadhesive tablets on aphthous lesions and found a significant reduction in pain intensity and lesion diameter of the lesions. Novianty *et al.* [20] investigated the efficacy of topical allicin on re-epithelialization of oral wounds caused by acetic acid in rats. According to their histopathologic results, allicin advances the wound healing process.

As mentioned, garlic and its bioactive compound, allicin, possess anti-inflammatory and antioxidant properties. Considering its lower price and more accessibility compared to the investigated substances in other studies, and the fact that no other study has ever evaluated the effectiveness of systematically administering garlic powder containing allicin on aphthous lesions, this study was designed to investigate the impact of tablets made of this substance on patients suffering from RAS.

Materials and Method

The present study received approval from the Medical Ethics Committee of Mazandaran University of Medical Sciences (IR.MAZUMS.REC.1403.372). Furthermore, it received approval from the Iranian Registry of Clinical Trials (IRCT20241208063988N1). Before any intervention, all information about the study design was sufficiently explained to participants, and a written consent form was obtained.

Participants and inclusion criteria

The sample size was calculated according to the findings of Xiao-Weng Jiang *et al.* [19] and was estimated to be 60 participants (30 in each of the groups) and was estimated to be 60 participants (30 in each of the groups). A total of 60 participants were selected according to the inclusion criteria and using a convenient sampling method of those who suffered from RAS and referred to the dental clinic of Mazandaran University of Medical Sciences. A random blocking method was used to divide participants into two groups. For this purpose,

numbers from 1 to 60 were assigned to participants. Participants were individually randomized using block randomization with a block size of two. Grouping was performed using random allocation software. Codes A and B were generated using the software and the codes were placed in a sealed envelope and the participant's number was written on it. All packages were identical in appearance, and a head nurse who was not involved in the study distributed the envelopes containing either the active medication or the placebo to the respective participants. The intervention took 7 days.

Participants were chosen based on the inclusion criteria and from those who had previously referred to the Mazandaran Dental School complaining from a single RAS lesion in lips and buccal mucosa. Patients were then examined by an experienced oral medicine specialist to confirm RAS lesions before enrolment. RAS is a disease manifested by the development of recurrent oral ulcers limited to the oral mucosa in patients without any other symptoms of disease. RAS was diagnosed by ruling out other diseases, such as hematologic disorders, immune disorders, and connective tissue diseases that form aphthous-like oral lesion [3]. The inclusion criteria of the present study were 18-50 years adults (men and women) who could follow instructions, patients were willing to enrol in the study and committed not to take analgesics during the study, all patients manifested aphthous lesions maximum 24 hours before referral [19, 21]. The selected lesions were located on the lips and buccal mucosa to have greater accessibility to measure their lesion diameter during follow-up sessions [22]. Exclusion criteria included allergy to garlic or allicin, hemorrhagic disorder [23], patients in their pregnancy or lactation period, patients with systemic diseases, such as hypertension and those forming aphthous-like lesions such as Crohn's disease, ulcerative colitis, Behçet's syndrome, or acquired immunodeficiency; consuming narcotics, non-steroidal anti-inflammatory drugs, immunomodulating agents, and administration of antibiotics (within 2 weeks before the study). Furthermore, patients taking any topical or oral medication within previous month, or those who had recently undergone dental procedures (within 2 weeks), and those using toothpastes with anti-inflammatory properties were excluded [19]. In addition, patients manifesting oral lesions caused by specific foods or showing gastrointestinal symptoms due to food intolerance and patients with traumatic oral lesions were excluded. Patients taking warfarin, statins, saquinavir, isoniazid, and cyclosporine were excluded from the study due to possible drug interactions with garlic [24].

Study protocol

In this study, blinding was impossible due to the strong scent of garlic. The patients were informed to refer to the dental clinic within 24 hours after the manifestation of the aphthous lesions. Their first referral was considered as the baseline (day 0). During this session the study process was explained to the participants and sufficient explanations about possible side effects were given. After patients were fully assured about the study's safety, a written consent form was obtained. A questionnaire including information about the medical and dental history of the participants was completed by them.

The intervention group received the garlic tablet 400mg (Dineh, Iran) containing 1100 µg of allicin and the control group received placebo; tablets were administered systemically. Previous studies administered 600-900 mg garlic powder daily, providing 3.6-5.4 mg of allicin (the active ingredient of garlic). Thus, in the present study the daily consumption of 4 tablets (4.4 mg daily) every 6 hours for 7 days was considered [25-26]. Garlic tablets used in the present study, are commercially available. The same procedure was conducted for the control group.

To evaluate the lesion diameter changes during the study, measurement was performed on days 0 (baseline), 3, 5, and 7 using a digital caliper by a calibrated examiner. Patients were also trained to measure their pain intensity according to the visual numerical scale (VNS) which is a numerical scale. VNS scale exhibits numbers from 0 to 10; in this scale, zero (0) means no pain and 10 means maximum pain [27-28]. Participants indicated the point that best signified their pain and used this numerical scale to measure their pain. VNS was measured 3 times daily after each meal. Patients with pain score of 1 and a lesion diameter smaller than 1mm were considered healed [3]. In case of any allergic reactions, including dyspnea, dermatitis, and itching, patients were asked to discontinue using the medication and refer to the closest health care center. Patients with a pain score of 1 and a lesion diameter less than 1mm

were considered recovered [3, 5, 29].

Developing the placebo

To develop placebo tablets, initially a uniform mixture was prepared containing various additives, including D-Mannitol (32.5% w/w) as a filler, Microcrystalline Cellulose PH-102 (Avicel®) (60% w/w) as the binder and filler, corn starch (5%w/w) as a disintegrant, Magnesium stearate (2% w/w) as a lubricant, and aerosil (0.5% w/w) as glidant. Following assessment and approving the produced powder's flowability, direct pressure was applied using an eccentric press device to develop tablets.

Data analysis

Data were reported using descriptive indices, including frequency, mean, percentage, and standard deviation. Kolmogorov-Smirnov and Shapiro-Wilk tests were used to examine the hypothesis of normality of the data. Independent t-test, Mann-Whitney, Friedman, generalized estimating equations (GEE) and Chi-square tests were used to assess the study's objectives. Data analysis was performed using SPSS22 software and a significance level of less than 0.05 was considered.

Results

A total of 60 participants (30 in each group), completed the study. In the present study, participants did not report any side effects, and all participants completed the study (Figure 1).

The mean age of participants was 29.82±6.41; 28.40±6.14 and 31.23±6.42 in the intervention and control groups, respectively. T-test results did not show any significant difference between the mean age of the two groups (*p* Value=0.087). Moreover, 51.7% of participants were female, and the Chi-square test showed no significant difference between participants' gender in the intervention group and the control group (p= 0.438). Table 1 reports the mean pain intensity of participants on measurement days; both groups followed a de- creasing trend. Comparing the mean pain intensity showed a significant difference only on the 3rd day of the study (p= 0.045). On the 3rd day of the study, the mean pain intensity of the control group was greater than the intervention group (Table 1). According to the Friedman test, the changes in pain scores in both groups across the study days were statistically significant. After adjusting for all variables, the GEE model indicated that the pain score was 0.38 units higher in the control group; however, this difference was not statistically significant (p= 0.154).

Table 2 shows the mean lesion diameter of both groups on measurement days, and a decreasing trend is observed in both groups. Comparing the mean lesion

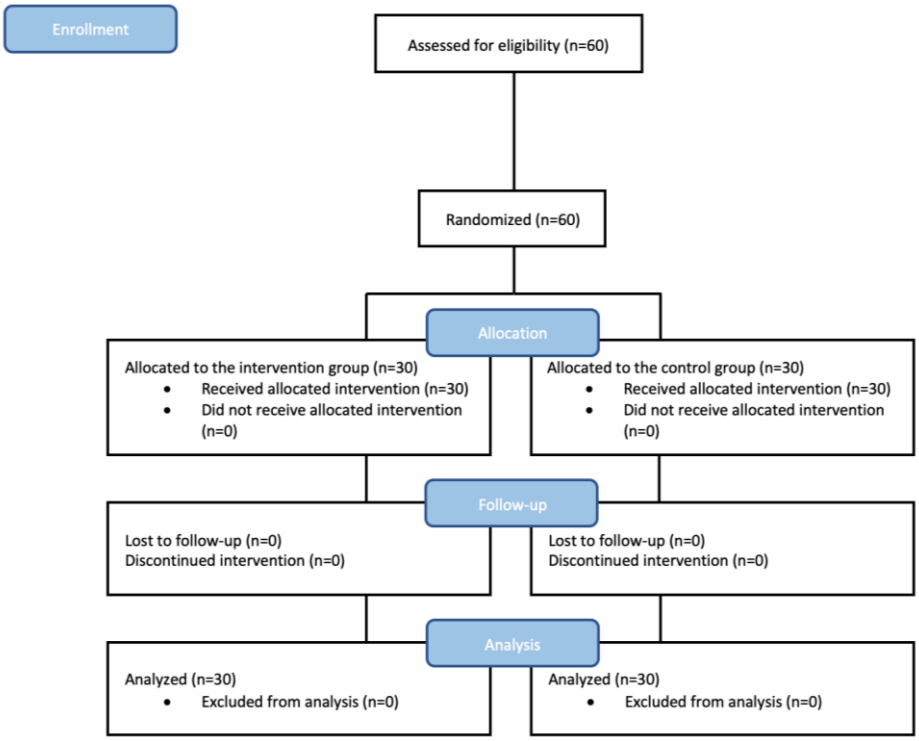


Figure 1: CONSORT flowchart

Table 1: Comparison of the mean pain intensity of groups on measurement days

Day	Intervention group	Control group	*p* Value+
0	7.23±1.55	7.40±1.67	0.695
3	4.57±1.17	5.27±1.57	0.045
5	0.47±0.68	0.90±0.96	0.069
7	0.10±0.31	0.27±0.58	0.252

+:Mann-Whitney test

diameter of the intervention and control groups on measurement days, showed a significant difference on days 3 and 5 of the study, and the mean lesion diameter was greater in the control group than the intervention group (Table 2). The changes in lesion diameter in both groups over the study days were statistically significant based on the Friedman test. After controlling for all variables, the GEE analysis showed that the lesion diameter in the control group was 0.19 units greater; however, this difference was not statistically significant (p= 0.274).

According to the independent t-test result, the healing period was shorter in the intervention group for approximately 1 day, but the difference was insignificant. However, the calculated (p= 0.056) was not statistically significant. Further studies with larger sample sizes are warranted to confirm this finding.

Table 3 indicates the mean pain intensity of each gender on the measurement days. The comparison showed a significant difference on the 3rd day. On the 3rd day, the mean pain intensity of men in the intervention group was significantly lower than that of the control group (Table 3).

Table 4 demonstrates the mean lesion diameter of each gender on the measurement days and compared the results by the Mann-Whitney test. No significant difference was observed between the mean lesion diameter of each gender (men and women) in both groups on measurement days.

According to the independent t-test result, in both genders (men (p= 0.470), and women (0.065)), the mean healing period was not significant (Table 4).

Table 2: Comparison of the mean lesion diameter of groups on measurement days

Day	Intervention group	Control group	*p* Value+
0	3.45±1.01	3.35±0.92	0.346
3	2.03±0.73	2.55±1.00	0.033
5	0.55±0.58	0.95±0.84	0.046
7	0.08±0.19	0.13±0.29	0.660

+:Mann-Whitney test

Table 3: Mean pain intensity of men and women in groups on measurement days

Gender	Day	Intervention group	Control group	*p* Value+
Women	0	7.12±1.69	7.50±1.87	0.194
	3	4.41±1.12	5.36±1.69	0.385
	5	0.35±0.61	1.00±1.04	0.222
	7	0.06±0.24	0.29±0.61	0.437
Men	0	7.38±1.39	7.31±1.54	0.982
	3	4.77±1.24	5.19±1.52	0.032
	5	0.62±0.77	0.81±0.91	0.135
	7	0.15±0.38	0.25±0.58	0.852

+:Mann-Whitney test

Discussion

The present study aimed to evaluate the efficacy of allicin, the primary bioactive compound derived from garlic (*Allium sativum*) in the management of minor RAS.

The primary analysis, conducted using GEE to account for the correlated nature of repeated measurements over time, revealed no statistically significant difference between the allicin and placebo groups in terms of pain reduction or lesion diameter. This finding indicates that, when considering the overall treatment trajectory across all time points, allicin did not demonstrate a superior therapeutic effect compared to placebo. Although individual day-by-day comparisons using the Mann–Whitney U test suggested potential differences at certain time points, these results must be interpreted with caution. Clinical evidence indicates that allicin, improves acute symptoms of minor RAS by diminishing ulcer size and pain. While conventional aphthous stomatitis therapies rely on corticosteroids or anti-inflammatory agents that present adverse effects under prolonged administration [30] preliminary trials demonstrate that it does not decrease the long-term recurrence rate of lesions [31-32]. In a double-blind, randomized,

Table 4: Mean lesion diameter of men and women in groups on measurement days

Gender	Day	Intervention group	Control group	*p* Value+
Women	0	3.53±0.96	3.21±0.87	0.570
	3	2.15±0.81	2.43±0.92	0.064
	5	0.50±0.59	0.86±0.84	0.061
	7	0.06±0.17	0.14±0.31	0.195
Men	0	3.35±1.11	3.47±0.97	0.928
	3	1.88±0.62	2.66±1.09	0.413
	5	0.62±0.58	1.03±0.87	0.583
	7	0.12±0.22	0.13±0.29	0.764

+:Mann-Whitney test

placebo-controlled clinical trial involving 96 participants with minor aphthous ulcerations, the application of 5 mg allicin adhesive tablets four times daily for 5 days achieved statistically significant reductions in both lesion dimensions and subjective pain scores on days 2, 4, and 6 compared to vehicle controls [31]. The application of multiple statistical tests across various days increases the probability of Type I errors, and the apparent significance observed in isolated comparisons may be attributable to chance rather than a genuine treatment effect. The GEE analysis, which provides a more robust and comprehensive assessment by incorporating all repeated measurements simultaneously, serves as the definitive analytical framework for this study. Accordingly, the absence of a statistically significant overall effect in the GEE model takes precedence over isolated significant findings from individual time-point comparisons. The lack of a significant therapeutic effect observed in this study may be explained by several factors. First, the self-limiting nature of minor RAS lesions, which typically resolve within 7–14 days, may have reduced the window of opportunity for detecting a measurable treatment benefit. Second, the relatively small sample size may have limited the statistical power to detect modest differences between groups. Third, the dosage and formulation of allicin used in this study may not have been optimal for achieving therapeutic concentrations at the site of the lesion. It is important to acknowledge the limitations of this study. The investigation was restricted to minor RAS lesions, which inherently limits the generalizability of the findings to major or herpetiform variants of the disease. These more severe forms may respond differently to therapeutic interventions and warrant separate investigation. Additionally, the relatively short follow-up period and the single-center design may further constrain the applicability of these results to broader clinical settings.

Conclusion

The findings of this study demonstrate that allicin, when administered at the studied dosage and formulation, did not produce a statistically significant improvement in pain intensity or lesion diameter compared to placebo in patients with minor RAS. The overall analysis using GEE, which appropriately accounts for repeated measurements, did not reveal a significant treatment effect. Future research should consider larger sample sizes, optimized dosing regimens, and inclusion of different clinical variants of aphthous stomatitis to evaluate the potential therapeutic role of allicin more comprehensively.

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Declaration of AI

The authors used ChatGPT version 5.3 (OpenAI, San Francisco, USA) to improve clarity and paraphrase some sentences. All conceptual content, data analysis, and interpretation were conducted solely by the authors.

Conflict of Interest

The authors declare that they have no conflict of interests.

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