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Evaluation of The Topical Effect of Calendula Officinalis Oral Paste in The Treatment of Recurrent Aphthous Stomatitis: A Randomized Double-Blind Controlled Trial

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ABSTRACT

Background and Objectives: Recurrent aphthous stomatitis is the most common recurrent ulceration in the oral mucosa. The anti-inflammatory and wound-healing properties of Calendula officinalis make it a medicinal plant. This study aims to evaluate Calendula Officinalis 10% and 15% oral pastes compared with triamcinolone acetonide 0.1% and placebo oral pastes, and to evaluate salivary Irisin as an inflammatory marker before and after treatment.

Materials and Methods: A randomised, double-anonymised case-control clinical study included 60 patients with minor recurrent aphthous stomatitis (age range: 16-50 years. Patients used (Calendula officinalis in 10 % and 15 %, triamcinolone acetonide 0.1 % and placebo) oral pastes for one week. Clinical observation for (ulcer size, pain and erythema) was done on days (0, 4 and 7). Salivary irisin level was measured before and after treatment.

Results: Significant differences were noted by topical treatment with 10% and 15% calendula officinalis and triamcinolone acetonide 0.1% oral pastes at the 4th and 7th day in reducing ulcer size, erythema and pain ($P<0.001$). The most significant reduction was achieved with 15% Calendula officinalis on the 4th day. A significant difference in salivary irisin levels was observed before and after treatment with all four group pastes ($P<0.001$).

Conclusion: Calendula officinalis 15% oral paste showed a significant effect on reducing ulcer size, pain, and erythema in Recurrent Aphthous Stomatitis patients over 4 days.

Keywords: Recurrent Aphthous Stomatitis, Calendula Officinalis, Inflammation, Irisin

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INTRODUCTION

The most prevalent oral mucosal disease, recurrent aphthous stomatitis (RAS), affects 5-25% of the general population.[1] RAS is characterised by recurrent, painful ulcers that are usually limited to the non-keratinised oral mucosa. Minor, major, and herpetiform are the three clinical forms of RAS. The minor variety of RAS accounts for around 85% of all cases and is the most prevalent type. Clinically, it manifests as a grey-white pseudomembrane with an erythematous halo and a painful, round or oval ulcer measuring less than 1 cm.[2,3]

The lesions typically resolve on their own within 7–10 days, leaving no scars. Numerous risk factors, including genetic predisposition, hematologic problems, immunologic defects, vitamin deficiencies, mental stress, and local trauma, have been suggested despite the condition's prevalence and lack of a known aetiology. It has been shown that immunological problems are linked to the pathophysiology of RAS. RAS ulcers may form as a result of multifunctional cytokines that mediate the immune response and are implicated in inflammation.[4,5,6]

Due to its unclear aetiology, RAS is difficult to treat, and there is not currently a recognised cure for the illness. The primary goals of RAS therapy are to reduce pain, accelerate healing, and decrease recurrence frequency. Many therapies have been proposed to achieve the goals, such as topical corticosteroids, antiseptic mouthwash, analgesics, anesthetics, antibiotics, and herbal medicines.[7,8]

There has been an increase in the use of herbal remedies to treat and prevent oral disorders. Because they are organic and non-synthetic, herbal extracts have attracted significant interest worldwide.[9] Asteraceae family member Calendula Officinalis (C. officinalis) is a therapeutic plant native to the Mediterranean region but now grown worldwide. It is known in English as marigold and pot marigold. Carotenoids, flavonoids, saponins, sterols, phenolic acids, lipids, and other biologically active components are found in Calendula officinalis. Historically, C. officinalis has been used to treat stomach ulcers, gastrointestinal cramps, peptic and intestinal mucosa cramps, inflammation of the mucous membranes, diaphoresis, analgesia, and as an antiseptic.[10,11] Irisin belongs to the family of adipo-myokines, released mainly by muscle and has anti-inflammatory properties. Irisin's anti-inflammatory function as part of a compensatory mechanism to reduce ongoing inflammation in RAS can explain the observed high irisin levels.[12,13] This study aims to compare the efficacy of 10% and 15% C. officinalis topical oral paste (new concentration and formulation) with 0.1% Triamcinolone Acetonide (TA) and placebo pastes in patients with RAS. To investigate the anti-inflammatory effect of Calendula officinalis oral paste in treating RAS disease by evaluating the level of salivary inflammatory marker (Irisin).

METHODS

This study was carried out at Hawler Medical University/College of Dentistry in Erbil City, Iraq, from January to September 2022. All participants were of Kurdish ethnicity and residents of Erbil Governorate/Iraq. The study protocol was submitted to the ethics committee at Hawler Medical University/ College of Dentistry. It was a randomized, double-blind clinical study (60 patients with minor RAS were enrolled; the age range was 16-50 years). Inclusion criteria: 1. Patients suffering from minor recurrent aphthous stomatitis (round or oval in shape). 2. Males and females aged 16-50 years old. Exclusion Criteria: 1. Patients who were pregnant and lactating women, or who had a history of allergy to the family of Asteraceae. 2. Treatment of ulcers with systemic steroids, vitamins, antibiotics, antihistamines, oral retinoids or immunomodulatory agents within three months before study entry and use of nonsteroidal anti-inflammatory drugs (NSAIDs) or mouthwash for ulcer treatment for at least 72 hours before study entry. Patients have been using nicorandil or have been under treatment with immunosuppressive and chemotherapy during the past year. 3. History of systemic diseases such as autoimmune blistering diseases, Crohn's disease, Behcet's disease, lupus, lichen planus, diabetes, PFAPA syndrome and HIV infection. 4. Patients have gingivitis or periodontitis.

RAS patients were divided into four groups to use four types of oral pastes (10% C. officinalis, 15% C. officinalis, 0.1% T.A. and placebo). They were given medications for seven days (day 0; start of drugs), then they were followed up on days 4 and 7. Clinical examination was carried out by ob-

serving the type (Minor), site (RAS ulcer in an easily accessible area), number (only 1 RAS in the mouth) and duration (less than 48 hours). The parameters, such as size (mm), pain (VAS score), and erythema (erythema score), were evaluated on days 0, 4, and 7. To determine the size of the ulcers, a calibrated William's periodontal probe with millimetre markings was used to measure the ulcers at their maximum diameter.[14] To measure pain and burning sensation, the Visual Analogue Scale (VAS), consisting of a 10 cm line containing equidistant subdivisions with the following associated notations: no pain, mild pain, discomforting pain, distressing pain, horrible pain and excruciating pain, was used. The investigators evaluated the level of erythema and exudation on a four-point scale ranging from 0 to 3: 0: No erythema; 1: Light red or pink; 2: Red but not dark; 3: Very red (dark). To determine the effectiveness of the treatment under study. EI was completed on days S4 and S7 to determine the extent of the ulcer size and the level of pain. The size and pain of RAS on day 0 are represented by S0 (baseline). EI was calculated using the following formula: EI = ((S0-S4 or S7) /S0) 100%. On a scale of 1 to 4, the EI was calculated as follows: 1: in which the RAS is fully recovered (EI ≥95%), 2: in which there is a noticeable improvement (EI < 95%) but EI ≥ 70%), 3: in which there is a moderate improvement (EI < 70% but EI ≥ 30%) and 4: in which there is no improvement (EI < 30%).[15]
Baseline lab tests, including salivary irisin, were performed for all RAS patients before and after medication use to evaluate and compare their anti -inflammatory effects.

Double Blind Technique

For the blinding method, all RAS patients were assigned to the study group (C. officinalis at 10% and 15%), the comparison group (Triamcinolone acetonide 0.1%), or the placebo-controlled group. The oral pastes (C. officinalis in 10% and 15 %, Triamcinolone acetonide 0.1% and placebo) were loaded in identical-looking white colored collapsible aluminium tubes. They were labelled with codes (A, B, C and D). A single investigator at Hawler Medical University/College of Dentistry, who was blind to the study protocol and subjects, randomly labelled these tubes and medications. They were then delivered to the clinical investigator who was blind to the treatment agents. The codes were kept until the end of the study's practical part. All participants in the study, including patients, staff, and the investigator, were blinded to the type of oral pastes used.

Preparation of Calendula Officinalis and Placebo Oral Pastes

A. Preparation of Calendula Officinalis Ethanol Extract Powder

The leaves of C. officinalis were initially gathered, cleaned with water, shade-dried at room temperature, then homogenised into a fine powder and stored. Additionally, taxonomic identification was carried out at the herbarium of the Department of Biology/ College of Science/ Salahaddin University-Erbil. The plant species named in the plant samples were then kept in the herbarium of the biology department. Ten grams of plant powder and 100 ml of the solvent (ethanol) were added to the Erlenmeyer flasks for the extraction and analysis. The plant-to-solvent (ethanol) ratio was 1:10. Microwave-assisted extractions were performed using the Microwave Milestone NEOS system, which is equipped with containers and an automatic temperature control system. This analysis procedure was carried out using a microwave for 60 minutes, up to the boiling point. Finally, the mixture was filtered and evaporated using a rotary evaporator to get pure calendula officinalis ethanolic extract.[16]

B. Preparation of Calendula Officinalis oral paste

The oral paste was prepared at Hawler Medical University/ College of Pharmacy. To prepare oral paste, powdered materials comprising (C. officinalis ethanolic extract, carboxymethylcellulose, pectin, methylparaben, gelatine powder and propylparaben) were mixed with beeswax and mineral oil. Beeswax was first melted in a water bath, then a measured amount of mineral oil was added, and the mixture was gently blended to achieve homogeneity. The powdered components were then carefully weighed using a delicate balancing scale and combined thoroughly using a mortar and pestle. At the same time, the beeswax and mineral oil mixture remained submerged in the water bath and was continued to be mixed until the paste was homogenised. Finally, sterile collapsible aluminium tubes containing the prepared mixture were filled and kept at room temperature until used, a few days after preparation.[17]

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ference compared with 10% C. officinalis, nor between 10% C. officinalis and 0.1% T.A (P>0.05). At the same time, there were significant differences between all other groups (P < 0.01; Table 2.

Table 1. Multiple comparisons for ulcer size (mm) between four group medications in RAS patients at fourth- and seventh-day assessment times

Day	Drug type	Drug type	Mean± SE	P value
Day 4	G1	G2	-1.02± 0.39	0.070
		G3	-1.354*± 0.30	0.009
		G4	-2.409*± 0.41	0.000
	G2	G3	-0.333± 0.41	0.856
		G4	-1.389*± 0.43	0.015
	G3	G4	-1.056± 0.43	0.088
Day 7	G1	G2	-0.788± 0.40	0.234
		G3	-1.232*± 0.40	0.024
		G4	-2.580*± 0.42	0.000
	G2	G3	-0.444± 0.42	.728
		G4	-1.792*± 0.44	0.002
	G3	G4	-1.347*± 0.44	0.022

G1: C. officinalis 15%, G2: C. officinalis 10%, G3: Triamcinolone Acetonide 0.1%, G4: Placebo, SE: Standard Error; post- Hoc test used
**P value<0.001: Highly Significant, P value >0.05: Non-significant

Table 2. Multiple comparisons for remarked changes in pain (VAS score) between four group medications in RAS patients at fourth- and seventh-**day** estimation times

Day	Drug type	Drug type	Mean± SE	P value
Day 4	G1	G2	-1.919*± 0.47	0.002
		G3	-1.919*± 0.47	0.002
		G4	-3.114*± 0.48	0.000
	G2	G3	0.000± 0.49	1.000
		G4	-1.194± 0.51	0.110
	G3	G4	-1.194± 0.51	0.110
Day 7	G1	G2	-1.000± 0.38	0.060
		G3	-1.111*± 0.38	0.031
		G4	-2.875*± 0.39	0.000
	G2	G3	-.111± 0.4	0.992
		G4	-1.875*± 0.41	0.000
	G3	G4	-1.764*± 0.41	0.001

G1: C. officinalis 15%, G2: C. officinalis 10%, G3: Triamcinolone Acetonide 0.1%, G4: Placebo, SE: Standard Error; post-Hoc test used;
**P value<0.001: Highly Significant, P value >0.05: Non-significant.

Preparation of a placebo oral paste

Placebo oral paste was prepared using the same method and ingredients as calendula officinalis oral paste, but without the active constituent (C. officinalis). The prepared placebo paste was also packaged in the same aluminium tubes as C. officinalis, oral paste.

Application of Calendula Officinalis (10 and 15%), Triamcinolone Acetonide 0.1 % and placebo oral pastes to Recurrent Aphthous Stomatitis ulcers

Patients who were examined as having RAS less than 48 hours were given detailed instructions on how to use the oral pastes correctly. All RAS patients were instructed to rinse their mouth with tap water before the administration of the medications (10 and 15 % C. officinalis, 0.1% T.A. and placebo) and apply a small amount of paste with the tip of a clean finger to the ulcer site three times per day (after meals) for 7 days (day 0 to day 7). They were advised not to eat or drink anything for 30 minutes after applying the pastes. Participants were requested to attend the clinic (follow-up) in the morning (9-11 am) on days 4 and 7 for evaluation of ulcer size (in mm), pain (VAS score), erythema (erythema score), and EI. They were strictly warned not to use any other products for RAS treatment during this study. At the end of therapy, all patients were asked to report any adverse effects of the medications, and their oral mucosa was examined for any abnormal changes or allergic reactions. The patients were advised to inform the researcher of any oral side effects, such as hypersensitivity, loss of taste, and a burning sensation. Patients should not use C. officinalis if they are allergic to ragweed, daisies, marigolds, or plants in the Asteraceae/Composite family.

Handling and Storing Saliva Samples

Unstimulated whole saliva was taken from all participants. For RAS patients, it was collected before and after treatment (at day 0 and day 7) in all four groups, and for the H.C. group, only at the first visit. Unstimulated saliva samples were collected in the morning between 9 and 11 A.M. by spitting. All participants were asked not to eat, drink, or brush their teeth for 90 minutes before the first sampling. The participants rinsed their mouths using distilled water. Then, they spit into the plastic tubes once or twice per minute for 10 minutes. The samples were centrifuged at 2000g for 10 min at 4°C; the upper layer was removed and stored in small aliquots at -80°C for further analysis.[18] Quantitative determination of salivary irisin using enzyme-linked immunosorbent assay (ELISA) was performed in RAS patients before and after treatment.

The Statistical Package for the Social Sciences (SPSS) version 23.0 was used to analyse the collected data. For multiple comparisons, A One-Way Analysis of Variance (ANOVA) was used. Two continuous variables were compared using the Student's test. The linear relationship between each pair of variables was examined using the Pearson correlation coefficient test. Results were deemed significant when the P value was < 0.05. A post hoc test was used for multiple comparisons.

RESULTS

Multiple Comparisons for Ulcer Size (mm) between Four Group Medications in RAS Patients at fourth- and seventh-day Assessment times

Table 1 shows multiple comparisons between four group oral pastes. On the fourth-day follow-up, there were statistically significant differences in the mean RAS sizes between the 15% C. officinalis, 0.1% T.A., and placebo groups ($P < 0.001$). In comparison, no significant difference was observed between 15% and 10% C. officinalis ($P > 0.05$). Pairwise comparisons revealed no statistically significant differences in the mean ulcer size on the seventh day between 15% and 10% C. officinalis, also between 10% C. officinalis and the T.A groups ($P>0.05$). In comparison, there was a statistically significant difference between 15% C. officinalis with 0.1% T.A and placebo groups ($P <0.001$), 10% C. officinalis with placebo group ($P <0.001$), and 0.1% T.A with placebo oral paste ($P <0.001$).

Multiple comparisons for remarked changes in pain (VAS score) between four group medications in RAS patients at fourth- and seventh-day estimation times

Pairwise comparisons revealed that the mean reduction in pain perception on the fourth day was statistically significant in the 15% C. officinalis group compared with the other three groups ($P < 0.01$). In comparison, there was no statistically significant difference between 10% C. officinalis and 0.1% T.A, or between 0.1% T.A and placebo groups ($P>0.05$). On the seventh day, follow-up 15% C. officinalis did not show a significant dif-

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Multiple comparisons for remarked changes in erythema score between four group medications in RAS patients at fourth- and seventh-day estimation times

Pairwise comparison between four group medications at fourth- and seventh-day assessment revealed a statistically significant difference between 15% C. officinalis with placebo, 10% C. officinalis and 0.1% T.A with placebo (P<0.001**); however, there was no statistically significant difference between 15% C. officinalis with 10% C. officinalis and 0.1% T.A (P>0.05), as shown in Table 3.

Table 3. Multiple comparisons for remarked changes in erythema score between four group medications in RAS patients at fourth- and seventh-day estimation times

Day	Drug type	Drug type	Mean ± SE	P value
Day 4	G1	G2	-0.162± 0.25	0.922
		G3	-0.384± 0.25	0.455
		G4	-1.148*± 0.26	0.001
	G2	G3	-0.222± 0.27	0.843
		G4	-0.986*± 0.27	0.006
	G3	G4	-0.764*± 0.27	0.046
Day 7	G1	G2	-0.242± 0.20	0.656
		G3	-0.465± 0.20	0.138
		G4	-1.409*± 0.21	0.000
	G2	G3	-0.222± 0.21	0.742
		G4	-1.167*± 0.22	0.000
	G3	G4	-0.944*± 0.22	0.001

G1: C. officinalis 15%, G2: C. officinalis 10%, G3: Triamcinolone Acetonide 0.1%, G4: Placebo; SE: Standard Error; post-Hoc test used;
**P value<0.001: Highly Significant, P value >0.05: Non-significant.

Comparison of the Effective Index (EI) of ulcer size among the Recurrent Aphthous Stomatitis groups

Regarding the healing effect of topical treatment by C. officinalis in 15 and 10%, T.A and placebo groups, EI was calculated for size of ulcers in (%), at day 4 the EI showed significant difference between groups (p<0.001**), the EI of 15% C. officinalis oral paste group revealed a marked healing of ulcers compared to 10% C. officinalis, T.A and placebo groups. At day 7, EI of 15% C. officinalis showed a higher healing effect, and a marked improvement was maintained for 10%C. Officinalis and T.A. groups, while the placebo-controlled group revealed moderate healing of RAS. EI at day 7 between groups was statistically significant (p<0.001**), as shown in Table 4.

Comparison of the Effective Index (EI) of pain moderation among the Recurrent Aphthous Stomatitis groups

The effectiveness index for pain with C. officinalis 15% oral paste treatment at day 4 showed a marked improvement compared to the 10% C. officinalis and T.A 0.1% groups, which showed similar moderate healing, while the placebo group didn't show healing. There was a statistically significant difference between groups (P < 0.001**) at the day 4 visit. At day 7 of treatment with topical application of C. officinalis 15% and 10%, there was complete healing of RAS. T.A. 0.1% revealed a marked improvement, while the placebo-controlled group showed only moderate healing. A highly statistically significant difference was observed between the four group oral pastes (P<0.001**), as shown in Table 5.

Table 5. Comparison of Effective Index (EI) of pain moderation among RAS groups

Time	Groups	N	Mean ± SE (%)	P Value
Day 4	15% C. officinalis	15	77.08 ± 6.13	0.0012**
	10% C. officinalis	15	54.03 ± 3.15	
	T.A	15	54.98 ± 1.75	
	Placebo	15	34.76 ± 4.56	
Day 7	15% C. officinalis	15	100 ± 00	0.001**
	10% C. officinalis	15	88.12 ± 4.92	
	0.1% T.A	15	86.58 ± 4.31	
	Placebo	15	60.01 ± 3.80	

C. officinalis: Calendula Officinalis; T.A: Triamcinolone Acetonide, SE: Standard Error, used one-way ANOVA test, **Significant at Level (P<0.001)

Comparison of salivary inflammatory marker Irisin level before and after treatment with oral pates among the Recurrent Aphthous Stomatitis patient groups

As shown in Table 6, comparing the mean Irisin level from baseline to 7 days after ulcer healing showed a statistically significant reduction in the C. officinalis 15% and 10% groups. After healing of RAS with 0.1% T.A oral paste, the mean irisin level reduction was statistically significant compared to day 0 (baseline). In the placebo-controlled group, salivary irisin levels decreased at day 7 after RAS treatment compared with day 0 before treatment, but the reduction was less than in other groups. Although the mean Irisin level was high across all groups before RAS treatment at day 0, a statistically significant difference was observed at day 7 after RAS treatment compared to day 0 (p<0.01**).

Table 6. Comparison of salivary inflammatory marker Irisin before and after treatment among RAS patient's groups

		Before Treatment (Day 0)	After Treatment (Day 7)	P Value
Treatment Groups	N	Mean ± SE (ng/ml)	Mean ± SE (ng/ml)	
15% C. officinalis	15	15.78 ± 2.40	9.36 ± 1.57	0.002**
10% C. officinalis	15	12.83 ± 1.85	6.87 ± 0.76	0.003**
0.1% T.A	15	19.14 ± 3.38	14.69 ± 2.86	0.001**
Placebo	15	17.25 ± 3.14	14.86 ± 3.11	0.02*

C. officinalis: Calendula Officinalis; T.A: Triamcinolone acetonide, SE: Standard Error, used paired t-test, **Significant at Level (P<0.001).

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DISCUSSION

At some point in their lives, up to 7 out of 10 people may experience recurrent aphthous stomatitis. RAS can be divided into three clinical categories: minor, major, and herpetiform. Although the precise aetiology of RAS is uncertain, several possible factors are likely to be involved. Among them are local factors like trauma, hematinic deficits, immunological factors, and psychological stress.[19,20] Additionally, ulcers could exist due to a hereditary susceptibility. Up to 40% of patients have a positive family history. Previous studies have suggested that some gene polymorphisms encoding proinflammatory cytokines, such as ILs, are responsible for the development of RAS. RAS has been treated with a variety of approaches, including topical agents, systemic drugs, physical modalities and natural herbal remedies.[21, 22, 23] The primary outcome parameters observed in this study were ulcer size, pain score, erythema, and healing effect. In the current study, topical administration of C. officinalis 15% oral paste significantly reduced ulcer size and erythema at the 4-day follow-up, and in some patients, full healing was achieved. Following the 7th day of treatment with C. officinalis 15% topical oral paste, the majority of patients had fully recovered, and their ulcers had vanished. The reduction in ulcer size and erythema on the 4th day and 7th day follow-up was also observed by using C. officinalis 10% and T.A 0.1% topical oral pastes, but it was less than that of C. officinalis 15% oral paste. while C. Officinalis 15% showed a much higher reduction than the placebo oral paste. Regarding the pain associated with RAS, most patients who used C. officinalis 15% oral paste reported complete alleviation after only 1 day of application, and the VAS score was significantly reduced on the 4th day of follow-up. They also reported improvements in their eating and speech. Compared with C. officinalis 10% and T.A 0.1%, the effects of all three groups were approximately the same, but C. officinalis 15% showed greater pain reduction, while the placebo oral paste group showed much less pain reduction than the other groups. In the current study, C. officinalis oral paste showed healing of RAS after three days of application and complete healing after seven days. This is in agreement with previous research,[24] which found that C. officinalis tincture at 10% was successful in treating oral infection. By reducing proinflammatory cytokines, C. officinalis has demonstrated strong anti-inflammatory efficacy. In vivo pharmacological testing has revealed that the triterpenoid fatty acid esters in calendula flowers are what cause the anti-inflammatory effects. Other categories of secondary metabolites in the plant that are correlated with its anti-inflammatory characteristics include alkaloids, tannins, flavonoids, essential oils, sterols, saponins, carotenoids, triterpene alcohols, mucilage, polysaccharides, and resin.[25,26] It has been determined that C. officinalis extract is risk-free, non-irritating, non-genotoxic, and non-mutagenic. Patients with compromised immune systems who had frequent and prolonged interactions with Calendula officinalis had allergy symptoms. Since calendula officinalis has the potential to hasten labour, it is essential for pregnant women to exercise caution while using any product containing C. officinalis.[27] In the current study, it was found that using T.A at a concentration of 0.1% was an effective way to treat RAS. This was also found in previous research,[28] where it was noted that 0.1% T.A. was more effective in reducing size, number, pain, erythema, and exudate levels on days 8 and 10, respectively. Triamcinolone acetonide is a synthetic corticosteroid. It can reduce the production of inflammatory mediators, including prostaglandin, neutrophils, eosinophils, monocytes, lymphocytes, and different cytokines (IL-2, IL-6, IFN-γ, and TNF-α) in minor RAS29. Regarding salivary irisin levels in RAS patients, the present study showed that levels before treatment with oral pastes (C. officinalis 10% and 15%, T.A 0.1%, and placebo) were high. However, after treatment with C. officinalis 15% oral paste, a high reduction in salivary irisin levels was observed, and this was greater in patients who used C. officinalis 10% and T.A 0.1% topical medications. After topical application of placebo paste, a decrease in salivary irisin was observed, but the reduction was less than that of the other three groups. The reduction was statistically significant in all four groups after treatment. Irisin has been linked to inflammation and has been researched in RAS patients earlier because RAS is the most prevalent inflammatory disorder of the oral mucosa. In the current study, we demonstrated elevated irisin levels in the saliva of RAS patients, which were higher than in controls without RAS, and, with regard to gender, similar results were observed in both male and female participants. This is consistent with a study by 13, who investigated the

significance of salivary irisin levels in patients with RAS and found that irisin levels were as high as IL-2 and IF-γ, and that there was a significant relationship between irisin and IL-2 and IF-γ. A correlation between salivary and serum irisin levels was observed, and it was found that both obese and normal-weight patients had considerably higher salivary irisin levels than their serum levels.[30]

CONCLUSION

Topical application of C. officinalis 15% oral paste has a significant effect in reducing RAS size, erythema and pain relief, and it has a better impact in healing RAS than triamcinolone acetonide 0.1%. C. officinalis oral paste was easy and acceptable to be used by patients without any allergic reaction or unfavourable odour. Detection of high levels of salivary irisin can be used as a marker of inflammation or inflammation severity in RAS patients.

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CONFLICT OF INTERESTS

The author has no conflict with any step of the article preparation

CONSENT FOR PUBLICATION

The author read and approved the final manuscript for publication.

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ETHICAL APPROVAL AND CONSENT TO PARTICIPATION

All patients gave written and verbal consent before participation.

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