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Research Article

PROPOLIS BUCCAL PASTE IN TREATMENT OF APHTHOUS ULCERATION: FORMULATION AND CLINICAL EVALUATION

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ABSTRACT

Background: Recurrent aphthous ulceration (RAU) is one of the most frequently encountered pathologic conditions in the oral cavity and does not have a well established etiology or treatment.

Methods: In the present study, propolis buccal pastes formulations were prepared and pharmaceutically and clinically evaluated for the treatment of recurrent aphthous stomatitis.

Results: Results indicated that the paste formulas were compliance with the pharmaceutical standard limits, stable at the ambient temperatures and free from any microorganisms during storage. On the other hand, the rate of aphthous ulcer healing was high with both formulas compared to placebo. The size of ulcer decreased during the first day of application of both formulas, being 45%, and 65% of the 1st group patients and 2nd group patients who treated with F1 and F2, respectively in comparison to 0.0% of the placebo group. The pain intensity decreased in patients through the first 24 hours in 90% of the 1st group patients, 97.5% in the 2nd group and 35% in placebo group. Besides, the pain disappeared during the first five minutes of the formulas application and its disappearance persists for more than four hours. The period of drug adherence to the oral mucosa in most patients was about 20-30 minutes.

Conclusion: The olive oil based propolis paste (F2) was significantly ($P<0.05$) more effective than sesame oil based formula (F1) thus it can be considered as a promising bioadhesive buccal formula for the treatment of recurrent aphthous ulceration.

Keywords: Aphthous Ulceration, Propolis Bucal paste.

INTRODUCTION

Recurrent aphthous ulceration (RAU) is a common, painful, and ulcerative disorder of the oral cavity of unknown etiology [1]. The lack of clarity regarding the etiology of aphthous ulcer has resulted in treatments that are largely empiric [2]. Many topical preparations have been employed for the treatment of RAU with varying effectiveness regarding reduction in the number of ulcers occurring, their duration and the frequency of recurrence of episodes of ulceration. Topical agents, such as anti-inflammatories, antimicrobials, immuno-modulative drugs, analgesics and corticosteroids are among topical agents that have been used to manage RAU [3-5].

Propolis is an ancient and important medicinal remedy that has been used in various clinical situations [6-8]. Recent reports of the biological effects of propolis indicated that it has antimycotic, antioxidant [9], and antimicrobial activities [10, 11], which have been utilized clinically, both systemically and topically. The UAE propolis is characterized by the presence of a high content of aliphatic acids (15.2%) and a low content of aromatic acids (4.3%) in addition to the high molecular compounds, as flavonoids, are present to the extent of about 33% [12]. The phenyilic acid found in propolis, for example, has powerful antimicrobial activity while flavonoids present anti-inflammatory and anesthetic actions, among other properties [13].

Propolis has already been employed to manage patients with RAU in forms of topical cream [14], oral capsules [15], alcoholic solution [16] and topical solution in propyleneglycol vehicle [17]. Although such dosage forms presented good results with a reduction in symptomatology and duration of lesions, without any side-effects but they need more frequent application which consequently may result in less patient compliance.

The aim of this study is to develop suitable buccal paste formulations for the treatment of aphthous ulceration using local UAE propolis alcololic extract in paste base to maintain satisfactory therapeutic level of the active ingredient in the mouth for prolonged duration of time. Formulas were assessed through in vitro evaluation of their pharmaceutical properties, stability and biological stability study. Also a clinical comparative study between these formulas had been conducted on different criteria including: duration of pain disappearance, duration of complete ulcer healing, onset of size reduction and duration of drug adherence to mucous membrane after pastes application.

MATERIALS AND METHODS

Propolis was kindly provided by Hajj Seed local farms (Dubai, UAE). Purified beeswax, pectin, carboxymethylcellulose (CMC), methylparaben, propylparaben, gelatin, ethanol (96% w/v) were purchased from Evans Drug Industry (USA). Olive oil and sesame oil were obtained from the local market (Dubai, UAE).

Preparation of buccal propolis paste formulations

The propolis buccal paste formulations were prepared under aseptic conditions. The required weight of dried pectin, CMC, gelatin, methyl paraben, propyl paraben were mixed together to form a homogenous mixture. Beeswax was melted in water bath at 70-80° C. The sesame or olive oil was added to the melted beeswax and continuously stirred with heating for 30 min. After that the homogenous mixture of the dried materials was gradually added to beeswax/oil mixture with continuous stirring and heating. Finally, the alcoholic extract of propolis (1propolis: 3 ethanol) 20% w/w was added to the base gradually with continuous stirring till homogenous propolis paste was attained. The paste was poured into the collapsible tubes, closed properly and stored in dry cool place. Control formula (placebo) was prepared as other formulas but free of the active constituent (alcoholic extract of propolis). Table 1 shows the composition of the prepared propolis buccal paste formulas.

Pharmaceutical evaluation of propolis paste formulations

The prepared formulations of propolis buccal paste were pharmaceutically assessed as per the international standards specifications [18], as shown in Table 2. Formulas color, taste and odor were tested by natural sense.

Determination of paste pH

One gram each of the paste formulations and the control was accurately weighed and dispersed in 10 ml of purified water. The pH of the dispersions was measured with a pH meter (Hanna Instruments, HI8417, Portugal) [19].

Stability of propolis paste formulations

The stability of the prepared formulas was performed by incubation of propolis paste tubes at different temperatures (4, 25, 35 and 50º C) over a period of 6 months. Samples (n = 5) were taken out at 30, 60, 90 and 180 days, and evaluated for the paste appearance, color, consistency and pH.

Table 1: Composition of the Propolis Buccal Paste Formulations

Formulation code	Formulation ingredients %(w/w)								
	Propolis extract	Pectin	Gelatin	CMC	Bees wax	Olive oil	Sesame oil	Methyl paraben	Propyl paraben
F1	20	10	2	10	10		4	0.3	0.1
F2	20	10	2	10	10	4		0.3	0.1
F3	-	10	2	10	10	4	-	0.3	0.1

Table 2: Pharmaceutical Evaluation of Propolis Buccal Paste Formulations

Test	Limit	Propolis buccal paste	Inference
Flow property at 10º C	Continuous	Continuous flow observed	Complies
Flow property at 20º C (orifices down)	Minimum length of 10 mm	More than 13 mm	Complies
Muccosal irritation test	Should not produce any irritation	No irritation observed	Complies
Attachment test at 20º C (orifices down)	Not less than 15 s	More than 25 s	Complies
Amount of soap	Not more than 20%	1%	Complies
Sieve test material transfer from US std sieve No. 100	100%	100%	Complies
Sieve test material transfer from US std sieve No. 200	98%	98.6%	Complies
Glass scratch test	Should not produce any scratch on glass	No scratch observed	Complies
Test for container	Use chemical resistant collapsible tubes having screw cap	Used chemical resistant collapsible tubes having screw cap	Complies
Orifice area	Not less than 13 and Not more than 36 mm^2	27 mm^2	Complies
Flavors and sweeteners	Distinct and pleasant	Distinct and pleasant	Complies

Table 3: Demographic Distribution of the Patients Groups

Patients groups	Age (year)							
	Sex	>19	20-29	30-39	40-49	50-59	60-69	>70	Total
1st group formula	Male	4	6	5	1	1	1	1	19
	Female	4	3	7	1	3	2	1	21
	Total	8	9	12	2	4	3	2	40
2nd group formula	Male	2	6	1	2	0	1	0	12
	Female	0	6	11	2	5	2	2	28
	Total	2	12	12	4	5	3	2	40
Placebo formula	Male	5	0	2	4	1	4	4	20
	Female	4	5	4	2	2	1	2	20
	Total	9	5	6	6	3	5	6	40
Total	Male	11	12	8	7	2	6	5	51
	Female	8	14	22	5	10	5	5	69
	Total	19	26	30	12	12	11	10	120
	%	15.8	21.7	25	10	10	9.17	8.3	100

Microiological stability studies

The probability of microbial growth in the formulated paste formulas was studied at the beginning and after six months of the storage. The formulations were incubated in nutrient agar and blood agar plates at 37º C for 48h-1wk in aerobic and anaerobic conditions for bacterial examinations. Alternatively for fungal examinations samples of paste formulas were incubated in Sabouraud's dextrose agar plates at 25º C for 1-4 weeks.

Clinical evaluation

The present clinical study was performed in accordance with the ethical standards formulated in the Helsinki Declaration version 2002 [20]. A single blind clinical study was carried out on 120 patients (mean age 39.5 years; 69 women and 51 men) randomly selected from the

outpatient clinic of the Dentistry Medical College, University of Khartoum over a period of 8 months. Ethical approval was achieved for this study by the Institutional Research Ethics Committee.

The patients having mouth ulcers were diagnosed by a dental specialist in oral medicine. The patients were informed not to take any drug that might help in treating the aphthous ulceration such as antibiotics, antiseptics and/or analgesics, during the study. Subjects were also informed that possible allergic reactions may occur and that they should terminate use of the product if any adverse reactions are noted. Patients were also aware that they may have received either the propolis or a placebo but they knew nothing about the type of the formula they used. Patients were given written and oral explanation of the study and risks and benefits.

The patients with mouth ulcers were randomized into one of the three following groups: Group 1 (40 patients) treated with the 1st formula containing sesame oil; 5 patients dropped out; Group 2 (40 patients) treated with the 2nd formula containing olive oil; one patient dropped out; Group 3, control group (40 patients) treated with placebo formula containing no active constituent (Propolis extract). Patients were asked to apply the paste directly on the lesion twice daily, stop eating for at least one hour after drug application and store the paste tubes in dry clean place at the room temperature. Patients groups were assessed for the parameters including: duration of pain disappearance after pastes application, duration of ulcer complete healing, onset of size reduction after drug application and duration of drug adherence to mucous membrane.

The results were collected, tabulated and statistically analyzed; the three studied groups were compared using Chi squire (X^2) test. A level of significance of $p<0.05$ was set to determine any significant difference among the groups. All statistical analyses were conducted in SPSS (V 6.0, SPSS©, Chicago, IL).

RESULTS AND DISCUSSION

Pharmaceutical evaluation of propolis pastes

Propolis buccal paste formulations had been evaluated according to the international standards specifications. The results for the performed tests were all complies with the standard limits, as represented in Table 2. Furthermore formulas were soft enough to be removed easily from their containers and hard enough to adhere to the mucous membranes for a time adequate to allow the active constituents of propolis paste to diffuse readily and smoothly through the membranes and achieve their healing action. The paste was viscous in consistency and had a yellowish to faint brown color. The taste and odor were palatable and characteristic of that of propolis.

pH of propolis pastes

The mean ±SD pH of propolis paste formulations was (5.6 ±0.61), (5.8 ±0.42) and (6.0 ±0.11) for F1, F2 and placebo formula, respectively. These pastes can be considered non irritant to the buccal cavity since the normal pH of buccal mucosa in healthy people is 6.78 ± 0.04 [21].

Stability of propolis paste formulations

The stability studies of the physical properties showed no change in color with increasing temperature except 50º C. A possible reason for this temperature effect was the loss of the oil media and aromatic materials from the tube. Slight changes in pH occurred when the product was kept at different temperatures indicating sufficient stability. Accordingly, the recommended temperature conditions for storing the paste were ambient. The stability in physical properties of the paste could be attributed to the antioxidant property of the propolis constituent flavanoid [22].

Microbiological stability

No bacterial or fungal growth was noticed in the incubated plates even after six months from the production time of the new formulas, which indicated that the formulas were completely free from any microbial growth for this specified period of time. This observation may be attributed to one, two or all of the following factors: (a) the high contents of solid materials and alcohol (b) the preservatives added to the formulas (c) the ability of the product itself to inhibit the growth of microorganisms [23, 24].

Clinical evaluation

In this clinical study, patients were divided into three groups. The demographic distribution of these groups is shown in Table 3.

a) Duration of pain disappearance after pastes application

Results showed that both formulas are effective and the pain intensity was significantly decreased ($p<0.05$) in most patients during the first day of application with 90 % in the 1st group and 97.5% in the 2nd group in comparison with the placebo formula only 35% of the patients showed a decrease in their pain intensity, Table 4.

Such result is due to the anti-inflammatory effect of propolis that had been preliminary assessed in the treatment of a variety of inflammatory and ulcerative conditions with low rates of minimal side effects [25]. The use of propolis for the treatment of mouth ulcers is a traditional therapy utilized by some communities in the Middle East. Samet et al. [15] reported that patients who took a 500-mg capsule of propolis daily supplement were shown to have a statistically significant decrease in the frequency of outbreaks of recurrent aphthous stomatitis.

The control base has a minor effect in treatment of aphthous ulceration but this effect was statistically in significant and it may be attributed to the presence of beeswax in the base which was found to have anti-ulcer and anti-inflammatory effects as stated in previous studies [26, 27]. Another possible explanation for this effect is the presence of CMC sodium in the control base which was used for mechanical protection of the oral and perioral lesions such as mouth ulceration [28].

In addition no significant difference was found between the propolis paste formulas over the first 6 h of the pastes application. While the 2nd formula (with olive oil) showed superior pain reduction over a period of more than 7 hours and in a percentage of 28.0% for 2nd group formula comparing to 17.5% for 1st group formula and (0.0%) for placebo group formula.

b) Duration of complete ulcer healing

The prepared formulas showed a high significant effect ($p<0.01$) comparing to the placebo group formula regarding the required duration for complete healing of the mouth ulcer. However F2 was appreciably more effective than F1 in complete ulcer healing during the first week of the treatment. The duration of complete ulcer healing for patients was less than one week in 60.0% for the 1st group formula and 80.0% for the 2nd group formula and the percentage for the placebo group formula was only 2.5% (Fig. 1).

c) Onset of size reduction after propolis pastes application

Significant reduction ($p<0.05$) in the size of the mouth ulcer had been achieved in 1st group patients during the first day of the paste application comparing to the 2nd group. Fig. 2 indicates that the use of propolis buccal paste decreased the size of the ulcer in 45.0% of the patients for the 1st group formula and in 65.0% for the 2nd group formula while for the placebo group formula no decrease in the size of the ulcer was observed.

These effects of propolis (size reduction and pain disappearance) are in agreement with the finding of Atta and Alkofahi [29] who found that chronic inflammation induced by cotton pellet granuloma was inhibited by ethanolic extract propolis. This extract has an antinociceptive effect against both acetic acid-induced writhing and hot plate-induced thermal stimulation in mice, indicating its central and peripheral effects.

The wide spectrum activity of propolis such as anti-inflammatory and antimicrobial effect explained the high effectiveness of propolis preparation in the treatment of mouth ulcer in comparison with other mouth ulcer preparations used for the same treatment of mouth ulcer. This could be due to its antimicrobial effect against a wide spectrum of microorganisms [24].

Table 4: Duration of Pain Disappearance after Pastes Application

Duration (h)	1st Group Formula* (%)	2nd Group Formula* (%)	Placebo Group Formula (%)
< 1	13	14	0
2	5	5	0
3	3.5	2.5	7.5
4	18	15	10
5	13.5	15	10
6	19.5	18	7.5
>7	17.5	28	0
Total	90%	97.5%	35%
No response	10%	2.5%	65%

*Significant difference from placebo group formula according to Chi squire test, p<0.05

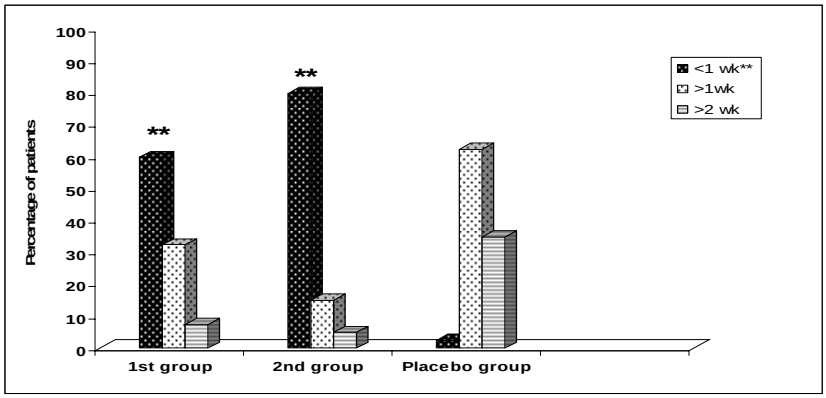

Fig. 1: Duration of complete ulcer healing after buccal pastes application (indicates a high significant difference from the placebo group patients, p<0.01)**

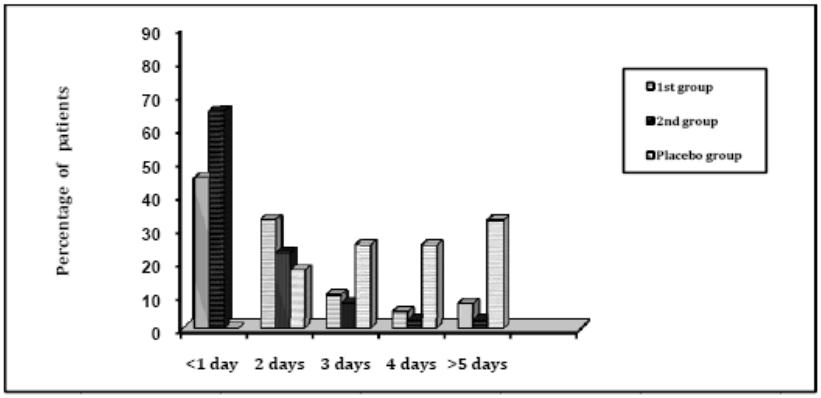

Fig. 2: Onset of mouth ulcer size reduction after buccal pastes application (* indicates significant difference between the 1st and 2nd groups patients, p<0.05)

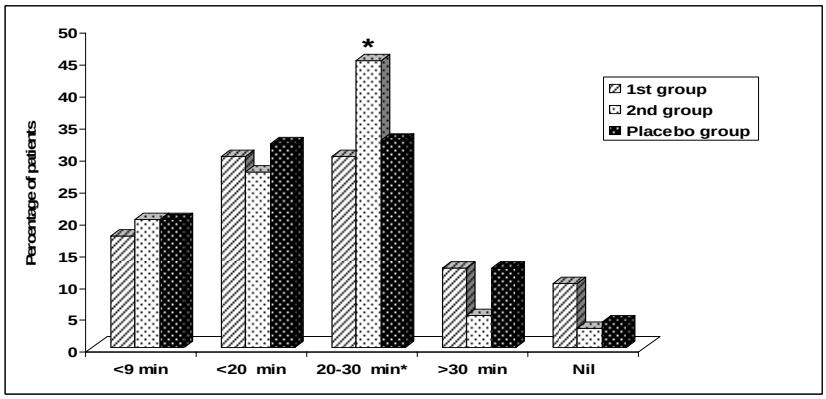

Fig. 3: Duration of propolis pastes adherence to buccal mucous membrane, (* indicates significant difference between the 1st and 2nd groups patients, p<0.05)

d) Duration of drug adherence to mucous membrane

By studying the duration of adherence of the paste to the affected area, it was shown that the period of drug adherence to the oral mucosa in most patients was about 20-30 minutes showing 30.0% for the 1st group formula, 45% for 2nd group formula and 32.5% for the placebo group formula as shown in Fig. 3.

As mentioned previously, the majority of patients who were treated by propolis buccal pastes show a significant difference between 1st group formula and 2nd group formula in the following criteria: pain disappearance, reduction in the size of aphthous ulcers and complete healing. The high effectiveness of the 2nd formula in comparison with the 1st formula could be attributed to the longer duration of adherence of its base to the site of ulcer that allows the active ingredients to diffuse completely and to act for a long period of time.

CONCLUSION

In conclusion, the use of olive oil based propolis paste formula for patients with RAU significantly effective in the lesions size reduction, duration of complete healing, pain disappearance and bioadhesion time to the buccal mucosa, comparing to sesame oil based formula (F1) and the placebo formula. No allergic reaction or any other side effects were observed with its use. Furthermore, pharmaceutical properties of the selected formula were fulfillment to the standard tests. Also it was stable and free of microorganisms over six months of storage thus it can be considered as a promising cheap and easily prepared medicinal preparation for the treatment of recurrent oral aphthous ulceration.

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