

Aqueous extract of propolis in the treatment of recurrent aphthous stomatitis (Double blind study)

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ABSTRACT

In this study, aqueous extract of propolis, which is a natural bee product, tested for treatment of recurrent aphthous stomatitis (RAS). A total of 40 patients with RAS divided into 4 groups. Each patient received one of following pre-prepared mouthwash:

Group A received aqueous propolis extract at 1% concentration.

Group B received aqueous propolis extract at 0.5% concentration.

Group C received dexamethosone at 0.1% concentration.

Group D received distilled water. (Control group).

The study was double blind and the patient instructed to use the mouthwashes for 5 min 3 times daily.

The results of study showed significant difference between groups in pain score assessed at day 2 of study. High percentage of no pain recorded in group A comparing to other groups. However at 5 days of drug therapy no significant difference noticed among the groups regarding pain score.

For assessment of healing after 5 days, although no significant difference noticed among the groups, group A reported to show high percentage of complete healing comparing to other groups. Also groups A, B and C showed 50% healing of ulcer comparing to only 20% in patient on group D.

In conclusion, aqueous propolis extract at 1% concentration showed good percentage of early reduction of pain score and rapid healing of ulcer with minimal side effect and further studies on other extract and concentration may be recommended.

Key Words: Propolis, recurrent aphthous stomatitis, dexamethasone.

الخلاصة

في هذه الدراسة، استخدم المستخلص المائي للعكبر - الذي هو أحد الراتجات الطبيعية المنتجة من قبل نحل العسل - اختبرت لعلاج تقرحات الفم القلاحية الراجعة. شارك في الدراسة 40 مريضاً يشكون من التقرحات القلاحية وقسموا إلى أربعة مجاميع، كل مجموعة استعملت أحد أنواع غسول الفم التالية:

المجموعة أ: استعملت غسول يحتوي على عكبر مائي بتركيز ١%.

المجموعة ب: استعملت غسول يحتوي على عكبر مائي بتركيز ٠.٥%.

المجموعة ج: استعملت غسول يحتوي على معلق الدكساميثازون بتركيز ٠.١%.

المجموعة د: استعملت غسول يحتوي ماء مقطر (المجموعة الضابطة).

أوصى المرضى باستخدام الغسول المعطى لمدة ٥ دقائق ٣ مرات يومياً.

أظهرت النتائج اختلافات معنوية لشدة الألم المسجل في اليوم الثاني لبدء الدراسة بين المجاميع المختلفة حيث ظهرت نسبة عالية من درجة الألم الخفيفة أو عدم وجود الألم عند المجموعة أ مقارنة ببقية المجموعات. أما في اليوم الخامس فقد كانت الفروقات من ناحية شدة الألم غير معنوية بين مختلف المجاميع.

بالنسبة لعلامات التئام التقرح لم تظهر اختلافات معنوية بين المجاميع بعد ٥ أيام من بدء الدراسة، لكن على الرغم من ذلك ظهرت نسبة عالية من المرضى في المجموعة أ وقد تم التئام التقرح مقارنة ببقية المجاميع، كذلك كانت نسبة ٥٠% من المرضى في المجموعات أ، ب، ج تم التئام التقرح مقارنة بنسبة ٢٠% فقط للمجموعة د التي استخدمت المادة الضابطة.

يستنتج من الدراسة أن لمستخلص العكبر المائي بتركيز ١% تأثير جيد على تقليص الألم الذي يصاحب التقرحات الفموية في الأيام الأولى لظهوره وعلى سرعة التئام التقرح وبتأثيرات جانبية قليلة. ينصح بعمل الدراسات مماثلة على تراكيز واستخلاصات مختلفة للعكبر.

INTRODUCTION

Recurrent aphthous stomatitis (RAS) is a disorder characterized by recurring ulcer confined to the oral mucosa in patient with no other signs of disease.⁽¹⁾ It is associated with a significant pain interfering with eating and drinking that may last 1 week to 1 month depending on the type of ulcer.⁽²⁾ Its precise etiology not clear and several possible causes determined include; here-ditary, hematological deficiencies, psycho-logy, trauma and hormonal changes.⁽²⁻⁴⁾

Treatments of RAS are largely symptomatic in nature. Most of preparations relief pain temporarily (e.g. topical anesthesia) but not enhance healing. Other preparations used include tetracycline mouthwash, doxymycin, diphenhydramine, levamisole, thalidomide, topical diclofenac, chlorhexidine and recently amlexanox oral paste.⁽³⁻⁶⁾ Topical and systemic steroids had been tested to reduce non infectious inflammatory origin of the RAS, but the side effects of opportunistic candidal infection must not be ignored.^(1, 2) However, overall current therapy don't carry a significant effect on reducing pain associated with RAS as well as not enhance its healing and new treatment with minimal side effects are clinically needed.⁽²⁾

Propolis is a natural resinous beehive product. It is collected by bees from certain trees, metabolized it and took it back to their hives to be used for sealing all fissures, protecting against introducers and sterilizing hive environment.⁽⁷⁾

Propolis used in folk medicine since ancient time. However its pharmacological activity established recently. It possess great antibacterial,⁽⁸⁻⁹⁾ antiplaque,⁽¹⁰⁾ antiviral,⁽¹¹⁾ antifungal,⁽¹²⁾ antioxidant,⁽¹³⁾ and anti-inflammatory⁽¹⁴⁻¹⁶⁾ activities.

Anti-inflammatory activity of propolis attributed to its amino acids, flavanoids, trepans, cinnamic acid and caffiec acid phenethyl ester (CAPE) that propolis contains. These substances make propolis exert dual lipooxygenase and cyclooxygenase inhibitory activities.⁽¹⁵⁻¹⁶⁾

Various studies performed to demonstrate the effect of different origin, prep-

aration and concentration of propolis on its pharmacological property.⁽⁷⁾

This study aim to determine the effect of aqueous propolis extract from Sinjar City at 1% and 0.5% concentration on the pain associated with RAS as well as on its healing

MATERIAL AND METHODS

This clinical study was carried out at Oral and Maxillofacial Surgery Department/ College of Dentistry/ University of Mosul

Patients participated fulfill following criteria:

- Patient should have recurrent oral ulceration without apparent causes.
- No history of systemic disease.
- Patient should not being on any drug therapy.
- Patient should not pregnant (For females).

Following verbal informed consent obtained from each patient on participation, each patient assigned randomly to one of the following groups:

- Group A: The patient received aqueous propolis extract at 1% concentration as mouthwash.
- Group B: The patient received aqueous propolis extract at 0.5% concentration as mouthwash.
- Group C: The patient received dexamethasone elixir at 0.1% concentration as mouthwash.
- Group D: The patient received distilled water as mouthwash.

The mouthwash given in pre prepared coded dark bottle to make the study double blind.

For groups A and B aqueous propolis extract prepared by chopping of propolis collected from Sinjar City in water at 7.2 pH at room temperature for 5 days. Extraction made using lypholyzer and final extract dried and suspended in water at 1% or 0.5% concentration^(10,15).

For group C commercially available dexamethasone elixir at 0.1% concentration were used (0.1 mg/ml).

The patients in all groups instructed to rinse the mouth thoroughly 3 times daily with 5 ml of given solution (after

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shaking). Patients also instructed to return at day 2 and day 5 of drug usage for evaluation. (every patient not follow these instructions excluded from the study).

For each patient following information recorded (Figure 1).

Statistical analysis performed to determine the significance of difference in pain and sign of healing between different group using χ^2 test.

☉ Name :	Age :	Sex:
☉ Frequency of aphthus attack:	Recurrent less than 2 week	
	1 month	
	3 months	
☉ Family history of aphthus:	Positive	Negative
☉ Number of lesion:	Single	Multiple
☉ Site of the lesion :	Lip	Tongue
	Buccal Mucosa	Palate
		Other.
☉ Size of the lesion:	mm.	
At each recall visit following informations recorded:		
☉ Grade of pain :		
	1. No pain.	
	2. Mild to moderate pain.	
	3. Severe pain.	
☉ Sign of healing:	Reduction in lesion size	
	No pain completely	
	No inflammatory zone at periphery	
(Recorded as healing or no healing)		
Post treatment side effects:		

Figure (1): Case sheet records

RESULTS

In this study, 40 patients participated and divided on to the 4 study groups (10 patients for each group). Mean age of patients were 29.2 ± 5 years. Ratio of male: female were 1:1.105. Patients with positive family history were 22(or 55%). Patients had single ulcer were 25 whereas the remaining 15 patients had 2 or more lesions at time of initiation of therapy. Frequency of RAS attack recorded as follow: 16 patients had RAS at less than 2 weeks, 13 patients had RAS at less than 1 month and 11 patients had RAS at less than 3 months. For the site of RAS; lesion recorded in lip of 19 patients, tongue of 11

patients, palate of 8 patients and buccal mucosa of 7 patients (Note that 15 patients had multiple lesion that may involve more than one site).

Mean size of lesion recorded at the time of initiation of the therapy was $0.43 \text{ mm} \pm 0.24 \text{ mm}$ at the maximum diameter of the lesion (No patients participated with major aphthus).

Table (1) and Figure (2) showed the number of patients at 4 groups according to pain grade assessed at day 2 from initiation of therapy. This result showed a statistically significant difference among the groups ($\chi^2 = 14.118$, $df = 6$, $p < 0.05$). High percentage of grade 1 pain record

noticed in group A comparing to low percentage of grade 3 record noticed in the

same group. Opposite results noticed in group D.

Table (1): Number of patients with different pain grade in 4 study groups at day 2

Pain Grade	Group A	Group B	Group C	Group D	Total
Grade 1	7	3	5	1	16
Grade 2	1	4	3	1	9
Grade 3	2	3	2	8	15

$$\chi^2 = 14.118 \text{ df} = 6$$

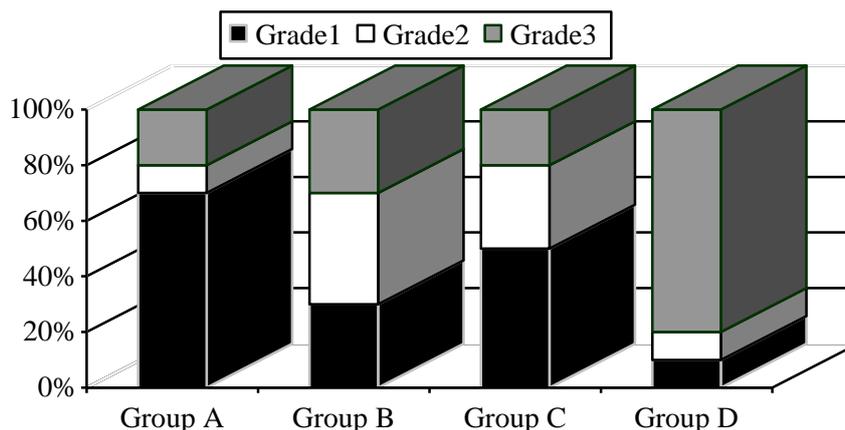


Figure (2): Percentage of patients with different pain grade in 4 study groups at day 2

Assessment of pain grade at day 5 from initiation of drug therapy showed no statistical significant difference among the groups ($\chi^2 = 4.08 \text{ df} = 6, p < 0.05$). How-

ever high percentages of grade 1 pain record reported in group A and C comparing to group B and D (Table 2 and Figure 3).

Table (2): Number of patients with different pain grade in 4 study groups at day 5

Pain Grade	Group A	Group B	Group C	Group D	Total
Grade 1	8	5	7	5	25
Grade 2	2	4	2	3	11
Grade 3	-	1	1	2	4

$$\chi^2 = 4.08 \text{ d.f} = 6$$

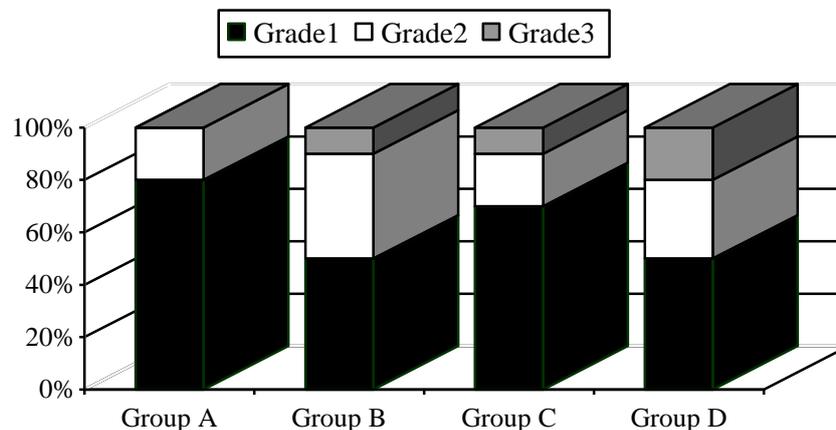


Figure (3): Percentage of patients with different pain grade in 4 study groups at day 5

For assessment of healing; at day 2 recall visit actually no case of complete healing recorded in 4 study groups.

At 5th day recall visit, the difference in between number of completely healed ulcer and that not show sign of healing

were statistically not significantly differ ($\chi^2 = 6.036$, $df = 3$, $p < 0.05$). However higher percentage of healed ulcer noticed in group A comparing to group D (70% and 20% respectively) (Table 3).

Table (3): Number of patients in 4 study groups according to the presence or absence of sign of healing at 5 day

Healing	Group A	Group B	Group C	Group D	Total
Yes	7	3	5	2	17
No	3	7	5	8	23

$\chi^2 = 6.036$ d.f = 3

When comparing percentage of patient according to pain recorded in 2nd and 5th day of therapy in each study group an increase in grade 1 record at 5th day of observation in group D was noticed. Other group show no statistical significant difference between 2nd and 5th day of

therapy ($\chi^2 = 0.48$ $df = 3$, $p < 0.05$). (Table 4)

Assessment of percentage of healing showed that of completely healed ulcer; 41% noticed in group A after 5 days therapy and only 12% in group D. (Table 4)

Table (4): Percentage of patients with grade 1 pain records at day 2 and day 5 and patient with complete healing at 4 study groups

Group	2 nd Day Grade 1 Pain	5 th Day Grade 1 Pain	5 th Day Complete Healing
A	44%	32%	41%
B	19%	20%	18%
C	31%	28%	29%
D	6%	20%	12%

For the side effects recorded; in group A, one patient show early sign of erythematous mucosal change and complain of itching. The patient instructed to stop the therapy at day 2 of therapy and given diphenhydramine mouthwash. Whereas following 5 day recall visit one patient in group C complain of moderate pain at dorsum of the tongue and show a hyperplastic white lesion confirmed as candidal infection when healed by stopping the therapy and proper antifungal administered.

DISCUSSION

As the etiology and pathogenesis of RAS were not well established, this disease continues to be difficult to treat. This led to introduction of drug therapy that is based primarily on symptoms management.⁽⁶⁾

In this study the use of topical steroid and propolis as mouthwash which are both anti-inflammatory agents provide good modality of treatment. As most of studies conducted state that 10 days at least necessary for complete healing,⁽²⁾ complete healing after 5 days of therapy reported in 50% of patient receiving 3 forms of drug therapy comparing to 20%

only in control group who receive placebo. This reduction in healing period in this study may raise the suspect of immunological base for RAS origin. This result agreed with several other studies conducted using various from of topical steroid therapy.⁽¹⁾

The exact mechanism of anti-inflammatory action of propolis is unknown, however a dual inhibition of lipo-oxygenase and cyclo-oxygenase are proved.⁽¹⁵⁾ In one study using aqueous propolis extract topically to suppress corneal neovascularization in rabbit, it showed a non-statistically significant difference between propolis 1% concentration and dexamethasone.⁽¹⁵⁾ Our study showed better result for group received aqueous propolis 1% than dexamethasone 0.1% in regarding of pain grade and healing (Table 4). Antioxidant and reparative properties of propolis may enhance healing of ulcer and provide stronger anti-inflammatory activity for propolis than dexamethasone.⁽¹³⁾ Also antibacterial activity may play a role by preventing secondary infection over the ulcer.⁽⁸⁻¹⁰⁾

The result showed a great difference in percentage of pain reduction and healing noticed between group received 1% propolis and that received 0.5% propolis extract (Table 4). This observation suggest that the functional dose to be at least 1% concentration and further study in more concentrated extract may be recommended.

No significant difference noticed in pain recorded at 5 day of therapy could be explained as that even without treatment, healing of ulcer started and completed at 7-10 days, so that minimal pain recorded at day 5 as healing was started.⁽²⁾

The emergence of candidal infection after dexamethasone topical uses was expected. However, when its uses are indicated and candidal infection suspected the use of proper antifungal can be initiated at the same time.⁽²⁾ On the other hand, propolis which actually have anti-fungal activity eliminate this side effect.⁽¹²⁾

Allergic reaction is the most important side effect of propolis usage and it is mild type allergic reaction causing contact dermatitis. This allergic reaction character-

ized by itching and mucositis and it may associate with ulceration, but it can be healed and disappeared by discontinuation of the therapy and proper topical anti-histamine or steroid therapy initiated. However, one must keep in mind such possible side effects and inform patient to stop the therapy and return for check up as soon as possible when sign of allergy occurs.⁽¹⁷⁻¹⁸⁾

The anti-inflammatory activity of propolis achieved in this study, as well as the anti-bacterial activity obtained *in vivo* in our previous study,⁽¹⁰⁾ make it desirable to suggest using propolis in the field of oral surgery as irrigating solution during operation or post operatively to reduce pain of dry socket.

CONCLUSION

In conclusion, aqueous extract of Sinjar City propolis at 1% concentration provide a good modality for management of RAS with minimal side effects and further investigation on other origin of propolis in Iraq and other concentration is recommended.

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