

## Efficacy of Propolis in Diabetic Foot Ulcer Management (or Treatment): A Randomized Controlled Trial

Mehdi Shekari<sup>a</sup>, Yaghob Koushan<sup>b</sup>, Ahmad Kachooie<sup>c</sup>, Hossein Khadem Haghighian<sup>a\*</sup>

<sup>a</sup>Department of Nutrition, School of Health, Qazvin University of Medical Sciences, Qazvin, Iran.

<sup>b</sup>Medicinal Plants Research Center of Islamic Azad University of Tabriz, Tabriz, Iran.

<sup>c</sup>Department of General Surgery, Qom University of Medical Science, Qom, Iran.

### ABSTRACT

#### Article History

Received:  
19/11/2017  
Revised:  
11/12/2017  
Accepted:  
24/12/2017

#### Keywords:

Diabetes mellitus;  
Diabetic ulcers;  
Propolis;  
Wound healing

**Background:** The impaired wound healing in diabetes mellitus is a major clinical problem. Propolis has pharmacological characteristics. This study aimed to evaluate the effects of oral, topical, and oral combined with topical administration of propolis on foot ulcers in diabetic patients.

**Methods:** Eighty patients with diabetic foot ulcers were divided into 4 groups. Group 1 received propolis ointment (3%, 1.5 g daily), Group 2 received three 500-mg propolis capsules per day, Group 3 received capsules and ointments, and Group 4 received the usual treatment of foot ulcer, including washing with physiological serum. The patients were treated for 4 weeks. Wound surface area changes were analyzed to determine the effectiveness of the compound in wound healing.

**Results:** The interventions decreased foot ulcer surface areas in all intervention groups (from  $685.13 \pm 286.16$  to  $74.89 \pm 26.40$  mm<sup>2</sup> [ $p=0.03$ ] in Group 1, from  $616.52 \pm 221.11$  to  $104.60 \pm 39.81$  mm<sup>2</sup> [ $p=0.046$ ] in Group 2, and from  $647.07 \pm 23$  to  $54.20 \pm 19.17$  mm<sup>2</sup> [ $p=0.01$ ] in Group 3). In Group 4, however, the reduction in ulcer surface area was not statistically significant. In the three experimental groups, the wounds were clinically improved in all subjects.

**Conclusion:** This study showed that in diabetic foot ulcers, treatment with either oral propolis capsules or combination therapy with oral and topical forms, significantly improves wound healing. Also, the application of these products does not seem to be associated with any unexpected adverse event.

### Introduction

Foot ulceration is one of the complications of diabetes, affecting 15% of people with diabetes and accounting for 15% to 20% of foot amputations [1]. A retrospective study from Tehran University of Medical Sciences found that of the 281 patients (39% female) hospitalized for diabetic foot ulcers between 1979 and 2000, about 30% were faced with amputation [2]. The rate of amputation caused by diabetes in Iran is higher than in other countries [3]. The risk factors for diabetic foot ulcers include infection, prior ulcers, peripheral neuropathy, peripheral vascular disease, and prior infection [4]. All diabetic ulcers cannot be prevented and treated, but proper care can greatly reduce its incidence [5]. Treatment for diabetic foot ulcers is very complicated and requires a lot of care [6]. All treatments used to

treat wounds have a relative effect on wound healing or the prevention of amputation. Therefore, new noninvasive drugs that have the most therapeutic effect in the shortest term should be investigated and evaluated [7].

Over the past decade, new treatments have been proposed that have been shown to reduce stroke and improve diabetic wound healing. To date, several topical medications have been introduced to improve diabetic wounds, including factoring gels, tretinoin, and Angi Pars [8]. In addition, several studies have shown positive effects of herbal medicines on diabetic foot ulcers. Honey and propolis have been used for the treatment of wounds for centuries. Propolis has antimicrobial, antioxidant, anti-inflammatory, and antitumor agents [9-10]. Antimicrobial activity of propolis against *Staphylococcus aureus*, *Streptococcus pyogenes*, gram-negative and gram-positive

\*Corresponding author.

E-mail address: khademnut@yahoo.com

© 2017 Tehran University of Medical Sciences. All rights reserved.

bacteria, *Candida*, *Streptococcus mutans*, anaerobic bacteria in human oral cavity, and *Salmonella* species has been reported [11].

The wound-healing effects of propolis have been reported in a case study. In this study, a female patient with diabetic ulcers was completely recovered after two weeks of intervention with propolis [12]. This substance has not been studied in the treatment of diabetic foot ulcers. Therefore, this study was conducted to evaluate the effects of propolis on diabetic foot ulcer healing and ulcer size..

## Subjects and methods

### *Subjects*

Patients with diabetes visiting the Metabolism and Endocrine Clinic of Shahid Beheshti Hospital in Qom were approached, and eligible patients were enrolled in the trial after being briefed completely and providing written informed consent.

### *Inclusion and exclusion criteria*

Inclusion criteria were having type 1 or type 2 diabetes with diabetic foot ulcers of grade 1-2 as determined using the Wagner Diabetic Foot Ulcer Grade Classification System, being 18-75 years old, and willing to participate.

Exclusion criteria included having drug or alcohol addiction, taking corticosteroids, being treated with radiotherapy, bone exposure at the wound site, using an effective medication in the wound healing process, or having another infectious ulcer near the diabetic wound.

### *Study design*

The sample size was calculated to be 80 patients (20 patients in each group). Participants in the study were randomly divided into four groups. Group 1 received propolis ointment (3% propolis, 1.5 g daily), Group 2 received three 500-mg capsules containing propolis, Group 3 received capsules and ointment, and Group 4 received the usual foot ulcer care (washing with physiological saline serum). The capsules and ointments were prepared by a researcher of traditional medicine at Tabriz Azad University.

The ointment was applied using a sterile 4 × 4-cm gauze dressing. The patients were advised to change their dressings every 24 hours and wash the wound completely with the serum before re-dressing. The control group was also advised to continue with the usual treatment of the wound.

The duration of the study, according to a pilot

study, was 30 days, during which a general surgeon made weekly assessments of the wounds by taking photos and measuring them with AutoCAD computer software.

The study was approved by the Ethics Committee of Qazvin University of Medical Sciences and registered with the clinical trials registry of Iran (IRCT2017040419669N3).

### *Statistical analyses*

SPSS software was used to analyze the data. After verifying the normality of the data distribution, the results of the evaluation of the wound area were presented as mean ± standard deviation. Mean changes in wound area were analyzed using paired t tests, and an ANOVA was used to compare the variables among the groups. Changes in wound area of below -10% were evaluated as worsening; between -10% and 10%, as no change; between 10% and 70%, relative improvement; and above 70%, complete recovery.

## Results

### *Basic patients' characteristics*

Among four parallel groups, no significant differences were observed in any of the appraised characteristics, such as age, weight, FBS, HbA1c, or wound area.

The primary ulcer grade, according to Wagner's classification system, was 2. All of the patients randomized to treatment completed the study.

### *Effects of propolis on wound healing*

Evaluation of wounds after the 4 weeks in intervention groups showed good clinical outcome in terms of wound healing. The mean wound area was compared among the four groups to evaluate the effects of the three treatment modalities against conventional therapy (Table 2). The interventions reduced foot wound area from  $685.13 \pm 286.16$  to  $74.89 \pm 26.40$  mm<sup>2</sup> ( $p = 0.031$ ) in Group 1, from  $616.52 \pm 221.11$  to  $104.60 \pm 39.81$  mm<sup>2</sup> ( $p = 0.046$ ) in Group 2, from  $647.07 \pm 23$  to  $54.20 \pm 19.17$  mm<sup>2</sup> ( $p = 0.01$ ) in Group 3, and from  $681.13 \pm 278.05$  to  $600.09 \pm 256.01$  mm<sup>2</sup> ( $p = 0.1$ ). The mean reduction in wound surface area was significantly greater in Group 3 (propolis plus ointment). The results of clinical assessments are summarized in (Table 3). In the three experimental groups, the wounds were clinically improved in all subjects.

**Table 1. Patient demographics and ulcer characteristic (mean ± SD)**

| Characteristic                   | Group 1<br>(n=20) | Group 2<br>(n=20) | Group 3<br>(n=20) | Group 4<br>(n=20) | P value |
|----------------------------------|-------------------|-------------------|-------------------|-------------------|---------|
| Age (year)                       | 57.11 ± 2.7       | 59.21 ± 4.09      | 61.90 ± 3.47      | 56.02 ± 3.21      | 0.20    |
| Weight (kg)                      | 78.24 ± 5.11      | 75.11 ± 6.04      | 72.46 ± 9.20      | 76.01 ± 4.32      | 0.41    |
| FBS (mg/dL)                      | 165.20 ± 49.17    | 159.48 ± 54.19    | 162.35 ± 48.10    | 158.07 ± 50.19    | 0.60    |
| HbA1c (%)                        | 9.01 ± 2.17       | 8.60 ± 1.98       | 8.80 ± 2.50       | 8.01 ± 2.43       | 0.08    |
| Wound<br>Size (mm <sup>2</sup> ) | 685.13 ± 286.16   | 616.52 ± 221.11   | 647.07 ± 23       | 681.13 ± 278.05   | 0.61    |

Group 1 = topical administration of propolis; Group 2 = oral administration of propolis; Group 3 = oral and topical administration of propolis; Group 4 = conventional therapy

**Table 2. Mean ulcers surface areas changes in both experimental and control groups before and after treatment**

| Variables  |                     | Group 1<br>(n = 20) | Group 2<br>(n = 20) | Group 3<br>(n = 20) | Group 4<br>(n = 20) |
|--|---------------------|---------------------|---------------------|---------------------|---------------------|
| Mean Ulcers<br>Surface Areas<br>(mm <sup>2</sup> ) | Baseline            | 685.13 ± 286.16     | 616.52 ± 221.11     | 647.07 ± 23         | 681.13 ± 278.05     |
|  | End                 | 74.89 ± 26.40       | 104.60 ± 39.81      | 54.20 ± 19.17       | 600.09 ± 256.01     |
|  | P1                  | 0.03                | 0.046               | 0.01                | 0.1                 |
|  | Mean<br>improvement | 89%                 | 83%                 | 91.6%               | 11.89%              |

Data are means ± SD. Group 1 = Topical administration of propolis; Group 2 = oral administration of Propolis; Group 3 = oral and topical administration of Propolis; Group 4 = conventional therapy

**Table 3. Clinical improvement outcomes in diabetic ulcers within experimental and control groups according to the percentage of wounds surface changes**

| Variables               | Group 1<br>(n=20) | Group 2<br>(n=20) | Group 3<br>(n=20) | Group 4<br>(n=20) |
|-------------------------|-------------------|-------------------|-------------------|-------------------|
| Worsening               | 0 (0%)            | 0 (0%)            | 0 (0%)            | 1 (5%)            |
| Ineffective             | 0 (0%)            | 0 (0%)            | 0 (0%)            | 17 (85%)          |
| Relative<br>improvement | 2 (10%)           | 3 (15%)           | 1 (5%)            | 2 (10%)           |
| Complete<br>improvement | 18 (90%)          | 17 (85%)          | 19 (95%)          | 0 (0%)            |

Worsening outcome: < -10% changes in wounds surface areas; Ineffective outcome: -10% to +10% changes in wounds surface areas; Relative improvement: +10% to +70% changes in wounds surface areas; Complete improvement: > +70% changes in wounds surface areas.

## Discussion

Disruption in the blood flow and oxygenation, along with other factors such as age, obesity, malnutrition, infection, some medications, and diseases such as diabetes, can delay the healing process of the wound [7].

There have been many studies on wound healing, all aimed at accelerating the recovery process. However, in diabetic patients, the process of repair is associated with failure [6]. In this study, we showed that diabetic foot ulcer surface area considerably decreased following 4 weeks of treatment with oral, topical, or the combination of the oral and topical forms of propolis. This finding is supported by the comparison of the improvement rates and the mean wound areas after the treatment. Treatment with propolis led to complete recovery in 85% of the patient in the group taking oral propolis (1500 mg per day for 30 days) and 95% of the participants taking oral supplements combined

with the topical (3% propolis) form.

Diabetic patients develop peripheral neuropathy in more than 80% of cases, which increases the risk of foot ulcers by 12 times. Poor glycemic control and the increase in oxidative stress promote the damage to neurons [13].

Honey and propolis have been used for wound healing for many years now. Several anti-inflammatory compounds have been derived from propolis, including caffeic acid, quercetin, naringenin, and caffeic acid phenethyl ester [14]. These compounds help macrophages for crushing the integrating of prostaglandins and leukotrienes and decrease inflammation by inhibiting myeloperoxidase, NADPH-oxidase, ornithine decarboxylase and tyrosine-protein kinase activity [15].

Propolis has been administered either topically or systemically in different vehicles, such as alcohol, propylene glycol, and water, with the aim of modulating the healing of surgical wounds [16-

18]. In this study, propolis was administered topically, as this substance has the capacity to penetrate the wound. This penetration capacity was demonstrated in a study by Sehn et al [19] using photoacoustic spectroscopy. Studies by Gulinelli et al [20] and Temiz et al [18] have demonstrated that the vehicle employed does not alter the effects of propolis.

Paulino et al [21] demonstrated that one of the main active ingredients in propolis that has an anti-inflammatory effect is artemillin-C, which can inhibit the inflammatory activity of leukocytes.

The findings of the present study are in agreement with those described by Sehn et al [19], who found that topical administration of propolis stimulated collagen regeneration. When treating wounds with caffeic acid phenethyl ester, a component of propolis, Serarslan et al [22] reported an increase in healing and epithelialization rates. McLennan and colleagues [23] reported similar results after administering propolis to wounds that were difficult to heal in rats with induced diabetes.

Two major causes of failure in diabetic wound healing are the increase in free radical generation and collagen decomposition, both of which can be attributable to deficiency in vitamins C and E or a lack of a potent antioxidant system [24]. The biological action of propolis is due to its active constituents including flavonoids with antioxidant properties [25]. Propolis, with its powerful antioxidant properties, is also active against oxidative stress and may induce a positive effect on diabetic metabolic abnormalities [15].

A clinical study showed that propolis decreased the levels of thiobarbituric acid reactive substances, by-products of lipid peroxidation, and increased the level of glutathione, a powerful antioxidant that protects the important cellular components against free radicals. The study showed that propolis may exert antioxidant effect by decreasing lipid peroxidation through reducing the production of oxygen radicals [26].

One of the principal biomarkers in wound fluid and a powerful mediator of foot ulcer healing in diabetes is matrix metalloproteinase-9 (MMP-9) [27]. Poor wound healing is associated with high levels of active MMP-9. A decrease in the expression of MMP-9 is correlated with improved wound healing and the percentage of collagen in wounds in a diabetic rat model [28]. Caffeic acid phenyl ester has potent ability to decrease the concentration of proinflammatory proteinase MMP-9 in vitro [23]. The mechanism underlying

this action of propolis is not understood, but effects on regulators of MMP activities, including the tissue inhibitors of MMPs, are possible mechanisms. Scientific reports indicate that propolis contains more than 180 ingredients and that while its components may vary based on plants in disparate geographic regions, the main active components are thought to be present in all forms of propolis [9].

### Conclusion

Our results show that propolis could reduce the wound size without any significant adverse effects. Therefore, the use of propolis may be extended to the clinical setting and prove an effective promoter of wound healing in patients with diabetes, where skin ulcers are often slow to heal. More detailed, in-depth studies should be carried out with the aim of studying and isolating each active ingredient of propolis and indicating its action in different ways of application.

### Disclosure statement

There are no competing financial interests.

### Acknowledgements

We thank the Deputy of Research, Qazvin University of Medical Sciences, Qazvin, Iran, for financial support. We also wish to thank the patients who participated in their study.

### Conflict of interest

None of authors have conflict of interests.

### Funding

None.

### References

1. Kiani J, Azizkhani H, Kosarifard S. The prevalence and associated risk factors of peripheral diabetic neuropathy in Hamedan, Iran. *Archives of Iranian medicine*. 2013;16(1):17.
2. Kiadaliri AA, Najafi B, Mirmalek-Sani M. Quality of life in people with diabetes: a systematic review of studies in Iran. *Journal of Diabetes & Metabolic Disorders*. 2013;12(1):54.
3. Al-Geffari M. Comparison of different screening tests for diagnosis of diabetic peripheral neuropathy in Primary Health Care setting. *International journal of health sciences*. 2012;6(2):127.
4. Bagheri M, Fayazi S, Rabee Z, Aarabi M, Latifi SM, Basiri G, et al. Barriers in controlling blood glucose in type 2 diabetes patients with diabetic foot ulcer, Ahvaz Razi Hospital, 2012. *J Chronic Dis Care*. 2012;2(2):17-25.
5. Sharifmoradi K, Karimi MT, Rezaeeyan Z. The

- Effects of Negative Heel Rocker Shoes on the Moment and the Contact Forces Applied on Lower Limb Joints of Diabetic Patients During Walking. *Physical Treatments-Specific Physical Therapy Journal*. 2016;6(3):129-36.
6. Chuan F, Tang K, Jiang P, Zhou B, He X. Reliability and validity of the perfusion, extent, depth, infection and sensation (PEDIS) classification system and score in patients with diabetic foot ulcer. *PloS one*. 2015;10(4):e0124739.
  7. Fard AS, Esmaelzadeh M, Larijani B. Assessment and treatment of diabetic foot ulcer. *International journal of clinical practice*. 2007;61(11):1931-8.
  8. Wieman TJ, Smiell JM, Su Y. Efficacy and safety of a topical gel formulation of recombinant human platelet-derived growth factor-BB (becaplermin) in patients with chronic neuropathic diabetic ulcers: a phase III randomized placebo-controlled double-blind study. *Diabetes care*. 1998;21(5):822-7.
  9. Lotfy M. Biological activity of bee propolis in health and disease. *Asian Pac J Cancer Prev*. 2006;7(1):22-31.
  10. Afsharpour F, Hashemipour S, Khadem-Haghighian H, Koushan Y. Effects of Iranian propolis on glucose metabolic changes, inflammatory factors, liver enzymes levels in type 2 diabetic patients: a randomized, double-blind, placebo-controlled, clinical trial. *Journal of Nutritional Sciences and Dietetics*. 2017;3(2).
  11. Fernandes Jr A, Balestrin E, Cunha M. Anti *Staphylococcus aureus* activity of bee propolis extracts prepared with different ethanol concentrations. *Revista de Ciências Farmacêuticas*. 2003;24(2):147-52.
  12. Khadem HH, Koushan Y, Asgharzadeh A. Treatment of Diabetic Foot Ulcer with Propolis and Olive Oil: A Case Report. 2012.
  13. Ghosh S, Saha S, Hossain S, Sengupta D, Roy S, Roy C. Healing of Diabetic Foot Ulcer by Homeopathic Therapeutic Aid: A Case Study. *American Journal of Homeopathic Medicine*. 2012;105(1).
  14. Farooqui T, Farooqui AA. Beneficial effects of propolis on human health and neurological diseases. *Front Biosci (Elite Ed)*. 2012;4:779-93.
  15. Guo X, Chen B, Luo L, Zhang X, Dai X, Gong S. Chemical compositions and antioxidant activities of water extracts of Chinese propolis. *Journal of agricultural and food chemistry*. 2011;59(23):12610-6.
  16. Abreu AM, Oliveira DWD, Marinho SA, Lima NL, de Miranda JL, Verli FD. Effect of topical application of different substances on fibroplasia in cutaneous surgical wounds. *ISRN dermatology*. 2012;2012.
  17. Panzarini SR, Nonato CC, Gulinelli JL, Poi WR, Sonoda CK, Saito CTMH, et al. Effect of the treatment of root surface-adhered necrotic periodontal ligament with propolis or fluoride in delayed rat tooth replantation. *Clinical oral investigations*. 2014;18(4):1329-33.
  18. Temiz M, Aslan A, Canbolant E, Hakverdi S, Polat G, Uzun S, et al. Effect of propolis on healing in experimental colon anastomosis in rats. *Advances in therapy*. 2008;25(2):159-67.
  19. Sehn E, Hernandez L, Franco S, Gonçalves C, Baesso M. Dynamics of reepithelialisation and penetration rate of a bee propolis formulation during cutaneous wounds healing. *Analytica Chimica Acta*. 2009;635(1):115-20.
  20. Gulinelli JL, Panzarini SR, Fattah CMRdS, Poi WR, Sonoda CK, Negri MR, et al. Effect of root surface treatment with propolis and fluoride in delayed tooth replantation in rats. *Dental Traumatology*. 2008;24(6):651-7.
  21. Paulino N, Abreu SRL, Uto Y, Koyama D, Nagasawa H, Hori H, et al. Anti-inflammatory effects of a bioavailable compound, Artepillin C, in Brazilian propolis. *European Journal of Pharmacology*. 2008;587(1):296-301.
  22. Serarslan G, Altuğ E, Kontas T, Atik E, Avci G. Caffeic acid phenethyl ester accelerates cutaneous wound healing in a rat model and decreases oxidative stress. *Clinical and experimental dermatology*. 2007;32(6):709-15.
  23. McLennan SV, Bonner J, Milne S, Lo L, Charlton A, Kurup S, et al. The anti-inflammatory agent Propolis improves wound healing in a rodent model of experimental diabetes. *Wound Repair and Regeneration*. 2008;16(5):706-13.
  24. Ahmed N. Advanced glycation endproducts—role in pathology of diabetic complications. *Diabetes research and clinical practice*. 2005;67(1):3-21.
  25. Bankova V. Chemical diversity of propolis and the problem of standardization. *Journal of ethnopharmacology*. 2005;100(1):114-7.
  26. Russo A, Longo R, Vanella A. Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. *Fitoterapia*. 2002;73:S21-S9.
  27. Hussain Z, Thu HE, Shuid AN, Katas H, Hussain F. Recent advances in polymer-based wound dressings for the treatment of diabetic foot ulcer: an overview of state-of-the-art. *Current Drug Targets*. 2017;18:000-.
  28. Leaper D, Meaume S, Apelqvist J. *Debridement methods of non-viable tissue in wounds*. Cambridge: Woodhead Publishing; 2011.