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Dose Topically Administration of Propolis Could Improve the Impaired Wound Healing in Diabetic Rats?

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Abstract

Background: The impaired wound healing in diabetes mellitus is a major clinical problem. Propolis is a mixture collected from trees by the honey bee, which has important pharmacological characteristic.

Objectives: This study investigated whether topical administration of propolis could improve the impaired wound healing in diabetic rats.

Materials and methods: Full-thickness skin wounds were created on the backs of streptozotocin (STZ) induced diabetic rats. Diabetic rats were then divided into 2 groups: propolis-treated group that was topically administered and a control group without propolis administration. Improvement percentage and morphometric parameters of skin wound were estimated.

Results: Wound closure was significantly accelerated by topical administration of propolis. In propolis-treated wounds there were significant increases in collagen regeneration, new blood vessel numbers, and epithelialization compared with the control group ($p<0.05$).

Conclusions: Propolis can improve the impaired healing of diabetic wounds. This effect might involve an increase in the collagen regeneration, new blood vessel numbers, and epithelialization. Therefore, the use of propolis may be extended to the clinical setting and prove an effective promoter of wound healing in patients with diabetes.

Keywords: Diabetes mellitus; Diabetic ulcers; Propolis; Wound healing

Introduction

Diabetes is one of the most common diseases in the world. About 220 million people in the world and 2 million people in Iran suffer from diabetes [1]. Its prevalence is increasing fast in Iran like other country [2].

Diabetes redounds to lower extremity amputation. Ischemia and neuropathy are the main causal factor for this problem. Dermal ulcers in diabetic patients remain a main cause of morbidity, loss of functional capacity, and health care costs. Foot ulcers create a difficult clinical management challenge. In diabetic patients, dermal ulceration and wound healing failure account for 72% of amputations is associated with gangrene [3]. Treatment of diabetic ulcers is costly and treatment failures are common. Average prices in excess of $40,000 have been reported for this problem treatment. The wound healing is dependent on interactions between cells and the extracellular matrix, an essential component of tissues. Ulcer complications have requirement amputation. Amputation does not end the morbidity cycle and high cost because the prognosis for the improvement leg in diabetic amputees is poor. In one study between 854 patients with diabetes, 12% of hospital admissions and 21% of total days of hospitalization were relevant to lower extremity ulcers. According to one study, 50% of surviving unilateral amputees undergoes improvement amputation in 24 months [3]. The normal healing wound process takes place at an optimal rate, but it is impaired in patients with diabetes. High blood glucose make difficult reproduction of cells and decreases production of collagen. Many studies have demonstrated that plant drugs have positive effective in diabetic foot ulcer treatment [4]. In the past decade, new methods such as Agni pars, growth factor gel, was introduced for diabetic foot ulcer treatment. Until now there aren't useful and effective treatments for diabetic foot ulcer [5]. Propolis is a mixture collected from trees by the honey bee, which has important pharmacological characteristic and it is useful for a wide range of purposes as

anti-inflammatory and hypotensive agent, immune system stimulant, and bacteriostatic. Many other properties of propolis are: cartilage, bone, and dental pulp regeneration, immunological properties, liver defense and antitoxic activity, antioxidant and immunomodulatory actions. Many studies had investigated the antibacterial activity of propolis [6]. Propolis samples have more than 150 components such as polyphenols, phenolic aldehydes, sesquiterpene quinines, amino acids, steroids and inorganic components [7]. Despite propolis has been implied in cicatricle phenomena, no study concerning the role played by Iranian propolis on wound healing in diabetes foot ulcer have been reported so far [8]. Thus, the aim of this study was to survey the effect of propolis on wound healing in streptozotocin-induced diabetic rats.

Materials and Methods

Crude samples of propolis were obtained from different region of Ahvaz, Iran. Propolis samples were cut into small pieces and stored in 4°C. Twenty eight grams of propolis were extracted by 100 ml of 70% (v/v) ethanol by orbital shaking at 150 rpm at room temperature for 5 days. Then ethanol extract of propolis was filtered and stored in the dark at 4°C and used within 2 months of preparation [8].

Study design

The experimental animals were divided into 2 groups (n=15) and received the following treatment: Group 1 (no treatment) and Group 2 (propolis). Animals in control group received normal saline as vehicle. During the intervention, animals were carefully monitored and weighed daily.

Animals

In this assay, 30 male albino rats of wistar strain (200-250 g), aged 6-8 weeks, were obtained from Physiology Research Center of Ahvaz Jundishapur University of Medical Sciences. The animals were maintained in the steel cages in an air condition room (22 ± 3°C, 55 ± 5% humidity and a 12 h light/dark cycle) and were maintained with free access to water and ad libitum standard laboratory diet.

Induction of diabetes mellitus

Diabetes was induced by an intraperitoneal injection of 55 mg/Kg streptozotocin (STZ) (Sigma, Aldrich, USA) dissolved in citrate buffer (0.1 M, PH: 4.6) was used for the induction of diabetes. Blood glucose levels were measured using a rapid glucometer (GR-102, TERUMO Co, Tokyo, Japan). One week after STZ injection, animals with blood glucose levels above 300 mg/dL were defined as diabetic and used in the study [9].

Full-thickness skin wound preparation

Two weeks after STZ administration, diabetic rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (45 mg/kg body weight). The dorsal regions were shaved with an electric clipper and the surgical area was disinfected with 70% alcohol (ethanol). A round section of full thickness skin (diameter 15 mm) was resected with scissors and hemostasis was obtained by direct pressure using sterile gauze. Wounds and surrounding areas were then covered with an adhesive-permeable dressing (Bioclusive; Johnson and Johnson Medical, Skipton, UK) [10].

Estimation of wound healing (wound closure)

Curative effect on the wound (wound closure) was evaluated by tracing the outer margins of the wound on each rat. Wound tracings were scanned using an image scanner (EPSON GT-8000; Nagano, Japan), and images were then exported to an image processing program (AutoCAD, version 2007, Public Domain Software). Wound areas calculated in square millimeters. Wound area was measured at 1, 7, 14 and 21 after wounding and the wound closure rate was expressed as the percentage of wound area compared with that on post-operative day (POD) 0 (100%). Rats were killed on PODs 1, 7, 14 and 21 using an overdose of sodium pentobarbital (300 mg/kg intraperitoneally) for histological evaluation.

Histological analysis

The wound and surrounding tissues were fixed with 10% formalin, embedded in paraffin, and sectioned. Sections of 5 mm thickness were stained using the naphthol AS-D chloroacetate esterase technique for neutrophil infiltration [11]. Vascularization and epithelialization were examined by immunohistochemical stains. Angiogenesis was assessed by immunohistochemistry (IHC) using a monoclonal antibody to the endothelial cell surface marker CD34 (mouse IgG1 QBEnd/10, Novocastra, UK).

Three separated sections of each wound were examined by light microscopy. The number of neutrophils, fibroblasts, and vessels were counted in 5 high power fields (100) over 3 separated sections. The stained sections of collagen and cytokeratin were displayed at 40 magnification on a monitor connected to a computer system. In treated group, the wounds were cleaned with normal saline solution and then dressed with 1 mL propolis paste twice daily. Measurement of the wound area (cm^2) was monitored planimetrically.

Statistical analysis

All the samples and standards were run in duplicate and the results were expressed as mean ± SD. The statistical comparison between the experimental groups was performed by independent-sample t-test using SPSS computer program. The probabilities of 5% or less ($p \leq 0.05$) were considered significant.

Results

No mortality was seen in the animals during the study. As general parameters for the evaluation of wound healing, histopathological examination and measurement of the wound area was used.

The wound healing effect of propolis extract was compared with the normal saline treated group on days 1, 7, 14 and 21 in the diabetic rat foot ulcer model. The results are shown in **Table 1**. On day 1, the baseline wound areas of control group and propolis extract group were similar, with no statistical difference (p=0.847, **Table 1**).

Table 1 Improvement percentage of skin wound at different times in two groups.

Day	1	7	14	21
Group		improvement percentage	improvement percentage	improvement percentage
No treatment	250 ± 10.7	204 ± 3.6	173 ± 4.7	149 ± 3.81
		18.4	30.8	40.4
Propolis	251 ± 10.05	132 ± 8.8	42 ± 13.42	2.4 ± 9.51
		49.42	83.9	99.08
Pv*	0.847	0.03	0.002	0.0001
*For comparison of between-group differences by independent t-test				

A trend of greater reduction of the ulcer area from days 1 to 21 was observed in the propolis extract group, with a statistical significant difference on day 7 as compared to the control treated group. Moreover, nearly all the rats in the propolis extract group had ulcers completely healed on day 21.

As shown in **Table 1** improvement percentage in the propolis group was better than control group throughout the study.

Histological analysis

The results of morphometric parameters were shown in **Table 2**. According to these results, epithelial diameter increasing in propolis group was better than control. Administration of propolis promoted increases in the epithelial diameter in treated group with statistical significance (**Table 2**).

Table 2 Morphometric parameters of skin wound at different times in two groups.

Morphometric parameters	days	0	7	14	21
	groups				
Epithelial diameter	No treatment	13.07 ± 3.9	23.02 ± 4.6	29.2 ± 5.02	32.01 ± 7.7
	propolis	12.9 ± 2.1	32.2 ± 9.9	39.8 ± 7.01	57.6 ± 4.7
Pv[1]		0.611	0.04	0.01	0.002
Healed wound cell numbers (in the 104 mm^2)	No treatment	78.2 ± 19.8	250.4 ± 55.04	492.02 ± 69.01	577.1 ± 48.8
	propolis	81.18 ± 25.4	603.2 ± 38.4	854.4 ± 37.9	998.025 ± 41.2
Pv[1]		0.820	0.03	0.002	0.0001
New blood vessel numbers (in the 104 mm^2)	No treatment	0	1.3 ± 0.7	1.9 ± 0.6	2.4 ± 0.5
	propolis	0	2.6 ± 0.3	4.9 ± 0.4	11.8 ± 0.7
Pv[1]			0.49	0.03	0.0001
New and relief collagen	No treatment	29.7 ± 2.5	47.05 ± 4.6	54.3 ± 4.8	761.03 ± 3.7
	propolis	32.7 ± 2.9	68.6 ± 4.3	84.6 ± 7.7	98.6 ± 9.4
Pv[1]		0.511	0.04	0.002	0.0003
[1]For comparison of between-group differences by independent t-test.					

Also, healed wound cell numbers, identified by immunohistochemical staining, was greater in the propolis group than in the control group, and a significant difference was seen. Vascularization was enhanced in the treated group. The number of new blood vessel in the subcutaneous layer after the wounding was significantly greater in the propolis group than in the control group. Moreover, the collagen regeneration area estimated by Azan–Mallory staining was significantly wider in the propolis group than in the control group. **Figure 1** shows healing of the injury in the 2 groups of study.

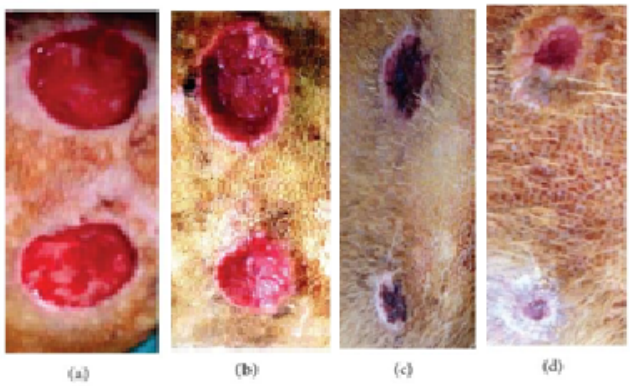

Figure 1 Representative skin wounds of the control group (upper wounds) and propolis group (lower wounds) at 0 (a), 7 (b), 14 (c) and 21(d) days after surgery.

Discussion

STZ selectively destroys pancreatic cells, inhibits the synthesis and release of insulin, and causes the onset of diabetes mellitus. It has been shown that blood glucose levels peak 1 to 3 days after a single high-dose injection of STZ, and then remain elevated. STZ-induced diabetes in rodents is considered to be a model of insulin-dependent diabetes mellitus and is widely used in the study of insulin-o penia and hyperglycemia [12]. In the present study, administration of propolis increased collagen regeneration, and epithelialization under hyperglycemic conditions.

Honey and propolis have been used for wound healing treatment from many years ago. In this study, results show that increasing epithelial diameter, cell number of healed wound tissue, new blood vessel numbers and percentages of new and relief collagen in the propolis group was more than control group. Decreasing of the wound tissue extent in the rats of the propolis group was more than control group.

Some anti-inflammatory substances found in propolis have been isolated. According to Mirzoeva and Calder, these substances are caffeic acid, quercetin, naringenin, and caffeic acid phenethyl ester (CAPE). These compounds contribute to the suppression of prostaglandins and leukotrienes synthesis by macrophages and have inhibitory effects on myeloperoxidase activity, NADPH-oxidase, ornithine decarboxylase and tyrosine-protein-kinase [13,14].

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 [15].

In this study, propolis was administered topically, as this substance has the capacity to penetrate the wound. Studies by Gulinelli has demonstrated that the vehicle that is employed does not alter the effects of propolis [16].

An alcohol vehicle was used in the present study. However, the use of an aqueous extract has been indicated for propolis [17].

According to Paulino artepillin-c, which is the main active ingredient in propolis, has an anti-inflammatory effect that is triggered by mechanisms associated with the inhibition of leukocyte activity. The authors state that the anti-inflammatory action of artepillin-c stems from the inhibition of nuclear factor kappa B, the reduction of prostaglandin E2, and the production and inhibition of nitric oxide production [18].

The findings of the present study are in agreement with those described by Sehn, who found that topical administration of propolis stimulated the collagen regeneration. When treating wounds with caffeic acid phenethyl ester, which is a component of propolis. Many studies demonstrated that propolis samples have antimicrobial activity for different microorganisms. All diabetic foot ulcer treatment has relative effect, so it is necessary that other noninvasive treatments with more efficiency will be created for this problem. Active components in propolis are flavonoids and phenolic acids [19].

In conclusion, our results indicated that propolis initially increases the collagen regeneration and epithelialization, which seem to be very important contributing factors to the improvement of diabetic wound healing. 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 lesions are often slow to heal. More detailed, in-depth studies should be carried out with the aim of investigating and isolating each active component of propolis, to determine its action in different vehicles of application.

Conflict of Interest

The authors declare that they have no conflict of interest.

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