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Original research article

Effect of topical mixture of honey, royal jelly and olive oil-propolis extract on skin wound healing in diabetic rats

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ABSTRACT

Background: Diabetic wound is a common complication of diabetes. The aim of this study was to evaluate the effects of topical application of mixture of honey, royal jelly and olive oil-propolis extract (Nika cream) on cutaneous wound healing in streptozotocin (STZ)-induced diabetic rats.
Materials and methods: In this interventional study, we prepared a new composition ointment entitled Nika cream. 42 Wistar male rats were divided randomly in normal and diabetic groups. Next, diabetes was induced by intraperitoneal injection of STZ (60 mg kg^{-1} body weight). The diabetic rats with marked hyperglycemia (serum glucose more than 200 mg/dl) were selected for the study. After anesthesia, full thickness skin of upper dorsal part of the rats was removed in 2 cm × 2 cm area. 24 h after the operation, wound in each animal was treated daily with Nika cream and phenytoin 1% in normal and diabetic groups. Control group received no treatment. Wound surface area was measured until 24th postoperative day. The time required for the completion of healing was also recorded.
Results: We demonstrated that Nika cream had distinct and non-exchangeable functions during the wound healing process. The progress of wound healing in diabetic animals was slower than in normal animals, and also healing effect of Nika cream compared with control and normal phenytoin 1% and diabetic test groups was significant ($P < 0.001$). General estimation of wound healing improvement of these groups is better than those of the control and phenytoin groups.
Conclusion: The results showed that Nika cream accelerate the wound healing in normal and diabetic subjects.

1. Introduction

Normal wound healing response begins at the very moment the tissue is injured. After the injury, an inflammatory response occurs and the cells below the dermis begin to increase collagen production. Afterward, the epithelial tissue is recovered and regenerated [1]. Diabetes mellitus (DM) is one of the common metabolic disorders associated with many complications in various organs [2]. It is epidemiologically and economically important in both developing and developed countries [3]. Nowadays it is known as a disabling disorder owing to a wide variety of irreversible complications such as renal failure, retinopathy, non-traumatic amputation, neuropathy and increasing risk of coronary heart disease, stroke and diabetic foot ulcer [4,5]. Wounds in diabetic patients typically show abnormal healing, characterized by hypergranulation, persistent inflammation, chronicity, increased bacterial load, copious exudate, and reduced ability to heal [6].

Many of the synthetic drugs used for treatment of wounds are not affordable because of their high cost. Some effective and new techniques, like recombinant growth factors or tissue-engineered wound dressings, are very expensive and not available for many patients in the developing countries [7]. Also, patients with chronic wounds require prolonged periods of dressings and this can cause a significant financial burden to the health-care system [8]. Nowadays, compared with synthetic drugs, traditional and herbal medicine is gaining popularity due to its widespread availability, moderate efficacy, no or fewer side effects and low cost [7].

Honey is a natural product made by honeybees. Previous reports from the literature show that honey has anti-inflammatory, antioxidant, and antimicrobial properties. In addition, honey has showed tissue-healing activities. It keeps wound moist, allowing epidermal migration, and provide trace nutrient that may aid healing [9,10].

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Propolis is a natural substance derived from plant resins gathered by honeybees. Propolis has antioxidative, antimicrobial, antitumor, and antiulcer effects [11]. In traditional medicine, propolis has been used for curing infections and healing wounds and burns [12].

Olive oil is extract from the olive (*Olea europaea*), a species from the *Oleaceae* family, a native plant, growing widely in the east Mediterranean region. Studies on animal showed that topical use of olive oil improves cutaneous wound and burns healing [7,9,13].

Considering the importance of diabetes and its costly complications such as foot ulcer, finding out the new medication with minimal side effects is essential. The aim of this study was to evaluate the effects of topical application of mixture of honey, royal jelly and olive oil-propolis extract on cutaneous wound healing in diabetic rats induced by streptozotocin (STZ) in male rats.

2. Materials and methods

2.1. The mixture of honey, olive oil-propolis extract, and royal jelly (Nika cream)

In this interventional study, first we prepared a new composition ointment entitled Nika cream (Patent register number: 79378, 2013 May 4). Ten grams of grinded propolis was mixed with 50 mL olive oil. This mixture was kept in a well-sealed glass container away from light for 3 months. Thereafter the mixture was gently heated in a water bath, having a maximum temperature of 40 °C, with continued mixing for 30 min. It was followed by filtration to separate the fine granules of propolis and to obtain olive oil propolis extract. Twenty milliliters of this extract was then mixed with 5 mL royal jelly in a liquid state and 35 mL honey. The mixture was then heated in a water bath with continued mixing to obtain a homogenous mixture of honey, olive oil propolis extract and royal jelly. The prepared mixture was then kept in dark well-sealed containers until use.

2.2. Drugs

Drug used were streptozotocin (STZ; Sigma, USA), phenytoin (Darou Pakhsh Company, Iran), ketamine hydrochloride (alfasan, Woerden-Holland, 50 mg kg^{-1} body weight) and xylazine hydrochloride (alfasan, Woerden-Holland, 10 mg kg^{-1} body weight). STZ was dissolved in 5 mM citrate buffer (pH 4.0) and immediately used after preparation. Phenytoin prepared as 1% ointment. Ketamine and xylazine used as anesthetic agent.

2.3. Experimental animal

The study protocol was approved by the Ethical Committee of Bu-Ali Sina University, Hamedan, Iran, according to the principles of laboratory animal care. 42 Wistar male rats (220–240 g) were housed in standard polycarbonate cages in a temperature-controlled room (22 ± 2 °C) on a 12-h light/dark cycle with free access to food and water and were acclimated at least one week before experiments. Animals were made insulin-dependent diabetic by intraperitoneal injection of 60 mg kg^{-1} body weight STZ. After 3 days following STZ administration, blood samples were taken from tail vein and hyperglycemia was confirmed by measuring blood glucose levels, using a glucometer (GlucoDr. Plus, AGM-3000, Korea). Animals showing fasting blood glucose higher than 250 mg/dl were considered as diabetic mice. Animals were divided randomly into non-diabetic (ND) and diabetic (DM) groups as follow (n = 7): control groups (without any treatment), groups which treated with phenytoin 1% and groups which treated with *Nika cream*.

2.4. Surgical wound model and planimetry

Two weeks after diabetes induction, dorsum of the rats was shaved and under general anesthesia and sterile condition, a square measuring of 2 cm × 2 cm was outlined in each animal using a marker. Then, the demarcated areas of skin were removed by scalpel. The wounds were left undressed after hemostasis. Animals were closely observed for any infection; those which showed signs of infection were separated and excluded from the study. Photographs were taken immediately after wounding and on days 3, 6, 9, 12, 15, 18, 21 and 24 post-operation by a digital camera while a ruler was placed near the wounds. The wound areas were analyzed by Measuring Tool of Adobe Acrobat 9 Pro Extended software (Adobe Systems Inc., San Jose, CA, USA).

2.5. Statistical analysis

Statistical analysis was performed using SPSS software (version 22; SPSS Inc., Chicago, IL, USA). Kolmogorov–Smirnov test was used to analyze the normality of the data distribution. The results were statistically assessed using one-way ANOVA analysis of variance. Following a significant P-value, post hoc analysis (Tukey's test) was performed for multiple comparison. The significance between before and after of each corresponding groups was assessed with the help of the paired sample t-test. The results are displayed as a mean value with standard error of mean (mean ± SEM). A level for $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of diabetes on blood glucose level and body weight

At first, there was not any difference between studied groups in terms of blood glucose level and body weight. In the end of study, non-diabetic groups showed body weight increment, whereas diabetic groups demonstrated decrement (Table 1). Diabetic animals had a significant increase in their blood glucose 3 days after diabetes induction (82.71 ± 3.637 mg/dl vs. 462.43 ± 16.533 mg/dl; $P < 0.001$) which was persistent afterwards (Table 2).

3.2. Effect of topical Nika cream on skin wound closure

As shown in Table 3 and Fig. 1, results demonstrate that Nika cream have distinct and non-exchangeable functions during wound healing. The progress of wound healing in diabetic animals was slower than in normal animals. Also, applying Nika cream accelerated wound closure in both non-diabetic and diabetic rats. In non-diabetic groups Nika cream significantly accelerated wound closure compared with both control (184.29 ± 0.12 mm^2 vs. 5 ± 0.03 mm^2; $P < 0.001$ in day 12) and phenytoin 1% (184.29 ± 0.12 mm^2 vs. 5 ± 0.03 mm^2; $P < 0.001$ in day 12). Also,

Table 1
Body weight of separate groups recorded at arrival and end of the study.

Treatment	Body weight (g)	
	Arrival	End of the trail
ND + Control	235.29 ± 1.66	266 ± 3.35
ND + Phenytoin	231 ± 2.66	260.29 ± 2.11
ND + Nika cream	233.29 ± 2.73	262.43 ± 3.34
DM + Control	226.86 ± 2.64	204.71 ± 3.62[a]
DM + Phenytoin	224.43 ± 2.94	200.29 ± 3.41[a]
DM + Nika cream	229 ± 3.81	232.71 ± 4.76[a,b]

Results are presented as mean ± SEM (n = 7).
[a] Significantly different vs. non-diabetic control at $P < 0.001$.
[b] Significantly different vs. diabetic control at $P < 0.001$.