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THE EFFECT OF TOPICAL HONEY DRESSING ON WOUND HEALING IN DIABETIC MICE

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Purpose: To assess the effect of topical application of honey on wound healing in diabetic mice.

Materials and Methods: Twenty-seven Swiss mice were used for the study, in which diabetes was induced via streptozotocin. Full-thickness skin defects 1 cm in diameter were created on both sides on the backs of the animals. The defects on the left side comprised the experimental group, whereas those on the right side were used as controls. Left-sided defects were treated with honey, while right-sided wounds were treated with isotonic sodium chloride. The mice were sacrificed on days 3, 6, and 9. All wounds were histopathologically evaluated according to the thickness of the granulation tissue and the distance of epithelization covered from the normal skin edge into the wound. The wound areas were calculated using 3D-DOCTOR (Trial Version, Able Software Corp., USA) software. A paired-sample t test was used for analysis.

Results: When the region treated with honey was compared with the control region on the same animal, the wound area was significantly smaller (Day 3 $p<0.001$, Day 6 $p<0.05$, Day 9 $p<0.01$) and epithelization advancement was significantly greater (Day 3 $p<0.05$, Day 6 $p<0.05$, Day 9 $p<0.001$), but the thickness of the granulation tissues was not significantly different (Day 3, 6, 9 $p>0.05$).

Conclusion: This study demonstrates that topical application of honey has a positive influence on wound healing, via facilitating wound contraction and epithelization in diabetic mice.

Key Words: Honey, wound healing, diabetes, mouse.

TOPİKAL BAL UYGULAMASININ DİYABETİK FARELERDE YARA İYİLEŞMESİ ÜZERİNDEKİ ETKİSİ

Amaç: Bu çalışmanın amacı, diyabetik farelerde topical bal uygulamasının yara iyileşmesi üzerindeki etkisinin araştırılmasıdır.

Araç ve Yöntem: Bu çalışmada, Streptozotosin ile diyabet indüklenmiş olan 27 adet İsviçre faresi kullanıldı. Hayvanların sırtlarında bilateral 1 cm çapında tam kat deri defektleri oluşturuldu. Sol taraftaki defektler deney grubunu, sağ taraftaki defektler ise kontrol grubunu oluşturdu. Deney tarafındaki defektlere bal, kontrol tarafındaki defektlere ise izotonik sodyum klorür tatbik edildi. Fareler 3, 6 ve 9. günlerde sakrifiye edildiler. Tüm yaralar histopatolojik olarak granülasyon dokusu kalınlığı ve yara kenarından epitel ilerlemesi yönünden değerlendirildiler. Yara yüzölçümleri 3D-DOCTOR (Deneme sürümü, Able Software Corp. USA) yazılımı ile hesaplandı. İstatistiki analiz için ikili sample-T testi uygulandı.

Sonuçlar: Bal ile tedavi edilen bölgeler, control bölgeleri ile kıyaslandığında, yara yüzeyi anlamlı olarak daha küçüktü (3, gün $p<0,001$, 6. gün $p<0,05$, 9. gün $p<0,01$), ve epitelizasyonda ilerleme anlamlı olarak daha fazlaydı (3. gün $p<0,05$, 6. gün $p<0,05$, 9. gün $p<0,001$). Ne var ki, granülasyon dokusu kalınlıkları arasındaki farklar anlamlı değildi. (3., 6. ve 9. günlerde $p>0.05$).

Sonuç: Bu çalışma göstermektedir ki, diabetik farelerde topikal bal uygulaması, yara kontraksiyonu ve epitelizasyonu üzerinden yara iyileşmesinde olumlu etkiye sahiptir.

Anahtar Sözcükler: Bal, Yara iyileşmesi, Diyabet, Fare.

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INTRODUCTION

It is well known that honey had been topically used for its positive effects on wound healing since ancient times. Its topical use was cited in the medical literature of Egypt, Greece, and the ayurvedic medicine of India.[1-3] As a dressing on wounds, honey is said to reduce inflammation, debride necrotic tissue, reduce edema, and promote angiogenesis, granulation, and epithelization.[2,4] Its use was also reported as an adjunct for acceleration of wound healing in ulcers, infected wounds, and burns.[5,6] The aim of this study was to assess the effect of the topical application of honey on wound healing in diabetic mice. We evaluated the effect of unboiled commercial honey (with no additives) on wound healing in a non-infected, healing-impeded acute wound model. Our review of the English literature revealed no experimental studies interpreting the effects of honey on a diabetic wound.

MATERİALS AND METHODS

The study was approved by the Ethical Committee of Ondokuz Mayıs University, and was performed in accordance with the standards of the ethical guidelines for the care and handling of labora-

Figure 1: Marking on the back of the mice.

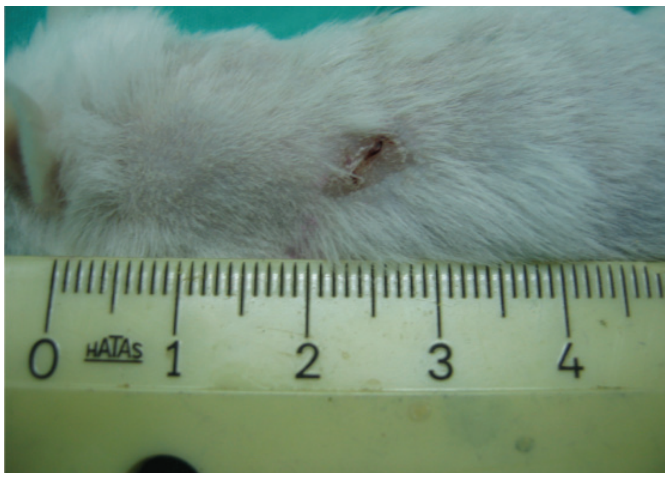

Figure 2a: Photograph of the trial defect by the end of 9 days.

tory animals. The male Swiss mice, which weighed 25-35 g, were housed in individual cages and fed a regular mouse diet and tap water. A single intraperitoneal dose of 80 mg/kg streptozotocin (Serva Electrophoresis GmbH, Germany) was used to induce destruction of pancreatic islet cells and persistent hyperglycemia. Blood glucose levels were monitored weekly using a blood glucose meter (Prestige Smart System; Home Diagnostics Inc., USA). Diabetes was defined as a random blood glucose level of more than 200 mg/dl for more than 4 weeks after injection, and 27 mice meeting these criteria were included in the study.

The animals were anesthetized with an intraperitoneal injection of 50 mg/kg ketamine, and full thickness skin defects with diameters of 1 cm down to the muscle layer were created on either side on the backs of the mice (Figure 1). The skin was held under constant tension during the process. The left-sided defects were used for the experimental treatment, while the right-sided defects served as controls.

Unboiled commercial honey with no additives was applied as a thin layer to the left-sided wounds. The right-sided defects were treated with physiological isotonic sodium chloride. No dressings were needed to cover the wounds as the mice were individually caged and it was observed to be impossible for

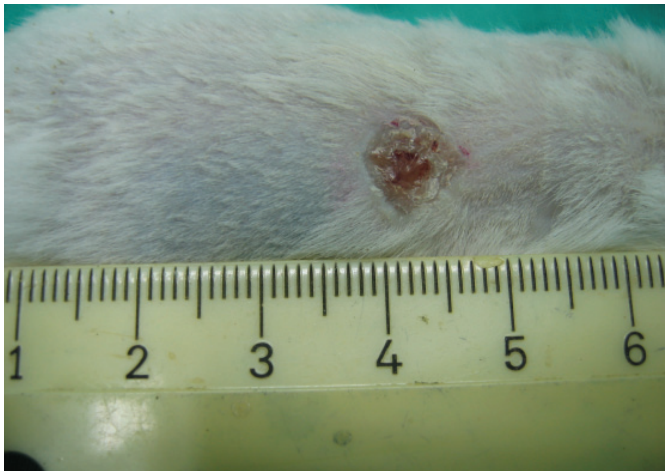

Figure 2B: Photograph of the control defect by the end of 9 days.

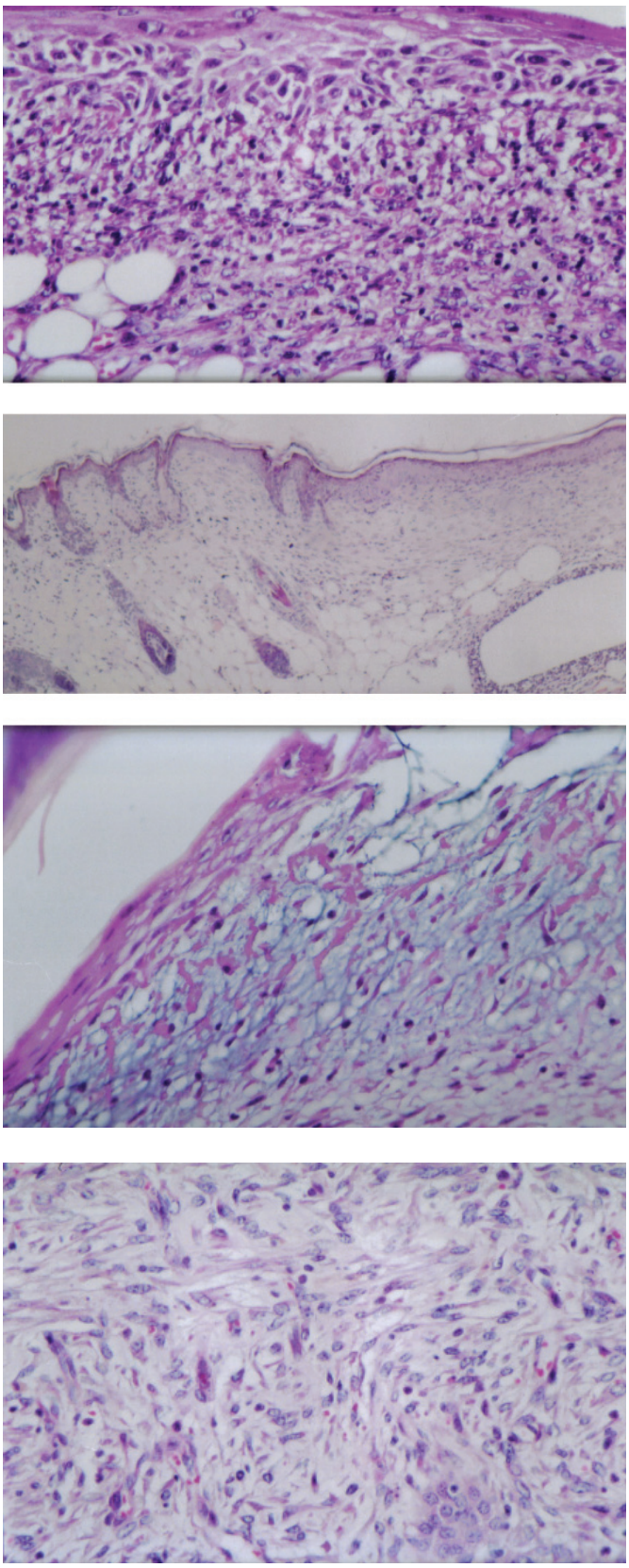

Figure 3: (a) Immature stratified squamous epithelium in the control group. (X200, H.E.). (b) Acanthotic reepithelization in the trial group. (X100, H.E.) (c) Myxoid degeneration based upon granulation tissue in the control group (X200, H.E.). (d) Mature granulation tissue in the trial group. (X200, H.E.)

the mice to reach the defects on their backs and cause self-leaking. All of the wounds were checked and locally treated with honey or isotonic sodium chloride twice daily at the same hour. Nine mice were sacrificed on each of the days 3, 6, and 9. Digital images of each pair of defects were taken (Digital IXUS 500 Canon, Japan) while the animal was lying prone on the table (Figure 2A,B). Afterwards, the animals were preserved in 10%-formalin solutions for 4 days. The wounds were then excised with 2 cm intact peripheral tissue from the wound edges and including the subcutaneous muscular layer. The specimens were prepared on the sagittal plane, close to the wound centre, and stained with haemotoxylin-eosin. The granulation tissue was examined under magnification (X40) with a light microscope and was measured in the centre of the wound. Epithelial advancement from the edges into the wound was evaluated. Areas of the wounds were calculated by 3D-DOCTOR software (Trial Version, Able Software Corp., USA). Statistical analysis of the data was performed with the paired-t test. All values were expressed as mean ± SD.

The main flowers involved in the collection of nectar for honey were thyme (*Thymus serpillum*) and astragalus (*Astragalus microcephalus*) available in central Anatolia (Turkey). The type of the bee producing the honey was the Anatolian honey bee (*Apis mellifera anatoliaca*).

RESULTS

On day 3, a comparison of the wounds revealed a 30% smaller wound area ($p<0.001$), and 10% more epithelial advancement ($p<0.05$) for the defects treated with honey. There were no differences ($p>0.05$) with regard to the thickness of the granulation tissue (Table 1).

On day 6, a comparison of the wounds revealed a 27% smaller wound area ($p<0.05$), and 19% more epithelial advancement ($p<0.05$) for the defects treated with honey. There were no differences ($p>0.05$) with regard to the thickness of the granulation tissue (Table 2).

On day 9, a comparison of the wounds revealed a 42% smaller wound area ($p<0.01$), and 42% more epithelial advancement ($p<0.001$) for the defects treated with honey. There were no differences ($p>0.05$) with regard to the thickness of the granulation tissue (Table 3).

The mean wound area was smaller (especially on day 3) and more epithelial advancement was observed (especially on day 9) in the honey-treated defects (Figure 3a,b). There were no differences between the two wound groups in terms of granulation tissue thickness (Figure 3c,d). No symptoms suggesting clinical infection were observed during the trial and no further treatment was warranted

Table 1: Results on day 3.

	Area of the wound (mm^2)	**Thickness of granulation tissue (mm)**	**Distance of epithelization (mm)**
Control	57.74 ± 9.90	0.27 ± 0.03	1.187 ± 0.164
Honey-treated	40.29 ± 7.74	0.26 ± 0.03	1.331 ± 0.187
	($p<0.001$)	($p>0.05$)	($p<0.05$)

Table 2: Results on day 6.

	Area of the wound (mm^2)	**Thickness of granulation tissue (mm)**	**Distance of epithelization (mm)**
Control	45.17 ±8.65	0.64 ± 0.09	1.323 ± 0.129
Honey-treated	32.89 ± 5.10	0.61 ± 0.07	1.585 ± 0.192
	($p<0.05$)	($p>0.05$)	($p<0.05$)

Table 3: Results on day 9.

	Area of the wound (mm^2)	**Thickness of granulation tissue (mm)**	**Distance of epithelization (mm)**
Control	19.66 ± 5.61	0.58 ± 0.07	1.983 ± 0.249
Honey-treated	11.29 ± 3.93	0.69 ± 0.07	2.834 ± 0.199
	($p<0.01$)	($p>0.05$)	($p<0.001$)

DISCUSSION

There are clinical and experimental trials claiming that honey accelerates wound healing and impedes bacterial colonization[1,7]. Although the exact mechanism for prevention of bacterial colonization is still unclear, there are data suggesting that the hyperosmolar structure of the honey is involved. Moore et al. reviewed the literature on the medical use of honey for dressing superficial burns, split-thickness graft donor sites, and infected postoperative wounds. They concluded that honey substantially accelerates wound healing[8]. Successful use of honey treatment was also reported for various wounds of diverse etiology, including venous leg ulcers, diabetic foot ulcers, pressure sores, and postoperative wound infection of the newborn[6].

Diabetes mellitus is one of the major metabolic disorders impeding wound healing and it is well known that diabetic individuals display a prolonged healing period of surgical wounds. In chronic diabetic wounds, traditional wet-dry wound dressing is generally unsatisfactory, and even modern dressings may sometimes be insufficient. Furthermore, prolonged use of special dressing products manufactured for optimal wound conditioning requires an extensive cost and special equipment. In this study, the effect of unboiled commercial honey with no additives on wound healing was evaluated in a non-infected, acute, healing-impeded wound model.

The findings of this study indicate that honey enhances epithelization and wound contraction in an acute diabetic wound. This effect can be attributed to the stimulating cytokines that honey contains. Additionally, the acidic structure of the honey provides a suitable environment for fibroblast migration, proliferation, and collagen organization. A lower level of hydrogen peroxide is also thought to induce early growth genes, which have an important effect on wound healing[3]. In a study assessing the effect of honey on cell culture, it was shown that the honey increases the concentrations of T and B lymphocytes, and accelerates immune response against infection by inducing release of a variety of cytokines from mastocytes[6]. In their study, Tonks et al. incubated monocyte cultures with commercial medical honey, and showed that release of TNF-α, IL-1β, and IL-6 was increased in culture supernatants[2]. These inflammatory cytokines would play a critical role in wound healing.

Subrahmanyam et al. demonstrated that acceleration of epithelization with topical use of honey occurs between days 6 and 9[1]. As a similar finding, we observed that the epithelization of the honey-treated defects is faster than the controls by the third day and this difference reaches a plateau between days 6 and 9. However, this study failed to show a positive effect for topical use of honey on granulation tissue formation.

In conclusion, topical application of honey to surgical defects in diabetic mice accelerates epithelization and decreases the defect size in comparison with the controls. The results of this study should be supported further with controlled clinical studies.

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