

Topical Application of Honey for Treatment of Skin Wound in Mice

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Abstract

Background: Honey has been shown to accelerate wound healing, which is especially important in the management of patients with full-thickness wounds of skin in dermatologic surgeries.

Objective: To evaluate the effects of honey in accelerating healing of full-thickness skin wounds in mice.

Methods: Two groups of male NMRI mice (n=12) were subjected to full-thickness skin wounds under general anesthesia. They were then randomly allocated to receive either single daily applications of placebo or honey (n=6 for each group). On each of the 4th, 7th and 10th days after operation, 2 mice from each group were sacrificed using an overdose of anesthetic. Macroscopic and microscopic characteristics of wounds were studied pathologically, histologically, and for resilience, ultimate tensile strength and toughness.

Results: Honey increased the formation of granulation tissue, density and activation of fibroblasts, keratinization in surface of wound, thickness of basement membrane and epidermis, and thickness of collagen fiber. It also decreased infection, inflammation, edema and dehiscence, and increased resilience, ultimate tensile strength and toughness of wound.

Conclusion: Honey accelerates healing of full-thickness skin wounds in mice.

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Keywords • Wound healing • Honey • Resilience • Mice

Introduction

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Maintaining skin integrity is vital for human and animals to protect against dehydration, bleeding and invasion of microorganisms. To do so, animals evolved a sophisticated mechanism of wound healing to quickly plug the gap, re-epithelialize over the defect, and rapidly replace the lost dermis with new matrix. Clearly, the speed of wound healing depends upon many factors including the size of the wound, blood supply to the area, presence of foreign bodies and microorganisms, and age, health and nutritional status of patients.¹ Management of patients with full-thickness skin wound continues to challenge physicians and surgeons in area of cosmetic dermatologic surgery. Currently, there are some medications that can be used to accelerate the healing of full-thickness skin wounds.

Table 1: Microscopic features of wounds in honey-treated (HG) and control (CN) groups at day-4, -7 and -10 of operation.

Parameters	Day 4 HG	Day 4 CN	Day 7 HG	Day 7 CN	Day 10 HG	Day 10 CN
Cell density	++	++++	-/+	++	-	+
Cell types (%)	PMN (40) L (60)	PMN (98) L (2)	PMN (1) L (99)	PMN (20) L (80)	-	PMN (15) L (85)
Granulation	+++	-	++++	++	-	+/-
Edema	+	++	+/-	+	-	+/-
Fibroblast density	++	-	++++	++	++	+++
Fibroblast activation	++	-	++++	++	++	+++
Basal cell layer/s	5	2-3	2	4	1	1-2
Edge thickness (layers)	9	7	6	5	3	3
Density and type of	++	+	+++	++	++	+++
Keratinization	O and P	P	O	O and P	O	O and P
Thickness of collagen fibers	ABN	ABN	60% of N	30% of N	85% of N	50% of N

PMN= Poly Morpho Nuclear; L= lymph; O= orthokeratotic; P= parakeratotic; N= normal; ABN= abnormal

- negative, +/- very mild, + mild, ++ mild to moderate, +++ moderate, ++++ severe

The use of honey as a medicine is referred to in the most ancient written records. Honey was prescribed by ancient physicians for a wide variety of ailments. The ancient use of honey as a wound dressing has been described by Forrest.² The ancient Egyptians, Assyrians, Chinese, Greeks and Romans, all used honey in combination with other herbs or on its own, to treat wounds and diseases of the gut³. In several reports, the rapid healing observed with honey dressings is noted.⁴⁻⁶

A number of authors, reporting the use of honey as a dressing on infected wounds, attributed its effectiveness, at least partly, to its antibacterial properties.⁷⁻⁹ Others reported that honey promoted the formation of clean healthy granulation tissue^{10,11}, and growth of epithelium over the wound.^{12,13} Thus, it helps skin regenerate, making plastic surgery unnecessary.^{14,15} Honey has been reported to reduce inflammation,¹⁶ edema and exudation.¹⁷ However, there is a tendency for some practitioners to dismiss any suggestion that treatment with honey is worthy of consideration as a remedy in modern medicine. An editorial published in the *Archives of Internal Medicine* assigned honey to the category of "worthless but harmless substances".¹⁸

In light of such controversies and consideration that honey improves the healing of burn wounds, this study was designed to examine whether honey could accelerate the healing of full-thickness skin wound.

Methods and Materials

Twelve male NMRI mice were randomly allocated to control (n=6) or honey-treated groups (n=6). Under general anesthesia a 2-mm full-thickness wound was made on the back skin of each animal. The control group received simple dressing with sterile gauze, whereas honey group received daily topical applications of natural Urmia (A city in North-West of Iran)

honey (1 g/day). On the 4th, 7th and 10th day of operation, two mice from each group were sacrificed with an overdose of ether anesthesia. Afterwards, a piece of treated skin was removed and fixed with 10% formalin solution. After fixation, routine processes of tissue preparation including dehydration, clearing and infiltration was performed. Specimens were then embedded in paraffin blocks. The paraffin blocks were trimmed, and thin serial sections (3–5 µm) were cut with a 2145 rotary microtome (LICA Company, Germany). Some sections were randomly selected and stained with hematoxylin and eosin (H&E). The sections were studied by a dermatologist and two pathologists, who were blind to the treatment used. The study of sections included; 1) gross pathology for the presence of infections, dehiscence and repair; 2) histological evaluations of wound site for the degree of healing; and 3) wound resilience, ultimate strength and toughness. The last ones were defined, respectively, as the ability of the wound to stretch and then resume shape without incurring any tissue damage, the maximum pressure it could tolerate before starting to weaken, and the total amount of pressure it could tolerate before rupturing.

Results

On day-4 of the operation, there was no dehiscence, infection or exudates in the honey-treated group, but scab was seen on the surface of wounds (Table 1). The control group did not have infection, but showed exudates and scab on surface of wounds. The mean distance between the two edges of the wound for honey-treated and control groups were 0.4 ± 0.03 and 0.2 ± 0.06 mm respectively. The surface of wounds was pinkish in both groups, however, wounds in the control group was more swollen and warmer than that of the honey-treated group. Relative to the control

group, the honey-treated group had increased formation of granulation tissue, density and activation of fibroblasts, keratinization in surface of the wound, and thickness of basal cell layer and epidermis. Moreover, inflammation, edema and dehiscence had decreased as compared to the control group (Table1). Most of inflammatory cells in the wounds of control group were polymorphonuclear cells (98%), while in the honey-treated group inflammatory cells found in the wounds were mostly lymphocyte (60%).

There was no dehiscence, infection and exudates, but scab on surface of wounds in the honey-treated and control groups on day-7 of operation (Table 1). The mean distance between two edges of wound in honey-treated group was 0.3 ± 0.04 mm, whereas in control group it was 2 ± 0.03 mm. In both groups the surfaces of wounds were pinkish; however, it was more swollen and warmer in the control group. Relative to the control group, the honey-treated group had increased formation of granulation tissue, density and activation of fibroblasts, keratinization in surface of the wound. Furthermore, thickness of basal membrane, epidermis and collagen fibers were more pronounced. Less inflammation, edema and dehiscence was observed in the honey-treated group than of control group. Most of the inflammatory cells in wounds of the control group were lymphocyte (80%), while inflammatory cells in the control group were mostly lymphocyte (99%).

There was no dehiscence, infection or exudates in both groups 10 days 10 after the operation (Table 1). The surface of wounds was normal in honey-treated group. While in the control group it was reddish and contained scar tissue. Relative to the wounds of control group, that of honey-treated group had increased density and thickness of collagen fiber (Table 1). There was no inflammation of wounds in honey-treated group, where the control group had some degree of inflammation, which was less than that on day-7. Inflammatory cells present in the wounds in control group were mainly lymphocyte (85%). In comparison to the control group in honey-treated group, wounds had increased resilience, ultimate tensile strength and toughness.

Discussion

Honey is not just mythological nourishment from the Gods. Actual Egyptian medical texts dating back to 2600–2200 BC mentioned that honey in at least 900 remedies. Many early cultures hailed honey for its sweetness, nutritional values and its topical healing properties

for wounds, sores, and skin ulcers. During wartime, honey was used as an antiseptic for wounds by ancient Egyptians, Assyrians, Greeks, Chinese, as well as modern Germans as late as World War I. From a scientific standpoint, there have been numerous studies over the last two decades verifying the power of honey to heal wounds and topical ulcers.^{3,7-9}

In agreements with previous reports^{10,13,19,20}, the finding of the present study indicate that honey increased the formation of granulation tissue, density and activation of fibroblasts, keratinization in the surface of wound, thickness of epidermis and thickness of collagen fibers. Moreover, in keeping with earlier reports,^{7-9,16,17,21-23} honey decreases infection, inflammation, edema and dehiscence. As well, honey increases the rate of wound healing, which confirms previous reports.^{4-6,24-27}

Some findings of the present study do not receive support from some previous studies. As an example, in one study honey failed to enhance wound healing in male Wistar rats, in which skin incisions on their back had been infected with *E coli*. In this study once the wounds presented abscess collection, the animals were assigned to one of the groups receiving topical applications of honey, hexachlorophene or honey and hexachlorophene. The study of Gutiérrez-Vega group was short of showing differences among the groups in terms of wound diameter, inflammatory response, and formation of granulation tissue, epithelialization and fibroblast population.²⁸

Three key ingredients in honey might be responsible for its wound-healing properties. Firstly, honey has a high sugar content, which absorbs moisture and makes it difficult for wound bacteria and fungi to survive. Secondly, many kinds of honeys contain glucose oxidase enzyme that in the presence of a little water, produces hydrogen peroxide, which is a common household disinfectant. Thirdly, honey contains bee pollen enzymes and propolis, all of which can stimulate new tissue growth. Honey may contain other medicinal compounds, including essential oils, flavonoids, terpenes and polyphenols, depending on the plant from which the pollen was taken.^{30, 31}

In conclusion, the findings of this study showed that honey can accelerate wound healing as well as increasing resilience, ultimate tensile strength and toughness of full thickness of skin wounds in mice.

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