Effects of Three Types of Honey on Cutaneous Wound Healing

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Abstract: Aim. To evaluate the effects of three types of honey (chestnut, blossom, and rhododendron) on the healing of full-thickness wounds. Methods. Twenty-four (24) New Zealand White female rabbits were used. Four 1.5 cm x 1.5 cm full-thickness skin wounds were created on the back of each animal and treated with pure honey or sterile saline, respectively. Wounds were assessed by wound measurements and collection of samples at 7, 14, and 21 days post wounding to evaluate the healing process. Variables of interest were hydroxyproline concentration and gross and microscopic morphological characteristics reflective of wound healing. Wounds of the honey-treated groups healed much faster than the control group. Results. On day 7, the formation of granulation tissue, epithelization, angiogenesis, and fibroplasia levels increased in the honey-treated groups (P < 0.05). A statistical difference between the honeys was not detected. Conclusion. The present results suggest that honey accelerates the inflammatory reaction and initiates healing early on in the treatment process.

Topical application of honey to full-thickness skin wounds has been recognized for centuries as effective in controlling infection and producing a clean granulating wound bed. The recorded observations show that inflammation, swelling, and pain are quickly reduced, unpleasant odors cease, sloughing of necrotic tissue occurs without the need for debridement, dressings can be removed painlessly (without causing damage to the re-growing tissue), and healing occurs rapidly with minimal scarring, eliminating the need for grafting.

Laboratory evidence suggest that honey has antibacterial properties that are due partly to its acidity and partly to phytochemicals from the nectar of particular plants. Honey is mildly acidic and has a pH between 3.2–4.5. Topical acidification of wounds promotes healing. The hydrogen peroxide produced by honey is responsible for the stimulation of tissue growth. Hydrogen peroxide has been shown to stimulate fibroblast growth in cell culture at micro- and nanomolar concentrations.
The medical and nutritional properties of honey depend on its chemical composition. The chemical composition of honey varies depending on the plant source, season, and production methods. There are many types of honey derived from different plant sources but currently only 2 honeys are approved for therapeutic use—Medihoney™ (Capilano, Australia) and Active Manuka Honey (New Zealand)—both of which are unifloral and derived from Leptospermum spp (tea trees). These honeys are thought to have additional therapeutic properties derived from the floral source. Pure chestnut honey (PCH) and pure rhododendron honey (PRH) are unifloral honeys, while pure blossom honey (PBH) is multifloral. Honey has an obvious potential for use in a variety of clinical settings, and while a few clinics and individuals are using honey therapeutically, further research is needed to determine whether the source of honey can affect wound healing.

This prospective, randomized study was designed to observe the clinical effects of three different types of honey and to correlate their biochemical and histopathological properties.

To the authors’ knowledge, nothing in the literature has compared the activities of pure chestnut honey, pure blossom honey, and pure rhododendron honey using a full-skin, cutaneous wound model.

Methods

Ethical approval. The study was carried out in accordance with the Canadian Council on Animal Care (CCAC) Guidelines and the Declaration of Helsinki. The experimental protocol was approved by the Experimental Animal Studies Ethics Committee of Ondokuz Mayis University and also by the local ethics committee approved by the Central Experimental Animal Studies Ethics Committee of the Republic of Turkey.

Study population. A total of 24, 6-month-old, New Zealand white female rabbits (2500 g ± 300 g body weight), which had been supplied by the Animal Unit at Ondokuz Mayis University, Samsun, Turkey, were used in the study. Upon arrival at the institution, the animals were housed in an environmentally controlled animal facility for 7 days for acclimatization. Each rabbit was positioned in sternal recumbency. Upon arrival at the institution, the animals were housed in an environmentally controlled animal facility for 7 days for acclimatization. Each rabbit was housed in its own standard cage and subjected to 12 hours of light and 12 hours of darkness. The room temperature and humidity were maintained at 19˚C ± 1˚C and 55% ± 10%, respectively. All rabbits were fed 160 g pelleted rabbit diet daily and water was available ad libitum. Care was taken to avoid unnecessary stress and discomfort to the animals throughout the experiment. The Ondokuz Mayis University Animal Care and Use Committee approved the study protocol.

A complete blood cell count was performed for each rabbit on days 0, 7, 14, and 21.

The types of honey used in this study were pure chestnut (Castanea sativa) honey, pure rhododendron (Rhododendron luteum) honey, and pure blossom (multifloral) honey. The honeys were procured from the Apiary of Ondokuz Mayis University and from local beekeepers in the Black Sea Region of Turkey. Standard rearing methods were applied to the colonies. In April, 7 kg–8 kg of syrup was given to each colony to ensure adequate nutrition. Beeswax cake, syrup, or chemicals to treat honeybee diseases were not given to the colonies during the main nectar flow period. The main honeycomb and supper were given to the colonies on an as needed basis. While chestnut and rhododendron honeys were produced in the Turkeli (41˚N and 34˚E) district of Sinop where these plants are common, the blossom honey was produced in the Kelkit district of Gumushane (39˚N 29˚E), which was rich in thyme (Satureja thymbra L), labiatae (Lamium album), alfalfa (Trifolium ambigu-um), and geyen (Astragalus microcebalus) blossoms. Honeys were produced in the months of August and September. The honeys were extracted and wrapped following filtration with a 0.2-mm filter.

Wound creation. Before the surgery was performed, the animals were premedicated using intramuscular (IM) xylazine (7 mg/kg, [Rompun®, Bayer, Istanbul, Turkey]) and anesthetized with IM ketamine (40 mg/kg, [Ketosal®, Richterpharma, Interhas, Ankara, Turkey]). A single dose of cephalolin sodium (50 mg/kg IM, [Cefozin® Bilim, Istanbul, Turkey]) was administered for antibiotic therapy preoperatively. Carprofen (4 mg/kg, subcutaneously [SC], [Rimadyl®, Pfizer Inc, Zaventem, Belgium]) was injected in all animals once just prior to the operation and every 24 hours for 3 days postoperatively.

Each rabbit was positioned in sternal recumbency. After clipping the hair on the back of the rabbits, the skin was sterilized with polyvidone-iodine (Betadine®, Kansuk, Istanbul, Turkey). The skin and the underlying cutaneous trunci muscle were excised with a #11 scalpel and scissors to create four 1.5 cm x 1.5 cm full-thickness wounds in 4-cm intervals. Hemorrhage was controlled by sterile surgical sponge compresses. One wound from each animal (test wound) was assigned randomly to receive PCH (0.5 mL), PRH (0.5 mL), or PBH (0.5 mL) with the fourth wound serving as the untreated control.
After application of the topical treatments, the wound areas were then bandaged with sterile nonadherent pads and porous adhesive tape. The wounds were cleansed with sterile saline solution and the topical applications were done every other day until complete epithelialization was achieved. Eight rabbits were evaluated on days 7, 14, and 21, respectively. Four wounds were created on each rabbit for a total of 32 wounds. Wound data were obtained at each application time; therefore, n values were accepted as 32 for histopathological, biochemical, and planimetry analyses. Five-millimeter (5-mm) punch biopsy instruments were used to obtain two skin specimens from the wound edge of each rabbit, using planimetry, immediately after measurement was performed. The skin specimens were sent to the laboratory for pathological and biochemical analysis.

**Subjective observations.** At every bandage change, the wounds were observed grossly for redness, swelling, odor, edema, vesiculation of the wound, marked accumulation of exudate, and scab formation. The wounds were also observed for formation of exuberant granulation tissue (more than the wound edges) during the study.

On days 14 and 21 any hair that had grown around the wounds was trimmed. The day that initial granulation tissue was observed, the day that the wound was covered, and the day the wound was completely filled with granulation tissue and epithelialized were recorded. The observations were performed in a nonblinded manner.

**Evaluation Parameters**

**Planimetry.** Planimetry was performed on days 0, 7, 14, and 21 on anesthetized animals (the anesthesia protocol used to create the wounds was repeated). The wound area of each lesion on each evaluation day was obtained by tracing the perimeter of the wound onto a sterile piece of clear acetate film with a special marking pen. The acetate was placed on the wound surface, smoothed, and held flat and immobile by an assistant, while the tracing was made by the examiner (Dr. Nisbet) wearing 2.5x loupes. The examiner traced the margin at the leading edge of the advancing epithelium. The area within the margin of the advancing epithelium was defined as the “unhealed wound area” (Figure 1). Wound tracings were digitized using digital scanning software and hardware (Sigma Scan® Pro 5.0, SPSS Science, Chicago, IL).14 The unhealed wound area was recorded at each day of measurement and was used for statistical analyses.

**Healing time.** The time between wound creation and the day that each wound was covered with epithelium was evaluated to compare the time to healing among the four study groups.

**Histopathological evaluation.** A 5-mm punch biopsy instrument was used to take skin specimens from the wound edge of each rabbit on days 7, 14, and 21 immediately following measurement. The specimens were fixed in 10% neutral buffered formalin and processed routinely for histopathological examination. Five-micrometer sections were stained with hematoxylin and eosin (H&E) and Masson’s trichrome. Although several histopathological parameters could be used to assess the progression of healing from the inflammatory to the repair stage, the progressive decrease in macrophages, fibrosis, and progressive increase in angiogenesis, epithelization, and collagen level were selected.

The four selected areas were examined under 400x magnification. The Abramov’s histological scoring system (modified Greenhalgh’s scoring system) was used for scoring epithelization, fibrosis, angiogenesis, and collagen level; the number of macrophages under this system was modified.15,16 While the Greenhalgh’s scoring system compiled several histological parameters simultaneously to create a single score, the Abramov’s system assessed each parameter independently and gave a score of 0–3. The collagen level was graded as: 0 (none), 1 (scant), 2 (moderate, [Figure 2]), and 3 (abundant, [Figure 3]). Epithelization was graded as either: 0 (none, [Figure 4]), 1 (partial), 2 (complete, but immature or thin), and 3 (complete and mature, [Figure 5]). Angiogenesis was graded as either: 0 (none), 1 (up to 5 vessels per high-power field [HPF]), 2 (6–10 vessels per HPF, [Figure 7]), and 3 (more than 10 vessels per HPF, [Figure 6]). Fibrosis was graded as: 0 (none to minimal fibroblasts), 1 (few fibroblasts), 2 (more fibroblasts, [Figure 3]), 3 (predomi-
Figure 2. PBH treatment group on day 7. Dermis showing mild collagen levels and dense fibrosis (Masson's trichrome stain). Bar = 200 µm.

Figure 3. PBH treatment group on day 21. Dermis showing abundant collagen levels and mild fibrosis (Masson's trichrome stain). Bar = 200 µm.

Figure 4. PCH treatment group on day 7. Epithelization was not seen (H&E stain). Bar = 200 µm.

Figure 5. PCH treatment group on day 21. Third level epithelization is seen (H&E stain). Bar = 200 µm.

Figure 6. PRH treatment group on day 14. Dense angiogenesis (arrows) was present in dermis (H&E stain). Bar = 200 µm.

Figure 7. PRH treatment group on day 21. Mild angiogenesis (arrows) was present in dermis (H&E stain). Bar = 200 µm.
nanty fibroblasts, [Figure 2]). The number of macrophages was scored as 0–25 = 1, 26–50 = 2 (Figure 8) and > 51 = 3 (Figure 9).

The following criteria were used to compare normal tissue morphology in different regions of the trunk. All the histological sections were blindly evaluated by the same investigator (Dr. Yarim).

**Biochemical evaluation.** The biopsy specimens were taken from the wound edges of each rabbit on days 7, 14, and 21. The samples were freeze-dried and stored at -80°C until use. Hydroxyproline was measured using the Bergman’s spectrophotometric method.17

**Statistical Analysis**

Data are presented as mean ± standard deviation (SD). First, the normality was investigated using the Kolmogorov-Smirnov test. Data on planimetry, biochemical measurements, and histopathological changes were considered to be nonparametric data; therefore, they were analyzed using the Kruskal-Wallis H-test. The differences between the groups were determined using the Mann-Whitney U-test. $P < 0.05$ was considered statistically significant.

**Results**

**Subjective observations.** Signs of mild inflammation (redness and swelling) were observed immediately after wounding. However, these signs subsided within 1 week of wounding. None of the wounds displayed gross evidence of strong antigenic reaction to any of the three different types of honey (eg, edema, wound vesication, marked accumulation of exudate). None of the wounds (treated or control) displayed exuberant granulation tissue.

The honey-treated wounds were covered with a translucent layer of possibly nonabsorbed honey. This layer, however, was not observed in the control group.

A few micropustules were found in the neo-epidermis of the control wounds and even less were found in the honey-treated wounds. On day 7 there was no dehiscence, infection, or exudate seen on the surface of wounds in the honey-treated groups. The surfaces of the wounds were a pinkish color in three groups; however, the wounds in the control group were more swollen and warmer to the touch than those in the honey-treated groups. In the honey-treated groups, granulation tissue formation was easily noticeable at all wound edges on day 7. The median time for the first observable granulation tissue was not significantly different than that of the honey-treated groups and control group.

On day 14, an increased amount of epithelial tissue at the wound edges was observed in the honey-treated wounds in addition to a small elevation of granulation tissue at the center of the wound. Filling of the open wound to skin level with granulation tissue was significantly slower in the control group when compared to the other three groups. On day 17, three rabbits in the PBH group had complete coverage of the wounds with granulation tissue and epithelization, whereas, wounds in the other groups were not completely epithelialized. On day 21, the wounds of all treatment groups were almost completely epithelialized.
Evaluation Parameters

**Planimetry.** There was no significant difference between the honey-treated groups and the control group on days 7 and 14. The unhealed area was the highest ($P < 0.05$) in the control and rhododendron groups on day 21 (Figure 10).

**Histopathological evaluation.** Histopathological examination results are summarized in Table 1. Although the collagen level was significantly higher in the honey-treated groups on days 14 and 21 ($P < 0.05$), the level was not significantly different between the honey-treated groups and the control group on day 7. On day 7, epithelization significantly increased in the honey-treated groups and the control group on day 7. On day 7, epithelization significantly increased in the honey-treated groups ($P < 0.05$) and a tendency to increase was observed later on. On day 21, the wounds of all treatment groups were almost fully epithelialized. A significant increase was noted in the honey-treated groups ($P < 0.05$) on days 7 and 21, whereas, no difference was observed regarding angiogenesis on day 14. When the macrophage counts were evaluated, although there were significant difference between the control and the honey-treated groups on day 7, the macrophage counts were significantly higher only in the PRH group on day 14 ($P < 0.05$).

Fibroplasia was significantly higher in the honey-treated groups ($P < 0.05$) on day 7, and a tendency for fibroplasia to decrease was observed later on. There was no significant difference that could be noted between the treatment groups during the rest of the study.

**Biochemical evaluation.** Hydroxyproline levels of all groups are presented in Figure 11. Hydroxyproline levels were higher in the honey-treated groups than in the control group on days 7 and 14. Highest hydroxyproline levels were observed in the PCH group ($P < 0.05$) on day 14.

Discussion

The medicinal properties of honey have been known for many years. Many different types of honey exist, but the differences are predicated on the floral source. Not many studies have compared the effects of different types of honey to those used in the present study.

Allen et al conducted a major study on the antibacterial activity of New Zealand honeys categorized according to their floral source. The present study did not evaluate the antibacterial activity of the honeys, but tested the wound healing properties of three honeys of varying botanical origins—one multifloral honey, the...
In the present study, significant differences were not detected, but less unhealed areas were seen in the pure blossom honey group wounds on days 7 and 14. On day 17 the wounds of three rabbits in the pure blossom honey group had completely granulated. Based on this evidence, the authors believe that multifloral honey initiates healing early on in the process. Gutiérrez-Vega et al\textsuperscript{20} fell short of determining differences among the groups in terms of wound diameter, inflammatory response, formation of granulation tissue, epithelization, and fibroblast population. Some ingredients in the honey might be responsible for its wound-healing properties. One type of 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.\textsuperscript{21–24} The present findings are in concordance with previous investigations.\textsuperscript{21–24}

There was no dehiscence, infection, edema or exudate in the honey-treated groups in this investigation, which seems to support the results of other studies.\textsuperscript{25–27} Additionally, honey increases the rate of wound healing, which confirms previous reports.\textsuperscript{28–30} This study affirms

### Table 1. Histopathological findings among groups. Collagen, epithelization, macrophage, fibroplasia, and angiogenesis of open wounds in rabbits treated with honeys of different botanical origin and untreated control wounds.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Collagen</th>
<th>Epithelialization</th>
<th>Angiogenesis</th>
<th>Macrophage</th>
<th>Fibroplasia</th>
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<tr>
<td></td>
<td></td>
<td>Day 7 (n = 32)</td>
<td>Day 14 (n = 32)</td>
<td>Day 21 (n = 32)</td>
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<td>Collagen</td>
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<td>1.50 ± 0.53†</td>
<td>1.88 ± 0.64†</td>
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<td>2.88 ± 0.35*</td>
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<td>2.50 ± 0.53*</td>
<td>2.75 ± 0.46*</td>
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<td>Epithelialization</td>
<td>Control</td>
<td>1.13 ± 0.35†</td>
<td>1.75 ± 0.71</td>
<td>3.00 ± 0.00</td>
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<tr>
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<td>Chestnut honey</td>
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<td>2.00 ± 0.76</td>
<td>3.00 ± 0.00</td>
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<td>3.00 ± 0.00</td>
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<td>2.00 ± 0.76</td>
<td>1.75 ± 0.71*</td>
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*†Indicate significant differences (P < 0.05)

Scoring method: The collagen level was graded as: 0 (none), 1 (scant), 2 (moderate), and 3 (abundant). Epithelization was graded as either: 0 (none), 1 (partial), 2 (complete but immature or thin), and 3 (complete and mature). Angiogenesis was graded as either: 0 (none), 1 (up to 5 vessels per high-power field [HPF]), 2 (6–10 vessels per HPF), and 3 (more than 10 vessels per HPF). Fibrosis was graded as: 0 (none to minimal fibroblasts), 1 (few fibroblasts), 2 (more fibroblasts), and 3 (predominantly fibroblasts). The number of macrophages was scored as 0–25 = 1; 26–50 = 2; and > 51 = 3.
that the honey accelerates wound healing and wound contraction and promotes epithelization. Observation of a positive effect by honey on wound healing is consistent with other studies.29-31

A few studies have used a model to assess the changes in the morphological and biochemical properties due to topical application of honey on cutaneous wounds.32-33 In the present study, the efficacy of honey in the healing of cutaneous wounds of rabbits was evaluated with a focus on histopathological and biochemical changes. Suguna et al34 and El-Banby et al35 report that honey-treated wounds show less neutrophilic infiltration and a greater formation of angioblasts and fibroblasts. The present study showed that angiogenesis and fibroplasia were significantly higher in the honey-treated wounds than in the control group on day 7. The macrophage level was the highest in the pure rhododendron honey group on day 14. These findings indicate that pure rhododendron honey might prolong the inflammation period.

Histopathologically, epithelization, angiogenesis, and fibroplasia level were the highest in the honey-treated groups on day 7, which may indicate that honey accelerates the inflammatory reaction and initiates the healing process in the early phases of healing.

Suguna et al36 demonstrated that honey accelerated the synthesis and maturation of collagen, thus resulting in an increased tensile strength of the healed skin. Tensile strength has commonly been associated with the organization, content, and physical properties of the collagen fibril network and it is one of the necessary parameters for determining the pharmacological effects of potential wound healing agents.37 Rozaini et al38 used a rat model to evaluate the effects of different types of honey on tissue tensile strength in burn wounds. They observed that the wounds treated with honey had an increased collagen concentration and stabilization of the fibers. The present study demonstrated that the collagen level and hydroxyproline level in the honey-treated groups were significantly higher than that in the untreated control group on day 14.

**Conclusion**

The present results suggest that the full-thickness wounds treated with honey experience increased granulation tissue formation, epithelization, angiogenesis, and fibroplasia in the early phases of healing. Although there are no significant differences between honeys, the source of the honey can affect the eventual wound healing process.

Existing evidence suggests that honey has the potential to reduce inflammation and scarring, and aids in cellular regeneration. Further research is required to understand the mechanisms of action regarding honeys from various sources in addition to comparing unifloral to multifloral honeys. The optimal method for applying honeys to various wound types such as ulcers, burns, and surgical incisions warrants further research.

**References**


