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Examination the effects of chestnut and Manuka Honey for wound healing on mice experimental model

Özcan Budak[1]*, Hüseyin Çakıroğlu[2]

1 Sakarya University, School of Medicine, Department of Histology - Embryology, Sakarya,TR
2 Sakarya University, Medical and Experimental Research Center, Faculty of Medicine, Sakarya, TR

*** Corresponding Author:** Özcan Budak **E-mail:** ozcanbudak@sakarya.edu.tr

ABSTRACT

Objective: In this study, the aim is to examine the effects of Chestnut and Manuka honey on wound healing in order to investigate the effectiveness of cost-effective alternative to current approaches in terms of wound care and treatment.

Material and Methods: In this study, we used 30 healthy male Balbc mice weighing 18-24 g. We randomly divided the rats into three groups. A control group, a group treated with only Chestnut honey, a group treated with Manuka honey cream. After the wounds were formed in groups, the tissue samples were gathered on the seventh and fourteenth days. Then these samples were examined histologically and immunohistochemically.

Results: When the study results were evaluated, statistically significant differences were seen between histological and immune-histochemical findings in wound tissue preparations. On the seventh day, tissue samples showed re-epithelialization (P=0,002), granulation cell density (P=0,003) and angiogenesis (P= 0,003). In the fourteenth day tissue samples, we found epithelialization (P=0,001), granulation cell density (P=0,002) and angiogenesis (P=0,001). In the tissue samples in the seventh and fourteenth days between the groups, we found immino-histochemically, Ki-67 and EGF dyeing percentages as P= 0,004 and P=0,003 respectively.

Conclusion: We think chestnut honey may contribute to a shorter wound healing process.

Keywords: Honey, Wound Healing, Re-Epithelialization

INTRODUCTION

Wound formation is defined as the permanent or temporary loss of histological and physiological properties when the tissues are exposed to a force greater than they can tolerate (1). Also, wound formation is a process that socially limits the quality of life of patients and increases the cost of treatment, affecting not only the patient but the whole society (2). In the past, the aim was to bring the wound lips closer together to allow the wound to heal quickly in the treatment of surgical wounds. In current approaches, it has been understood that a moist and warm environment to be created around the wound is more effective in wound healing (2). Thus, it is based on the idea that this situation creates an ideal environment for wound healing and that epithelial cells can move freely (3). In addition to being a topical agent, honey has a low treatment cost. Along with its antibacterial properties, honey is also used as a wound healer. By keeping the wound moist, it allows epidermal migration and it provides trace elements that are effective in healing. It also stimulates the release of inflammatory cytokines from macrophages. All kinds of honey can be effective in wound healing. Jellybush or Manuka honey is obtained from the plant named ocean sturgeon and it is known to have high antibacterial properties together with Jambhul honey from India (2). In a systematic review by Yaghoobi et al., it was observed that honey has a similar effect compared to conventional treatments in the treatment of acute wounds and superficial-partial thickness burns (4). In another study on mice; Acacia honey was used in wound treatment and a positive increase was found in granulation tissue, collagen synthesis and wound healing (5). Studies showing that the use of honey is effective in the healing of chronic wounds and accelerates healing have been reported (6, 7).

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Considering the plant existence, endemic species, medicinal aromatic plants and wide range of varieties in our country, there are many possibilities that can be used for the benefit of medicine. In the literature review, no findings or studies were found regarding the effectiveness of Manuka Honey and Chestnut Honey of Western Black Sea Origin on wound healing. However, the comparison of the effects of Chestnut Honey from Western Black Sea, which is likely to be among the natural treatment alternatives in wound healing, with Manuka Honey on wound healing performance will be a unique study.

In this study, the aim is to examine the effects of Chestnut and Manuka honey on wound healing in order to investigate the effectiveness of cost-effective alternative to current approaches in terms of wound care and treatment.

MATERIAL and METHODS

This study was conducted by Sakarya University Animal Testing Local Ethics Committee approval in Sakarya University Experimental Research and Medical Centre (Dated 12/01/2022 and numbered 02). The procedures on the rats were humane, and the standards of the study were pertinent to the standards of the existing ethical animal testing procedures.

Experimental Animals

In this study, 30 healthy male BALB/c mice weighing between 18-28 grams were used as experimental animals. This experiment was conducted in Sakarya University Experimental Research and Medical Center (Sakarya, TURKEY). The mice were fed with water and ad libitum according to standard diet (Arden Research& Experiment, Ankara, TURKEY). Mice were kept at 21±1 °C in well-ventilated places within 12- hour day-night cycle.

Experimental Protocol

We randomly divided the mice into three groups as spontaneous recovery, Manuka honey and chestnut honey groups. The decubitus ulcer model was used by Stadler et al. (8). The rats were anaesthetized by first Ketamine HCL 100 mg/kg IM (Ketasol %10, 10ml) (Ketasol, Richterfarma, Austria) and then Xylazine 10 mg. The hair between the two blade bones of the rats was shaved. Following the cleaning of the wound site, each mouse was wounded in a circular shape with a 5 mm diameter sterile punch biopsy instrument excisionally, into the superficial fascia to include all layers of the epidermis and dermis. The wound creation was done by the same person each time. After the skin defect was created in mice, the wounds were wiped with serum physiological before dressing, and this process was repeated during each dressing change. Wound care was performed every day for 14 days following the application while the day of wound formation was accepted as the day zero of the application. The standardized forms of animal and herbal products to be applied to wounds were used. In addition to these, the tissue samples from all groups were taken for the examination in the light microscope on the 7th and 14th days of the surgical intervention.

Histopathological Evaluation

Tissue samples were collected from wounds on the seventh and fourteenth days of the decubitus ulcers. The tissue samples were immobilized in the formaldehyde buffered 10% for 48 hours. The tissue sample was soaked in paraffin-embedded blocks after the tissue embedding processes. The preparations were stained with Hematoxylin-Eosin (H-E) for histological examination. According to "The Wound Healing Points Evaluation Criteria" a histologist evaluated histopathological examinations (9).

Immunohistochemical Staining Method

After the deparaffinization of the tissues placed on a slide with “Poly-L-Lysine”, 4 µm thick sections from tissues fixed in neutral formaldehyde solution were boiled in a citrate buffer for antigen retrieval for 20 minutes in a microwave oven. For endogenous peroxidase inactivation, they were incubated with 3% hydrogenperoxide (H_2O_2) for 20 minutes after PBS bath. After cooling, Kİ-67 (sc-52746, Santa Cruz, USA),IL-6 (sc-32296, Santa Cruz, USA), EGF (sc-52012, Santa Cruz, USA), primary antibodies were used as primary antibody and the rat and rabbit specific HRP/DAB detection IHC kit was used as secondary antibody. Immuno-positivity in samples was evaluated after counterstaining with hematoxylin by giving

a semi-quantitative number for positive cells. In all groups, at least 200 cells were marked within each x40 magnifying area. In incisions, the percentage of the stained cells and staining level were the criteria to be chosen. For each incision, immune-histochemical staining scoring was calculated according to H-SCORE, which is a scoring algorithm formulated as (I x PC), (I: the level of staining, PC: the percentage of stained cells in each level) (10).

Statistical Analysis

Statistical analyses were performed using the SPSS 24.0 package program (SPSS Inc. and Lead Tech. Inc. Chicago. the USA). Shapiro Wilks test was used in compliance with normal distribution. Kruskal Wallis test was used for numerical data of the subgroups that did not show normal distribution. Intergroup evaluations for statistically different parameters were performed using the Mann-Whitney U test and comparing them in pairs. Results were given as mean ± standard deviation. For all statistical analyses, a two-tailed P-value <0.05 was considered statistically significant.

RESULTS

When we compared the 7th and 14th day tissue samples of the control, Chestnut and Manuka honey groups in terms of reepithelialisation, statistically significant differences were found between the tissues of both days for the groups (P values in order p=0.002; p=0.001). On the 14th day, it was observed that the reepithelialisation was completed in the chestnut and Manuka honey groups, but not in the control group. On the 7th day, it was observed that the formation of reepithelialisation in chestnut honey was quite ahead of Manuka honey group (p=0.006). It was observed that reepithelialisation was more advanced on the 7th day in chestnut and Manuka honey groups than in the control group (P values in order p=0.002; p=0.001) (Figure1).

When we compared the control, Chestnut and Manuka honey groups in terms of granulation cell density on the 7th and 14th days, it was seen that the granulation cell density was lower in the Chestnut honey group than the Control and Manuka

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honey on both the 7th and 14th days. Statistically significant differences were observed between the groups. (P values for the 7th and 14th days, respectively, p= 0.004; p=0.002). When compared with Chestnut honey group (p=0.000) and Manuka honey group (p=0.000), the granulation cell density the control group tissue samples were observed to be more on the 7th and 14th days This showed that wound healing was slower in the control group. The same situation was also observed in the Chestnut honey and Manuka honey groups on the 14th day and 7th day. Statistically significant differences were seen on the 7th and 14th days (P values p=0.000 and p=0.006, respectively) (**Figure1**). Likewise, statistically significant differences were observed in Chestnut honey and Manuka honey groups on the 14th day compared to the 7th day. P values, on the 7th day p=0.000 and on the 14th day p=0.006 (**Figure1**).

When we compared the tissue samples of the control, Chestnut and Manuka honey groups in terms of angiogenesis rates, it was observed that the tissue samples on the 14th and 7th day created statistically significant differences between the groups on both days (P value respectively p = 0.003; p = 0.001). In the chestnut honey group, angiogenesis was found to be higher than Manuka honey and control groups on the 7th day (P values respectively p=0.005 and p=0.001 (14). The intensity of angiogenesis in the chestnut honey group on the 14th day was lower than Manuka honey and control groups (P values respectively p=0.006 and p=0.001). Manuka honey group had a higher angiogenesis rate on the 7th day than the control group, but on the 14th day, angiogenesis was observed at higher rates in the control group than Manuka honey group. Statistically significant differences between the two groups were observed on the 7th (p=0.002) and 14th (p=0.000) days. (**Figure1**).

We stained the tissue samples on the 7th and 14th days of the standard and Western diet groups with Ki-67 and EGF antibodies separately and evaluated the results. The percentage of Ki-67 staining in the tissue samples of the Chestnut honey group on the 7th day was higher than that of Manuka honey and control groups. P values were p=0.015 with Chestnut-Manuka honey and p=0.001 with chestnut honey-control group, respectively. On the 7th day, the least percentage of Ki-67 staining was seen in the control group. On the other hand, the percentage of Ki-67 staining in the chestnut honey group was seen at a lower rate in the wound tissue samples on the 14th day compared to the 7th day. The highest percentage of Ki-67 staining on the 14th day was seen in the control group. Statistically significant differences were observed between the control group and Chestnut honey group (p=0.003). There was no statistical difference between the percentage of Ki-67 staining on the 14th day of Chestnut honey and Manuka honey groups (P>0.05). Statistically significant differences were also observed in the percentage of EGF staining between the groups on the 7th and 14th days (p=0.000). On the 7th day, the highest percentage of EGF staining was observed in Chestnut honey group. The lowest was observed in the control group. Statistically significant differences were observed in Chestnut honey- Control group (p=0.000), Chestnut honey- Manuka honey groups (p=0.011). On the 14th day, the highest percentage of EGF staining was seen in the Control group while no statistically significant differences were observed between Chestnut and Manuka honey groups (p>0.05). Statistically significant differences were observed between the control group- chestnut honey group (p=0,002) and the control group- Manuka honey group (p=0,003) (**Figure1**).

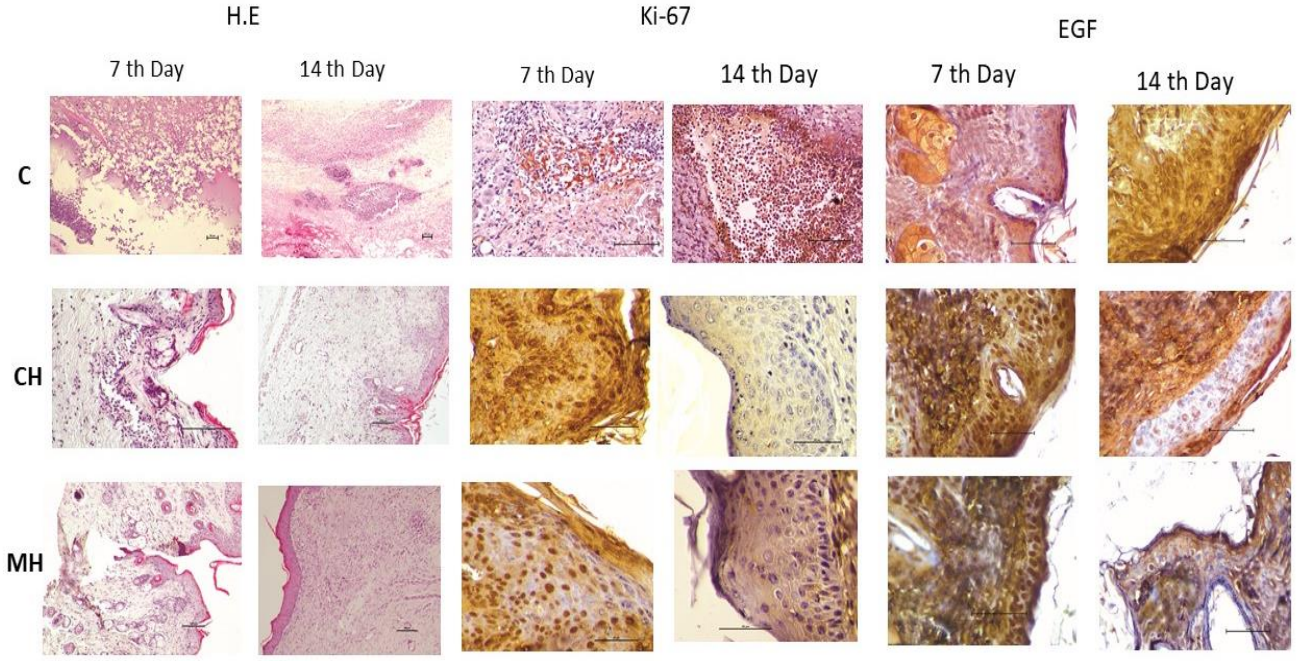

Figure1. Histopathological comparison between groups, X100. As a result of both H.E and imminohistochemically coloring KI-67 and EGF; In the CH group, a faster remodeling occurs in the 7th days than in groups MH and C. In group C, recovery and remodeling were seen at the latest. C: Control, CH: Chestnut Honey, MH: Manuka Honey. H.E.: Hematoxylane Eosin. EGF: Epidermal Growth Factor.

DISCUSSION

Honey contains sugar, enzymes, flavonoids, minerals and other nutrients. It has antioxidant, anti-inflammatory properties and it has been used in wound treatment to promote rapid healing or healing support (11, 12). In addition to the epithelium in the wound area, myofibroblasts, collagen and angiogenesis play important roles in wound healing (11, 12, 13, 14). Collagen is the main structural component of the extracellular matrix and it plays a vital role in maintaining the integrity of all tissues and wound healing (14, 15). Angiogenesis refers to the formation of new blood vessels at the wound site and it is a necessary component of the healing process due to increased nutrient requirements (16). In our study, we evaluated the healing process of Chestnut and Manuka honey in the wound area. For this purpose, we performed our observations by mutually evaluating the histological properties of epithelialization, granule tissue density and angiogenesis parameters in wound tissues and the intensities of Ki-67 and EGF staining immunohistochemically.

In a previous study, it was reported that reepithelialisation in the wound area occurred more quickly in the wound healing phase of Manuka honey and Indonesian honey compared to the control group (17). In a study conducted with rabbits, chestnut, flower and rhododendron honeys were used in wound treatment. Very high reepithelialisation rates were observed on the 7th day, and it was observed that the epithelialization was completely realized on the 21st day (18). In our study, there was no difference in reepithelialisation between Chestnut and Manuka honeys on the 14th day. However, on the 7th day, reepithelialisation was more advanced in Chestnut honey than in Manuka honey group. We observed that reepithelialisation was faster in Chestnut honey group. On the 14th day, except for the control group, epithelialization was completed in the honey groups. From this perspective, our study is similar to the studies in the literature (17, 18). It is reepithelialisation that starts a few hours after injury in wound healing, but it shows more pronounced activity in the proliferative phase and it can continue until the extracellular matrix remodelling phase (19). Epidermal growth factor (EGF) is an important indicator of this phase and has mitogenic and migratory activity in border keratinocytes (20). In our study, the highest intensity of EGF positive staining was observed in Chestnut honey group on the 7th day in the scar tissue samples, and the lowest staining was observed on the 7th day in the control group. On the other hand, EGF positive staining intensity was the same in Chestnut and Manuka honey groups on the fourteenth day. Epithelization was completed in these two groups. EGF positive staining results were lower and epithelialization was not completed fully in the control group on the fourteenth day. Our IHC staining results showed parallelism with our H.E. staining results.

In literature, it has been reported that honey and especially Manuka honey have positive effects on tissue healing, tissue formation, capillary vessel modelling and increasing collagen synthesis in wound healing stages (17, 21). In our study, on the 7th day, granulation and angiogenesis in the chestnut honey group showed differences compared to Manuka and the control groups. An intense formation was observed in Chestnut honey group. On the 14th day, no difference was observed in Chestnut and Manuka honey groups while a slow progress was observed in the control group. The rapid recovery status of chestnut honey group on the seventh day differed from studies in the literature (5, 18, 21). The formation of granulation tissue formed by macrophages, fibroblasts and neoforms is essential for the reepithelialisation process and tissue restructuring (19). Increased cell proliferation is an important aspect of wound healing in general, and Ki-67protein is an important indicator of this cellular event.

Ki-67 expression is also commonly an indicator of cell growth in a total cell population (22). In our study, it was observed that KI-67 positive staining was quite high in Chestnut honey group on the 7th day. It was also high in Manuka honey group, but still lower than Chestnut honey group, and the lowest level was observed in the untreated control group. On the 14th day, Ki-67 expression was in the control group because the wound healing process continued. On the 14th day, Ki-67 level was quite low in Chestnut and Manuka honey groups. We think that this is due to the fact that the healing process is faster in honey groups. Ki-67 protein positivity results were found to be similar to the granulation and angiogenesis results.

CONCLUSION

The results obtained from this study show that honey used for therapeutic purposes in wound healing is effective in the healing process. In addition, Chestnut honey was found to be more effective than Manuka honey in wound healing. For this reason, the botanical origin of honey is extremely important in terms of productivity. Application time also affects wound healing. Based on the results from this study, new prospective studies with different honey types, animal models and dosages can be planned.

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Ethical approval: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by Local Ethical Committee. All procedures performed in studies with human participants met the ethical standards of the Institutional Research Commission and the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards.

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