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Investigation of the effects of Pine and Chestnut Honey on wound healing

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Abstract: This study aimed to investigate the effect of creams containing pine and chestnut honey on wound healing in rats. The animals were divided into the following four groups: a control group (A), a group treated with only basic cream ingredients (B), a group treated with pine honey cream (C), and a group treated with chestnut honey cream (D). Full-thickness skin wounds were created on the back of each rat (14 per group) with a 10-mm diameter punch instrument. In each group, skin samples were taken from seven rats on day 7 and from the remaining seven rats on day 14. The effects of the creams on wound healing were assessed by histopathological examination and hydroxyproline assays. The histopathological examination showed that chronic inflammation significantly decreased in group D compared to the control group ($P<0.05$). Furthermore, the levels of granulation tissue and granulation tissue/fibroblast maturation in skin samples taken on day 14 were also significantly lower in group D compared to the control group ($P<0.05$). It was observed that the neovascularization values of skin samples taken on day 7 were lower in group D than in the control group ($P<0.05$). Also, the difference in hydroxyproline levels between groups B and D was statistically significant ($P<0.05$). The results showed that the cream containing chestnut honey had a positive effect on wound healing.
Keywords: Apitherapy, chestnut honey, pine honey, rat, wound healing.

Çam ve Kestane Balı'nın yara iyileşmesi üzerine etkilerinin araştırılması

Özet: Bu çalışmada, ülkemize özgü çam balı ve kestane balı içeren krem formülasyonlarının yaralar üzerine iyileştirici etkilerinin inclenmesi amaçlanmıştır. Çalışmada, herhangi bir uygulama yapılmayan kontrol grubu (A), sadece krem taşıt maddesi uygulanan grup (B), Çam Balı kremi uygulanan grup (C) ve Kestane balı kremi uygulanan grup (D) olmak üzere 4 farklı denek grubu oluşturuldu. Her grupta (n=14) yer alan sıçanların sırt kısmına 10 mm çapında punch aparatı ile tam kat yara oluşturuldu. Farklı gruplarda yer alan sıçanların yarısının deri örnekleri yara oluşumunu takip eden ilk 7. günde alınırken geri kalanların deri örnekleri 14. günde alındı. Alınan bu deri örneklerine histopatoloji incelemesi ve hidroksiprolin analizi yapıldı. Histopatolojik değerlendirme sonucuna göre, kronik yangının, kestane balı kremi uygulanan grupta kontrol grubuna göre önemli derecede azaldığı tespit edildi ($P<0,05$). Buna ek olarak, granülasyon doku ve granülasyon dokusu/fibroblast olgunlaşmasının da kestane balı kremi uygulanan ve 14. gün alınan deri örneklerinde, kontrol grubuna göre önemli derecede azaldığı belirlendi ($P<0,05$). Kestane balı kremi uygulanan grubun 7. günde alınan deri örnekleri neovaskülarizasyon değerlerinin, kontrol grubu neovaskülarizasyon değerlerine göre daha düşük değerlerde olduğu gözlendi ($P<0,05$). Ayrıca, hidroksiprolin düzeyleri çalışma grupları yönüyle değerlendirildiğinde B ve D grupları arasındaki farklılık istatistiksel açıdan önemli bulundu ($P<0,05$). Sonuçlar bütün halinde ele alındığında, kestane balı içeren kremin yara iyileşmesi üzerine katkısının olumlu yönde olduğu belirlendi.
Anahtar sözcükler: Apiterapi, çam balı, kestane balı, sıçan, yara iyileşmesi.

Introduction

Honey contains sugar, enzymes, flavonoids, minerals, and other nutrients that have exert antioxidant, antibacterial, and anti-inflammatory effects and has been used as a wound dressing to support quick and improved recovery (2, 43). Honey effectively treats foot and mouth wounds in cattle and horses and chemical eye injuries in rabbits (47). In addition to the epithelium, myofibroblasts, collagen, and angiogenesis play important roles in wound healing (1, 12). Fibroblasts play a crucial role in wound healing; the division and migration of epidermal cells into the surrounding wound area is of secondary importance (22). Collagen is the main structural component in the extracellular matrix and is vital in ensuring all tissues' integrity and wound healing (9, 12). Angiogenesis refers to forming new blood vessels from existing blood vessels

and is a necessary component of the healing process due to the increased nutrient requirement (10). Activated platelets, neutrophils, and macrophages play a significant role in wound healing (16). Wound care gels, creams, and dressing materials containing honey that are approved by the U.S. Food and Drug Administration (FDA) are being used in wound healing. Products containing Manuka honey are especially recommended to treat minor wounds, cuts, burns, diabetic foot ulcers, leg ulcers, pressure ulcers (bed sores), partial- and full-thickness wounds, first- and second-degree partial burns, and traumatic and surgical wounds (37).

Honey is important for health since it possesses antimicrobial properties and contains antioxidants varyingly depending on its botanical source. Its antimicrobial effect results from its high osmotic pressure and low pH, as well as the presence of compounds, such as hydrogen peroxide, flavonoids, and phenolic compounds (caffeic and ferulic acids) (23, 25, 46). The antimicrobial properties of honey are determined by the diversity and concentrations of these components that depends on various factors, such as its floral origin and nectar combination (flower type, single/many), source (flower/honeydew), climatic characteristics (dry/humid), and color (light/dark) (3, 24, 44). Besides its antibacterial activity, honey has antifungal and antiviral effects (11, 25, 31). Chestnut honey (CH), important flower honey, is obtained from trees belonging to the Castanea genus of the Fagaceae family. CH is distinguished from the other types of honey by its taste, aroma, and color (21). A study in Spain established that the taste and aroma of CH collected from different regions differed (7). Another study found that the number of essential elements in dark-colored honey, such as CH, was higher than in light-colored honey (6). The pine scale *Marchalina hellenica*, which plays an important role in the production of pine honey (PH), is found in Turkey on the following pine species: *Pinus brutia* (Turkish red pine), *P. halepensis* (Aleppo pine), *P. silvestris* (Scotch Pine), and *P. pinea* (Stone pine) (48).

Studies on treating wound bacteria with specific regional honey indicate that the antimicrobial activity of honey depends on the dose (11, 25). Another scientifically promising finding is that honey inhibits the growth of antibiotic-resistant bacteria and also prevents the development of resistance to antibiotics (25). The antibacterial and healing effects of honey can lead to less painful, quicker, natural, and cost-effective treatment of wounds. This study examines the effects of creams containing honey from Düzce and Muğla provinces on wound healing in rats.

Materials and Methods

***Animal materials*:** Fifty-six healthy male Wistar albino rats (250–350 g) aged 6–8 weeks were used in the study. Ethical consent was obtained from the Animal Experiments Local Ethics Committee of Kırıkkale University on 31/03/2016 (Meeting Number: 16/03, Meeting Decision: 16/44). Food (standard rat feed) and water were provided ad libitum for the duration of the experiment. The animals were kept in rooms with a 12-hour light/dark cycle at a suitable humidity and temperature.

***Obtaining and analyzing honey samples*:** The CH and the PH were collected from the Beekeepers Association in Düzce and Muğla Province for this study.

The properties and antibiotic residue (sulfacetamide, sulfadiazine, sulfamethoxazole, sulfamerazine, sulfisoxazole, sulfamethizole, sulfabenzamide, sulfamethazine, sulfachloropyridazine, sulfadimethoxine, sulfathiazole, sulfameter, sulfamethoxypyridazine, sulfadoxine, methacycline, epitetracycline, doxycycline, tetracycline, oxytetracycline, epioxytetracycline, chlortetracycline, and chloramphenicol) analysis were performed at the Muğla Sıtkı Koçman University Food Analysis Application and Research Center.

DIN 10760 was used in pollen analysis, while the in-house method was used to measure the moisture, conductivity, pH, free acidity, (hydroxymethiylphurfural (HMF), sugar profile, concentrations of proline, diastase, and naphthalene, and antibiotic residues; the TS 13262 method was used in protein and raw honey analysis.

***Sterilization of honey*:** The honey samples were sterilized using Cobalt-60 (^{60}Co) gamma radiation at a dose of 5 kGy.

Preparation of cream formulation: The process of the sterilized honey into a pharmaceutical product in cream form was carried out by Nehar Chemicals, Istanbul, Turkey. Glycerin, methylparaben, sodium benzoate, liquid paraffin, and cetearyl alcohol were used as auxiliary substances in the preparation of the cream formulations; honey was added to 25%.

***Formation of experimental groups and the experimental protocol*:** The model defined by Park et al. (34) was used as an experimental wound model. After the rats were anesthetized with diethyl ether, the prospective wound area was shaved and cleaned with povidone-iodine (betadine). Using the punch biopsy instrument, two 10-mm diameter full-thickness excision wounds, each approximately 1 cm away from the midline, were created on the dorsal thoracic region in all rats. At the same time, the animals used in the study were divided into four groups, with 14 in each group. Group A (control) did not receive any treatment to the wound area. Plain (common) cream (group B) was treated with a cream containing basic ingredients. Group C was treated with a cream containing PH. Group D was treated with a cream containing CH. The animals were kept in individual cages throughout the test period, and the wounds were fully covered with the appropriate creams. Two subgroups of seven animals were

formed in each group. At the end of the first seven days, the animals in one subgroup from each group were sacrificed, and skin samples were taken for histopathological evaluation. The animals in the second subgroup of each group continued to be treated with the creams for a total of 14 days. All animals were sacrificed with an overdose of general anesthetics at the end of day 14, and skin samples were collected from the wound area. Depending on the nature of the tests, the samples were fixed in buffered formalin or kept at -80ºC until analyzed.

***Morphometric parameters*:** The wounds on the rats were checked daily from the beginning of the study until the practical applications were completed. The skin samples collected from the animals sacrificed on days 7th and 14th of the study were placed in cryotubes and stored in freezers with dry ice. All clinical findings in rats were also noted during the experimental studies. The Greenhalgh method was modified for histopathological evaluation (35).

***Calculation of the wound area*:** To determine the healing process, the rats were anesthetized, and the size of the wounds was drawn on acetate paper with a permanent marker with a diameter tip of 0.3 mm; this application was repeated at intervals of two days. The drawings were transferred to a computer and evaluated using the SketchAndCalc program. The image was scaled and measured using the ruler feature, and the size was recorded in mm/cm.

***Hydroxyproline analysis*:** A Hydroxyproline Assay Kit (MAK008) from Sigma was used to measure hydroxyproline levels and assess the collagen level in tissue samples taken from the wound areas.

***Statistical analysis*:** All variables were examined using the Shapiro–Wilk test of normality, followed by the Levene test for the homogeneity of the variances, before performing significance tests. Descriptive statistics were calculated and shown as "Arithmetic mean ± Standard error of mean (SEM)" or "Median (Minimum-Maximum)" where necessary. The effects of the group (A, B, C, D), time (Day 7 and Day 14), and their interactions on wound measurement area were analyzed using the MIXED procedure. The animals in the groups were included in the model as random effects, and the group, time, and their interactions were included as fixed effects. The post-hoc Bonferroni test was used for multiple comparisons. Histopathological score changes between 7-day and 14-day were analyzed using the Wilcoxon signed-rank test. The Kruskal–Wallis test was performed to determine the differences in scores between the groups on each day. Dunn-Bonferroni test was used as a post-hoc test for parameters that were found to be significant. In the hydroxyproline analysis, statistical control of the differences between variables was measured using the analysis of variance (one-way ANOVA). The Duncan test was used as a post-hoc test for variables that showed significant differences between groups. $P<0.05$ was the criterion used for all statistical analyses. All analyses were performed using the SPSS (V22.0) software package.

Results

***Honey analysis*:** The PH and CH analyses did not find antibiotic and naphthalene residues. The content analysis results were confirmed to be within the limits specified in the Communiqué on Honey; the origin of the honey was also confirmed. Pollen analysis results for CH and PH are presented in Table 1. The CH and PH contents are presented in Table 2.

Table 1. Pollen analysis results of chestnut and pine honey.

Analysis performed	Results		Measurement Limit (LOQ)	Analysis Method
	Chestnut	**Pine**		
Isolated Dominant Pollen (>15%)	*Castanea sativa* Miller (Fagaceae)	*Pinus brutia* (Pinaceae)	50%	DIN 10760
	-	Astragalus subsp. (Fabaceae)	18%	DIN 10760
Isolated Significant Pollen (>1%)	*Helianthus annuus* L. (Asteraceae)	-	10%	DIN 10760
	-	*Erica manipuliflora* Salisb. Ericaceae)	12%	DIN 10760
	Astragalus odoratus L. (Asteraceae)	Asteraceae	5%	DIN 10760
Isolated Pollen (<1%)	Apiaceae	Apiaceae	1%	DIN 10760
	-	Asteraceae	1%	DIN 10760
	Poaceae	Poaceae	1%	DIN 10760

Table 2. Content analysis results of chestnut and pine honey.

Analysis performed	Result		Measurement Unit	Analysis Method
	Chestnut	Pine		
Moisture	20.28	17.96	%	IHC
Conductivity	1.72	1.24	mS/cm	IHC
Proline	855.08	801.9	mg/kg	IHC
pH	4.78	4.61	pH	IHC
Free Acidity	19.82	21.85	mmol/kg	IHC
Diastase	9.80	19.97		IHC
HMF	24.05	1.09	mg/kg	IHC
Fructose+Glucose	59.87	62.44	g/100g	IHC
Fructose/Glucose	1.62	1.19		IHC
Saccharose	N.D.	N.D.	g/100g	IHC
Naphthalene	N.D	N.D	mg/kg	In-House Method
Protein (Delta 13C)	-26.40	-26.34		TS 13262
Raw Honey (Delta 13C)	-26.56	-27.19		TS 13262
Difference in protein and raw honey delta C13 values in honey	0.17	0.85		TS 13262
C4 Sugar Ratio calculated from Delta C13 Value	N.D	N.D	%	TS 13262

N.D. None detected.

***Histopathological examinations*:** On day zero, 14 tissue samples taken from groups A, B, C, and D were examined. It was observed that the epidermis was intact, and the blood vessels were hyperemic in all tissue samples. The histopathological examinations of all groups on days 7 and 14 are presented in Figures 1, 2, and 3, and the evaluation scoring is presented in Table 3. The change in acute inflammation over time between days 7 and 14 was statistically significant ($P<0.05$), although the differences in acute inflammation between the groups on days 7 and 14 were not statistically significant ($P>0.05$). Similarly, the change in chronic inflammation over time between days 7 and 14 was statistically significant ($P<0.05$), although the differences in chronic inflammation between the groups on day 7 were not statistically significant ($P>0.05$); however, the differences between the groups on day 14 were statistically significant ($P<0.05$). Group D was similar to groups B ($P>0.05$) and C ($P>0.05$), but it was statistically different from group A ($P<0.05$). Chronic inflammation decreased significantly in the group treated with CH-containing cream (group D) compared to the control group ($P<0.05$).

The change in the amount of granulation tissue over time between days 7 and 14 was statistically significant ($P<0.05$). Although the differences in the amount of granulation tissue between the groups on day 7 were not statistically significant ($P>0.05$), the differences between the groups on day 14 were found to be statistically significant ($P<0.05$). Group D was similar to groups B ($P>0.05$) and C ($P>0.05$), but it was statistically different from the control group ($P<0.05$). The change in granulation tissue/fibroblast maturation over time between days 7 and 14 was statistically significant ($P<0.05$). Although the differences in granulation tissue/fibroblast maturation between the groups on day 7 were not statistically significant ($P>0.05$), the differences between the groups on day 14 were found to be statistically significant ($P<0.05$). Group D was similar to groups B ($P>0.05$) and C ($P>0.05$), but it was statistically different from the control group ($P<0.05$).

The change in the amount of collagen over time between days 7 and 14 was statistically significant ($P<0.05$), although the differences in the amount of collagen between the groups on days 7 and 14 were not statistically significant ($P>0.05$). The change in epithelialization over time between days 7 and 14 was statistically significant ($P<0.05$), although the differences in epithelialization between the groups on days 7 and 14 were not ($P>0.05$). The change in neovascularization over time between days 7 and 14 was statistically significant ($P<0.05$). Although the differences in neovascularization between the groups on day 14 were not statistically significant ($P>0.05$), the differences between the groups on day seven were found to be statistically significant ($P<0.05$). Group D was similar to groups B ($P>0.05$) and C ($P>0.05$), but it was statistically different from the control group ($P<0.05$).

Table 3. Histopathological examination in all groups.

Groups		A		B		C		D		P
Variable	**Time**	**Mean ± SEM**	**Median (Min - Max)**	**Mean ± SEM**	**Median (Min - Max)**	**Mean ± SEM**	**Median (Min - Max)**	**Mean ± SEM**	**Median (Min - Max)**	
Acute Inflammation	Day 7	0.57 ± 0.2	1 (0 - 1)	0.29 ± 0.18	0 (0 - 1)	0.43 ± 0.2	0 (0 - 1)	0.29 ± 0.18	0 (0 - 1)	0.662
	Day 14	0 ± 0	0 (0 - 0)	0 ± 0	0 (0 - 0)	0 ± 0	0 (0 - 0)	0 ± 0	0 (0 - 0)	1
	P	0.046		0.157		0.083		0.157		
Chronic Inflammation	Day 7	2.57 ± 0.2	3 (2 - 3)	2 ± 0	2 (2 - 2)	2.14 ± 0.26	2 (1 - 3)	2 ± 0.22	2 (1 - 3)	0.144
	Day 14	1.14 ± 0.26	1 (0 - 2)^{a}	0.29 ± 0.18	0 (0 - 1)^{ab}	1.14 ± 0.51	1 (0 - 3)^{ab}	0 ± 0	0 (0 - 0)^{b}	0.012
	P	0.023		0.014		0.038		0.014		
Granulation Tissue	Day 7	2.57 ± 0.3	3 (1 - 3)	2.57 ± 0.2	3 (2 - 3)	2.86 ± 0.14	3 (2 - 3)	2.43 ± 0.3	3 (1 - 3)	0.636
	Day 14	1.29 ± 0.36	1 (0 - 3)^{a}	0.71 ± 0.47	0 (0 - 3)^{ab}	0.71 ± 0.42	0 (0 - 3)^{ab}	0 ± 0	0 (0 - 0)^{b}	0.032
	P	0.024		0.026		0.024		0.016		
Granulation Tissue/ Fibroblast Maturation	Day 7	2 ± 0.31	2 (1 - 3)	2.14 ± 0.14	2 (2 - 3)	2.29 ± 0.29	2 (1 - 3)	2.29 ± 0.29	2 (1 - 3)	0.824
	Day 14	1.43 ± 0.37	1 (0 - 3)^{a}	0.71 ± 0.47	0 (0 - 3)^{ab}	0.57 ± 0.43	0 (0 - 3)^{ab}	0 ± 0	0 (0 - 0)^{b}	0.024
	P	0.157		0.04		0.04		0.016		
Collagen	Day 7	0.86 ± 0.34	1 (0 - 2)	1.29 ± 0.18	1 (1 - 2)	1 ± 0.31	1 (0 - 2)	1.29 ± 0.42	1 (0 - 3)	0.744
	Day 14	2.71 ± 0.29	3 (1 - 3)	2.57 ± 0.3	3 (1 - 3)	2.43 ± 0.43	3 (0 - 3)	3 ± 0	3 (3 - 3)	0.476
	P	0.026		0.034		0.047		0.026		
Reepithelization	Day 7	1 ± 0	1 (1 - 1)	1.71 ± 0.36	1 (1 - 3)	1.86 ± 0.4	1 (1 - 3)	2 ± 0.38	2 (1 - 3)	0.158
	Day 14	2.71 ± 0.29	3 (1 - 3)	3 ± 0	3 (3 - 3)	3 ± 0	3 (3 - 3)	3 ± 0	3 (3 - 3)	0.392
	P	0.014		0.034		0.046		0.059		
Neovascularization	Day 7	3 ± 0	3 (3 - 3)^{a}	2.86 ± 0.14	3 (2 - 3)^{a}	2 ± 0.31	2 (1 - 3)^{b}	1.86 ± 0.34	2 (1 - 3)^{b}	0.007
	Day 14	0.71 ± 0.29	1 (0 - 2)	0.43 ± 0.3	0 (0 - 2)	0.71 ± 0.47	0 (0 - 3)	0 ± 0	0 (0 - 0)	0.208
		0.016		0.014		0.071		0.017		

a,b indicates the differences between groups in the same row at $P<0.05$.

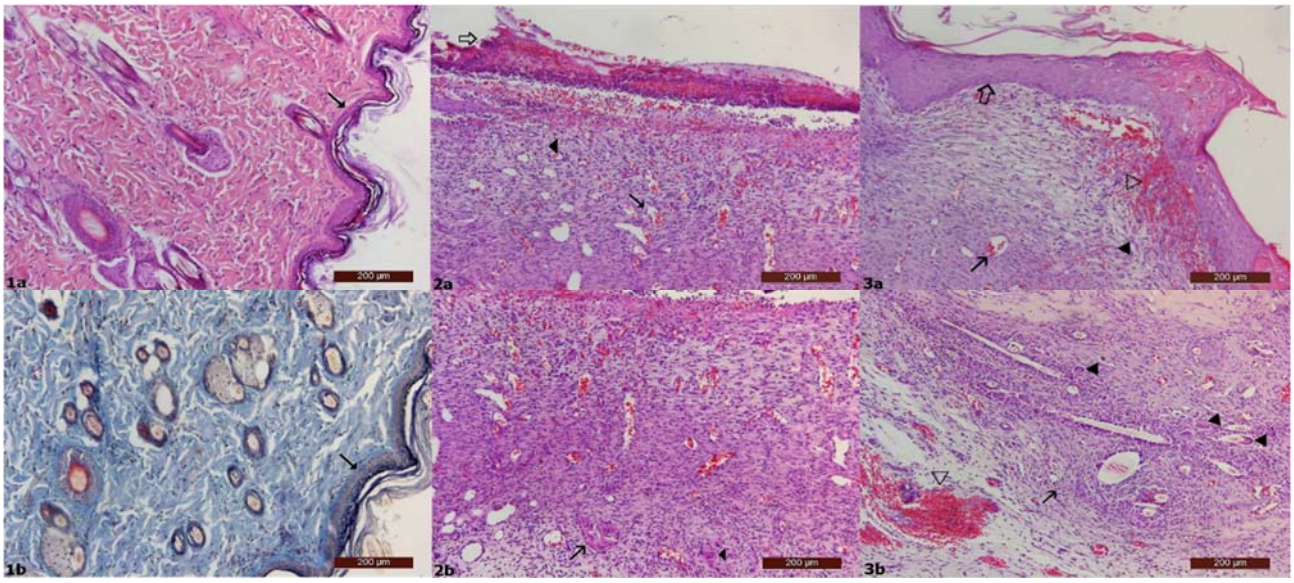

Figure 1. 1a,b: Histopathological examination of rat tissues on day zero. Intact epidermis (arrows) and dermis tissue. a: HxE b: Masson Trichrome (day 0). **2a,b:** Group A Histopathological examination on day 7. Ulcer in the epidermis (White arrow), mononuclear inflammatory cells (black arrowhead) and newly formed blood vessels, neovascularization (Black arrow). b: Foreign body giant cells (Black arrowhead) and amorphous substance with grayish material in the middle (Black arrow), HxE. **3a,b:** Group B Histopathological examination of the wound area on day 7. a: Reepithelialization in the epidermis (White arrow), mononuclear inflammatory cells (black arrowhead), bleeding site (White arrowhead) and newly formed blood vessels, neovascularization (Black arrow). b: Foreign body giant cells in free form and around the hair follicles (Black arrowheads), bleeding site (White arrowhead) and mononuclear inflammatory cells (Black arrow), HxE.

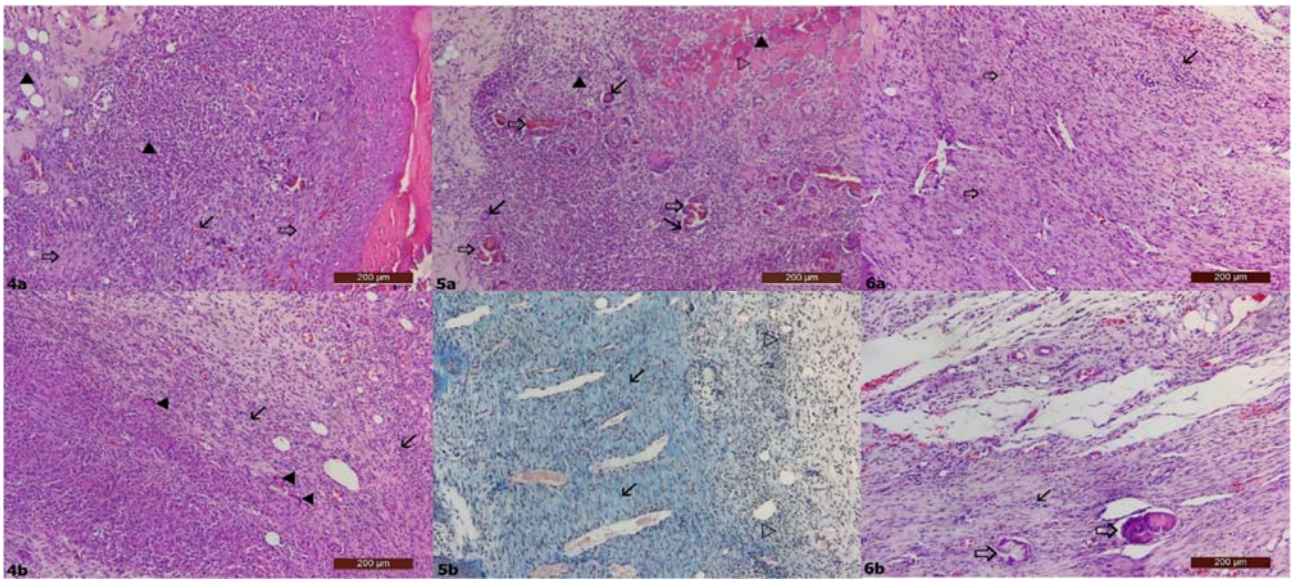

Figure 2. 4a,b: Group C Histopathological examination of the wound area on day 7. a: Mononuclear inflammatory cells infiltrated up to the muscles in the dermis (black arrowheads), granulation areas (White arrows) and newly formed blood vessels, neovascularization (Black arrow). b: Free foreign body giant cells (Black arrowheads) and diffuse mononuclear inflammatory cells (Black arrow), HxE. **5a,b:** Group D Histopathological examination of the wound areas on day 7. a: Mononuclear inflammatory cells infiltrated up to the muscles in the dermis (black arrowheads), foreign body giant cells in free form or around a pinkish amorphous structure (White arrows) (Black arrows), HxE. b: Increase in granulation tissue and collagen amount (Black arrows) and diffuse mononuclear inflammatory cells (White arrowheads), Masson Trichrome (x200). **6a,b:** Group A Histopathological examination of the wound area on day 14. a: Ongoing mononuclear inflammatory cells (black arrows) in the dermis and the increase in the amount of granulation tissue and fibroblasts (White arrows). b: pinkish/grayish amorphous structures (White arrows) and diffuse mononuclear inflammatory cells (black arrows), HxE.

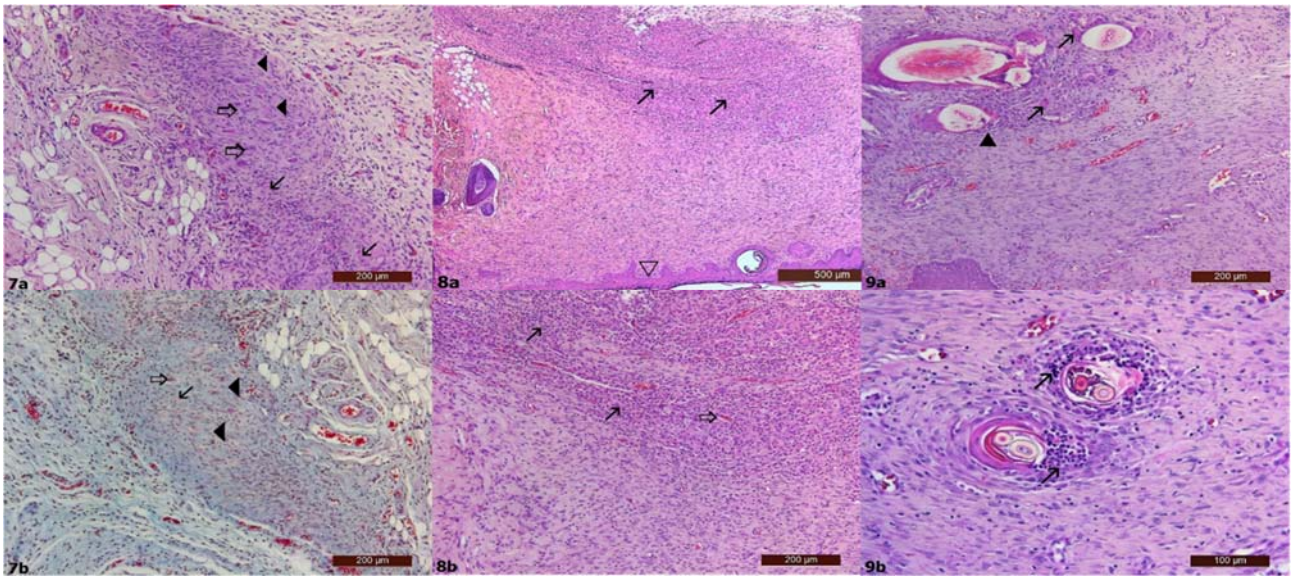

Figure 3. 7a,b: Group B Histopathological examination of the wound area on day 14. a-b: Initiation of reparation in the muscles and the placement of connective tissue cells between the muscles (black arrowheads), multinuclear cell formations (regenerative muscle cells) (White arrows) and the separation of muscle bundles and loss of myofibril lines (Black arrows), a: HxE. b: Masson Trichrome. **8a,b:** Group C Histopathological examination of the wound area on day 14. a: Reepithelialization shaped in the epidermis (white arrowhead) and mononuclear inflammatory cells infiltrated up to the muscles in the dermis (black arrows). b: Newly formed blood vessels, neovascularization (White arrows) and diffuse mononuclear inflammatory cells (Black arrows), HxE. **9a,b:** Group D Histopathological examination of the wound area on day 14. a: Mononuclear inflammatory cells (black arrowhead) and foreign body giant cells (black arrows) formed around the hair follicles in the dermis. b: Mononuclear inflammatory cells formed around the hair follicles in the dermis (Black arrows), HxE.

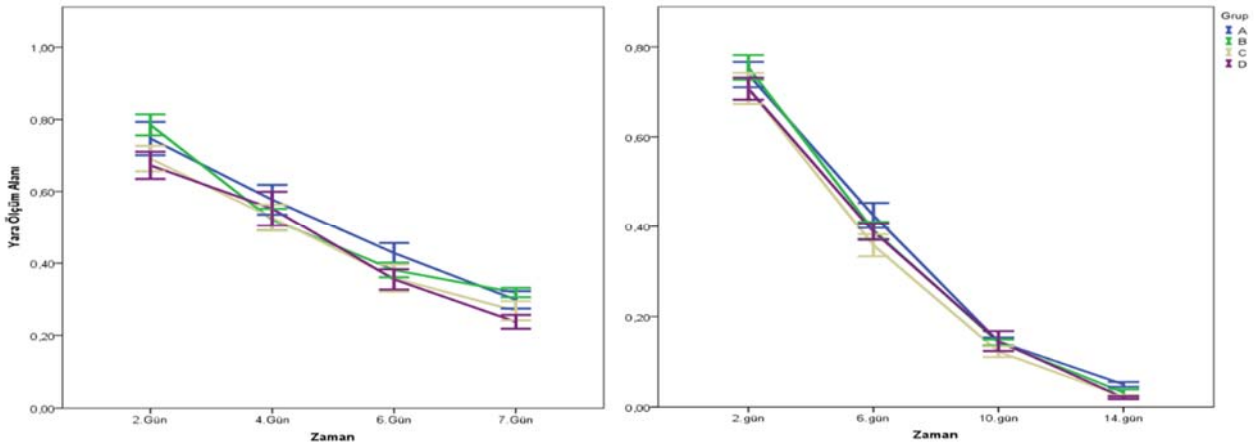

Figure 4. Display of the first 7-day and 14-day wound measurement areas of the experimental groups.

Table 4. Hydroxyproline levels in the experimental groups (µg/µl).

Number	Intact skin	Group A	Group B	Group C	Group D
Mean±SD	103.86±5.13^{d}	59.34±4.52^{c}	42.14±2.41^{b}	39.53±4.16^{b}	24.63±2.61^{a}

The values refer to mean ± standard deviation. (a,b,c,d) indicates the differences between groups in the same row, P<0.05

***Follow-up of wound areas in rats*:** After day 7, the incision area was covered with scabs in the control animals, and the epithelialization was insufficient. Skin repair was better in all rats treated with the creams, and it was difficult to distinguish the wound area from normal skin. Concerning the wound area, an observable difference was detected between the groups treated with creams for 14 days and the control group.

***Analysis of the wound areas in rats*:** The changes in the wound areas in rats on different days are presented in Figure 4. No significant differences were found between the groups regarding wound measurement areas

(P=0.424). The measured wound areas similarly decreased with time in all groups (P<0.001). There were differences between the groups for each time. Although the least mean wound measurement on day 7 was in group D, this difference was insignificant (P<0.005).

***Determination of hydroxyproline*:** Significant differences (P<0.005) were observed in hydroxyproline levels between the groups (Table 4). When hydroxyproline levels were evaluated on day 14, the highest level was found in intact skin, followed by group A (no cream), group B (treated with basic-ingredients cream), group C (treated with PH cream), and group D (treated with CH cream). The difference between groups B and D was found to be statistically significant (P<0.005).

Discussion and Conclusion

In the experimental burn model study conducted by Zohni et al. (26), the inflammatory response decreased significantly on day 7 in the group treated with hydrogel dressing preparation containing honey. In the current study, it was determined that inflammation decreased significantly in all groups on day 7. The results revealed that CH has an anti-inflammatory effect. The antibacterial activity of honey results from its high osmolarity, low pH (3.2-4.5), and the presence of hydrogen peroxide (5, 33, 42). Hydrogen peroxide creates free radicals that mediate the entry of leukocytes into areas of inflammation. It promotes the production of pro-inflammatory cytokines by leukocytes (17). It was reported that Manuka honey significantly increased the production of pro-inflammatory cytokines (45).

A previous study indicated that the epithelialization of wounds treated with Manuka honey and Indonesian honey (18) was higher than that of untreated wounds in control groups. Zohni et al. (26) reported that honey-based hydrogel dressing preparation accelerated epithelialization in the experimental burn model in rats. In the full-thickness wound healing study performed in rabbits, three types of honey (chestnut, flower, and rhododendron) were applied to the wound, and epithelialization increased significantly (P < 0.05) on day 7 in the groups treated with honey; the wounds of all trial groups were almost completely epithelialized on day 21 (32). In the current study, no differences were observed in epithelialization between the groups on days 7 and 14. Epithelialization was complete on day 14. Similar results have been reported by Nisbet et al. (32) and Haryanto et al. (18). Based on the day seven results, no differences were observed between the groups regarding epithelialization in the current study, unlike the results reported by Nisbet et al. (32) and Zohni et al. (26).

Several studies have reported that materials containing Acacia honey (19), Malaysian honey (26), Indonesian honey (18), Manuka honey (18, 30), and other types of honey (32, 38) enhance granulation tissue formation, capillary formation, and collagen synthesis in wound areas. In the current study, although the differences in the amounts of granulation tissue and granulation tissue/fibroblast maturation between the groups on day 7 were not statistically significant, the differences between the groups on day 14 were found to be statistically significant. The granulation tissue in the CH group decreased rapidly by day 14 day compared to the other groups. In this study, the differences in the amount of collagen between the groups on days 7 and 14 were not statistically significant. The collagen results of this study are similar to the results of Zohdi et al. (26), Nisbet et al. (32), Mukai et al. (30), and Iftikhar et al. (19). Sugar in honey can be used as an energy source for the synthesis of collagen, which can be demonstrated by fibroblast proliferation and collagen synthesis on day 8 (43). In the current study, the thickness of the wound tissue did not significantly increase; studies by Ghaderi and Afshar (14) and Haryanto et al. (18) also showed the effectiveness of Indonesian and Manuka honey on the formation of collagen fibers was almost the same. Honey is mildly acidic, with a pH between 3.2 and 4.5 (28). Topical acidification of wounds also supports healing (20). The hydrogen peroxide produced by honey stimulates tissue growth. It was demonstrated that hydrogen peroxide stimulates fibroblast growth in cell culture at micromolar and nanomolar concentrations (39). Furthermore, honey is a rich source of carbohydrates. It can provide the environment and energy required for fibroblastic proliferation and maturation and collagen formation, increasing wound shrinkage and consequently increasing the tensile strength of wounds (5, 11). Honey in low concentrations delivers hydrogen peroxide to the wound area in a slow-release manner, which promotes angiogenesis and the growth of fibroblasts (27, 28). The increase in collagen production and fibroblast maturation with the CH-containing cream may be due to the hydrogen peroxide produced by the honey and the energy it provides to the wound area. In this study, the differences between the groups on day seven were found to be statistically significant. Group D was similar to groups B and C but statistically different from group A. It is possible that the lower level of neovascularization in group D on day 7 compared to the other groups and the complete disappearance of vascularization on day 14 were due to the healing effects of CH. The increased healing rate can also be attributed to the lymph-draining osmotic effect of honey and may result from a deeper flow of nutrients from working capillaries (29).

Nisbet et al. (32) evaluated the effects of three different types of honey (chestnut, flower, and rhododendron) on the healing areas of full-thickness wounds in rabbits on days 7 and 14; they could not find significant differences in the wounds during the healing

process. The flower honey was found to be the least effective in this regard. In another study, the effects of Manuka honey and Indonesian honey on full-thickness wounds in rats were compared; according to the study, wounds did not differ significantly between the Indonesian and Manuka honey groups 11 days after wound creation. In the control group, the decrease in the wound area was very slow for the first five days; however, the reduction in the control group's wound areas was the same as that for the honey-treated groups on day 11 (18). In the current study, wound healing at day 14 was found to be similar in all groups, similar to the results reported by Nisbet et al. (32) and Haryanto et al. (18).

Increased hydroxyproline content in granulation tissue is indicative of an increased collagen cycle and shows the maturation and proliferation of collagen during wound healing (22). Although some studies have shown that applying honey to the wound area increases the production of hydroxyproline (19, 32, 36), one study has indicated that honey has no effect on hydroxyproline production (40). In the current study, the hydroxyproline levels of the groups treated with CH- and PH-containing creams were found to be significantly lower on day 14 compared to the control group. These results differ from the results of previous studies. Furthermore, the current study found that auxiliary substances in creams also decreased the hydroxyproline level in the wound area. The histopathological examination showed no significant differences in the amount of collagen between the groups on days 7 and 14.

More oxygen is released from oxyhemoglobin at an acidic pH (41). Therefore, the absence of necrosis of the wound edges in treated animals may be associated with improved tissue oxygenation due to the lower pH of the honey. The lack of necrosis provides increased wound contraction, lower inflammatory responses, better tissue organization, and improved mechanical properties of treated wound lesions. Thus, honey shortens the inflammatory phase of wound healing due to its antibacterial effect, while its acidic pH supports the delivery of O_2 to the healing tissue. The high concentration of carbohydrates in honey provides a rich source of nutrients and energy for the healing tissue, while amino acids play a significant role in collagen formation and maturation, improving contraction and epithelialization, helping in fibroblastic division and maturation, reshaping and organizing collagen fiber, and consequently increasing tensile strength. The organization of collagen fibers may be due to reduced edema and inflammatory exudates, which may result from the high osmolarity of this high-energy source (8, 33). Furthermore, honey can contain many medicinal compounds, including essential oils, flavonoids, terpenes, and polyphenols, depending on the plant from which the pollen is taken (4, 13, 15). These various components of honey also affect wound healing.

In conclusion, the results from this study show that creams containing CH were more effective in wound healing than those containing PH. Thus, the botanical origin of honey is significant in terms of efficiency. The application period also affects wound healing. Based on the results obtained from this study, new forthcoming studies with different types of honey, animal models, and dosages can be planned.

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Ethical Statement

This study was approved by the Kırıkkale University Animal Experiments Local Ethics Committee (Meeting Number:16/03, Meeting Decision: 16/44).

Conflict of Interest

The authors declared that there is no conflict of interest.

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