doi.org/10.36721/PJPS.2021.34.3.SUP.1063-1067.1

Effect of propolis on wound healing in sacrococcygeal pilonidal disease: A randomized controlled clinical trial

Mehmet Kubat[1]*, Zülfikar Karabulut[2] and Serkan Şengül[2]
[1]Department of General Surgery, Alanya Training and Research Hospital, Alanya, Antalya, Turkey
[2]Department of General Surgery, Alaaddin Keykubat University, Alanya, Antalya, Turkey

Abstract: Wound healing and recurrence are the leading problems encountered in sacrococcygeal pilonidal sinus disease. Propolis has a place in both traditional and complementary medicine, and in vitro and in vivo studies have reported its anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal and immunostimulant properties. In the present study, we discuss the effect of propolis on wound healing in sacrococcygeal pilonidal diseases treated with marsupialization. Patients who were admitted to our clinic with sacrococcygeal pilonidal disease were analyzed prospectively, with a total of 33 patients divided into study and control groups. All patients underwent marsupialization surgery, and the wound areas were analyzed post-operatively, on the 0, 7th, 14th, 28th days and on the day of complete recovery. An acceleration of wound healing was observed from the first week that was found to be even faster between days 14 and 28. The complete recovery score in the study group was significantly lower. Propolis can be used to accelerate wound healing when the marsupialization method is preferred in patients diagnosed with uncomplicated sacrococcygeal pilonidal cyst due to its low cost, good patient compliance, low side effect profile, lack of toxicity and high efficacy.

Keywords: Pilonidal cyst, wound healing, regeneration, propolis, biological product, biopolymers.

INTRODUCTION

Wound healing is a multifactorial complex repair process, the various stages of which can be affected by factors such as age, gender, presence of infection, nutritional status, accompanying diseases and medications (Mills *et al*., 2020). There are numerous products and methods used fort he immediate treatment of chronic wounds that minimize scar formation and complications. However, many of these products are not cost-effective (Dhivya *et al*., 2015). The high cost of chronic wound treatment is placing an increasingly significant burden on healthcare systems. Since bacterial resistance against antimicrobials is increasing day by day, studies conducted on natural and traditional products with healing potential are increasing (Sell *et al*., 2012).

Sacrococcygeal pilonidal sinus disease is common disorder in which wound healing and recurrence are the leading problems, and for which many surgical approaches have been developed. Techniques requiring secondary wound healing are among the most frequently used procedures due to their ease of application and the decreased prevalence of early wound complications (Wysocki, 2019).

Propolis (bees-wax), which has been a staple ingredient in traditional and complementary medicines for 1000s of years, is a botanical resinous bee product. The anti-inflammatory, anti-oxidant, anti-bacterial, anti-fungal and immunostimulant properties of which have been reported in both in vitro and in vivo studies. (Oryan *et al*., 2018, Corrêa *et al*., 2020). The properties and bio-activities of propolis may vary, however, depending on the flora of each geographical region (Jobir and Belay, 2020). Propolis is known to have more than 300 different components, although the main components that are thought to play an active role in wound healing are caffeic acid esters and flavonoids (Russo *et al*., 2002).

The present study compares the rate of wound healing between well-assessed, standardized propolis (Anatolian propolis) use and routine wound dressings in patients with sacrococcygeal pilonidal sinus treated with marsupialization, as a secondary healing process, and to evaluate the effectiveness of propolis.

MATERIALS AND METHODS

Included in this randomized, prospective study were 33 patients aged 18-45, who underwent marsupialization surgery due to sacrococcygeal pilonidal sinus in Alanya Training and Research Hospital between September 2019 and December 2019. Patients with recurrent and complicated disease, patients with accompanying morbidities, patients who did not give consent for marsupialization as a surgical technique, and those allergic to bee and bee products were excluded from the study. The demographic data of the patients, and the results of wound area analyses post-operatively, on the 0, 7th, 14th, 28th days, and on the day of complete recovery were recorded. The patients were divided into two groups

**Corresponding author:* e-mail: dr.m.kubat@gmail.com

as propolis and control. A gauze soaked with 1 ml of a 15%-propolis water solution was applied to the entire wound surface for each dressing in the patients in the propolis group. In the control group, the gauze was soaked in a 0.9% NaCl solution and administered to the entire wound surface.

The surgical procedures were performed by the same team, and all-surgery was performed under spinal anesthesia. Typically, sacrococcygeal pilonidal sinus tissue was excised using an ellipse-shaped skin incision measuring 5x3 cm, after which marsupialization was performed by stitching the sacrococcygeal fascia with a 0-vicryl suture material, reducing the wound size. The remaining gap was left to secondary healing. Dexketoprofen Trometamol 25 mg twice daily was given to all patients as routine for 5 days for postoperative pain control.

The wound dressings of all patients were changed every other day. Wounds were photographed postoperatively, and on the 7th, 14th and 28th days after the operation, and wound areas were measured and recorded using the "ImageJ" (https://imagej.nih.gov/ij/download.html) program.

The propolis solution used in the study was water soluble containing 15% propolis extract, and water and vegetable glycol as the carrier. (Bee'o® Up (conf. No & date: 004464-13.07.2018)) A laboratory analysis of the solution revealed: Total Phenolic Content: 66.3 mg GAE/ml, Total Flavonoid Content: 47.9 mg TE/ml, Antioxidant Capacity: 254.9 mg TE/ml, Galangin: 3150 µg/ml, Caffeic Acid: 1275 µg/ml and CAPE: 3150 µg/ml. The product used in the study was produced using a mixture of local propolis (Anatolian propolis) varieties, well standardized and highly preferred due to their high levels of flavonoids and caffeic acid phenyl ester (CAPE).

STATISTICAL ANALYSIS

Mean, standard deviation, median lowest, median highest, frequency and ratio values were the descriptive statistics used in the data analysis. The distribution of variables was measured with a Kolmogorov-Smirnov test. An Independent sample t test and a Mann-Whitney U test were used for the analysis of quantitative independent data, and a Wilcoxon test was used for the analysis of the dependent quantitative data. A Chi-square test was used for the analysis of qualitative independent data. The SPSS 26.0 program was used for the statistical analysis.

Ethical approval

The collection of data and the analysis were carried out after approval was obtained from the local ethics committee (date: 01/Agu/2019 no: 7-26). The study protocol followed the ethical guidelines of the 1975 Declaration of Helsinki, as reflected in the approval granted by the ethics committee. Informed consent was obtained for experimentation with human subjects.

RESULTS

All of the patients participating in the study were male and were aged 18-45 (mean 25.5±6.0) years. Complete recovery was observed in all patients (mean 52.0 days ± 10.5). Wound dehiscence developed as a complication of wound healing in the first week in 10 patients in the entire sample. The average wound area sizes were 4.7cm2 on day 0, 5.0 cm2 on day 7, 4.3cm2 on day 14 and 2.8cm2 on day 28 (table 1).

The age and gender distribution of patients did not differ significantly ($p>0.05$) between the study and the control groups. In the study group, the complete recovery score was significantly lower ($p<0.05$) than in the control group (table 2). The complication rate in the study and control groups did not differ significantly ($p>0.05$) (table 2).

The wound area on day 0, 7th and 14th in both groups did not differ significantly ($p>0.05$). The 28th day wound area score in the study group was significantly lower ($p<0.05$) than in the control group. In the study group, the decrease in wound area recorded on the 28th day was significantly higher ($p<0.05$) than in the control group (table 2) (fig. 1)

DISCUSSION

Due to healing difficulties and treatment costs, chronic wounds are placing an increasing significant burden on the health economy every day (Fetterolf, 2019). Most chronic wounds exceed planned treatment times, and a significant number requiring long-term wound care and additional surgery may not heal at all. There have been many multifaceted studies carried out to identify the optimum treatment process (Pushparaj *et al*., 2019). While many of these studies take advantage of the possibilities of modern medicine, many evaluate the effectiveness of traditional complementary medicines (Mujica *et al*., 2019).

Deroofing, marsupialization and wide-open excision methods based on secondary wound healing in sacrococcygeal pilonidal sinus diseases are in common use due to their ease of application and reduced prevalence of early wound complications. Marsupialization has become a frequently adopted approach since it was first described in 1938, with better recurrence rates and recovery rates recorded than with wide open excisions (Stauffer *et al*., 2018). Healing occurs with wound contraction and re-epithelialization. Most wounds progressing to the chronic stage recover in 2–4 months, despite the optimal treatment processes, although long-term treatment may be required (Wysocki,

2019). In this observational study the marsupialization method was used on the sacrococcygeal fascia in patients who gave their consent for the surgical technique, being associated with better patient compliance, better outcomes and the availability of experienced surgeons. The infectious complications associated with the marsupialization technique, which has a 58% lower recurrence rate than primary closure methods, do not differ from those associated with primary closure methods. The advantage of primary closure over marsupialization is the shorter time for wound healing and return to work, although recurrence rates, wound infection development and wound dehiscence are controversial issues. It is not the surgical technique that determines the long-term satisfaction of patients, but the lack of recurrence (Doll *et al*., 2015).

Propolis, a bee (apis mellifera) product, has anti-microbial, anti-oxidant, mast cell regulatory and anti-inflammatory properties that support chronic wound healing, along with flavonoids and caffeic acid phenyl ester (CAPE) (Przybyłek and Karpiński, 2019, Curti *et al*., 2019, Wolska *et al*., 2019). Propolis solutions containing high flavonoid and CAPE levels have thus been used for wound healing. Flavonoid compounds have also been reported to reduce lipid peroxidation and to prevent tissue necrosis (Olczyk *et al*., 2013), and this antioxidant potential is also believed to be directly related to wound healing (Mujica *et al*., 2019, M Afonso *et al*., 2020). Propolis has also been found to reduce bacterial colonization and prevent biofilm formation (Meto *et al*., 2020), and it has been well documented that biofilm formation affects immune response and has a negative effect on chronic wound healing (Goldberg and Diegelmann, 2020). In the present study, faster wound healing was observed in the propolis group, while no local infectious complications were observed in any of the patients. This finding reduces the difference between marsupialization and primary repair techniques in terms of the recovery time, as stated previously (McCallum *et al*., 2008).

Factors such as the source of the propolis, dosage and propolis extraction solvents may have an effect on antimicrobial activity and recovery (Miguel and Antunes, 2011). Since ethyl alcohol extraction solutions have a bactericidal effect and alter the wound healing process, we used a propolis solution in a water-soluble form using propylene glycol, which had a high flavonoid content. Previous studies have suggested that a high flavonoid content can accelerate recovery (De Almeida *et al*., 2013), while further studies suggest that it is not possible to control the total flavonoid content of propolis in wound healing (Batista *et al*., 2012). This does not negate the fact that propolis clinically facilitates wound healing, although it does suggest that the content may lead to minimal differences in recovery.

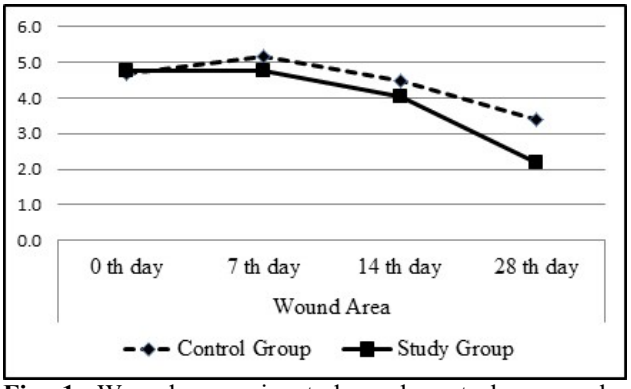

Fig. 1: Wound areas in study and control groups by healing days

The effectiveness of propolis has been demonstrated in many in vivo and in vitro studies (Martinotti *et al*., 2019, Sahib *et al*., 2020), and in the few clinical studies in literature, propolis has also been found to be effective (Mujica *et al*., 2019, Henshaw *et al*., 2014). The present study concluded that the improvement seen in wounds treated with propolis was statistically significant, with an acceleration in wound healing observed from the first week. In the experimental study conducted by Eyarefe *et al*. (Eyarefe *et al*., 2019), the effects of propolis on wound healing were evaluated, and as was observed in the present study, propolis was found to accelerate wound healing. In their study, Mujica *et al*. reported that propolis was found to be effective in diabetic foot wounds by reducing oxidative stress and increasing cicatrization (Mujica *et al*., 2019). Furthermore, in a group of patients with similar diabetic foot disorders, Henshaw *et al*. also reported on the effectiveness of propolis (Henshaw *et al*., 2014). In the present study, the positive effect of propolis on wound healing was observed through an evaluation of an uncomplicated patient group rather than a patient group with comorbidities with infectious and vascular pathologies such as diabetic foot. In a study of burn patients, a natural rubber membrane with additional propolis was used, and was reported to accelerate wound healing (Krupp *et al*., 2019). Propolis is known to shorten the inflammatory phase of wound healing by reducing the number of inflammatory cells, and it also increases collagen organization, leading to faster wound contraction. It is known that propolis accelerates wound closure from the first day and wound contracture is faster after 14 days (Abu-Seida, 2015). Similarly, we found in the present study that the effect of propolis on wound healing was significantly faster after day 14.

In the present study, statistically significantly fewer complications such as wound dehiscence and infection were identified, and the difference between the two groups was insignificant in this regard. All of the wound dehiscences in the present study occurred in the first postoperative week, and was thought to be due to mechanical effects rather than wound healing.

Table 1: Characteristics of all patients

		Min-Max	Median	Avg.±sd / % (n)
Age		18.0 - 45.0	25.0	25.5±6.0
Complication	(-)			69.7% (n=23)
	(+)			30.3% (n=10)
Complete Recovery (day)		34.0 - 72.0	52.0	52.0±10.5
Wound Areas	0 day	3.3 - 7.9	4.5	4.7±1.2
	7 th day	2.4 - 9.4	4.4	5.0±1.7
	14 th day	2.2 - 7.3	4.0	4.3±1.4
	28 th day	1.0 - 4.8	2.9	2.8±1.0

Table 2: Comparison of control group and study (propolis) group, and evaluation of wound healing

		Control Group (n=18)		Study Group (n=15)		*p value*
		Avg.±sd /n (%)	Median	Avg.±sd /n (%)	Median	
Age		25.8 ± 5.9	25.0	25.3 ± 6.3	25.0	0.637[m]
Complication	(-)	13 (72.2%)		10 (66.6%)		0.730 [X²]
	(+)	5 (27.8%)		5 (33.3%)		
Complete Recovery		59.7 ± 6.6	58.5	42.7 ± 5.6	42.0	*<0.001*[t]
Wound Area	0 th day	4.7 ± 1.1	4.5	4.8 ± 1.3	4.2	0.871[m]
	7 th day	5.2 ± 1.7	4.3	4.8 ± 1.8	4.8	0.329[m]
	14 th day	4.5 ± 1.3	4.1	4.0 ± 1.6	4.0	0.311[m]
	28 th day	3.4 ± 0.8	3.1	2.2 ± 0.9	2.4	*<0.001*[m]
0th-7th healing	(area)	0.5 ± 1.9	-0.2	0.0 ±	1.7 -0.5	0.158[m]
p value		0.744[w]			0.307[w]	
0th – 14th healing	(area)	-0.2 ± 1.5	-0.7	-0.7 ± 1.6	-1.2	0.138[m]
p value		0.133[w]			0.069[w]	
0th- 28th healing	(area)	-1.3 ± 1.2	-1.6	-2.6 ± 1.2	-2.6	*0.010*[m]
p value		*0.001*[w]			*0.001*[w]	

[t] t test / [m] Mann-whitney u test / [w] Wilcoxon test / [X²] Chi square test

Furthermore, there were no allergic reactions or side effects in the patients treated with propolis. There has to date been no large cohort study into the potential side effects of propolis. When the side effect profile is evaluated based on studies performed to date, local side effects have been observed in topical applications (de Groot, 2013). We encountered no such side effects in our study group. The toxicity of propolis has not been reported in literature.

CONCLUSION

Based on the presented results, propolis may be preferred for the acceleration of wound healing in patients with uncomplicated sacrococcygeal pilonidal sinus who are treated with marsupialization, due to its anti-bacterial, anti-fungal, anti-oxidant and anti-inflammatory activity. It is a biomaterial that can be used in acute and chronic wound care products due to its low cost, good patient compliance, low side effect profile, low expectation of toxicity and high efficiency. It can expand the perspective of wound healing products and technologies, which is an important problem in the health economy.

REFERENCES

Abu-Seida AM (2015). Effect of propolis on experimental cutaneous wound healing in dogs. *Vet. Med. Int.,* 672643.

Afonso AM, Gonçalces J, Luis A, Gallardo E and Duarte AP (2020). Evaluation of the *in vitro* wound-healing activity and phytochemical characterization of propolis and honey. *Appl. Sci.,* **109**(5): 1845.

Batista LLV, Campesatto EA, Assis MLBD, Barbosa APF, Grillo LAM and Dornelas CB (2012). Estudo comparativo do uso tópico de propolis verde e vermelha na reparação de feridas em ratos. *Rev. Col. Bras. Cir.,* **39**(6): 515-520.

Corrêa JL, Veiga FF, Jarros IC, Costa MI, Castilho PF, De Oliveira KMP, Rosseto HC, Bruschi ML, Svidzinski TI and Negri M (2020). Propolis extract has bioactivity on the wall and cell membrane of *Candida albicans*. *J. Ethnopharmacol.,* **256**: 112791.

Curti V, Zaccaria V, Sokeng T, Jorel A, Dacrema M, Masiello I, Mascaro A, D'Antona G and Daglia M (2019). Bioavailability and *in vivo* antioxidant activity of a standardized polyphenol mixture extracted from brown propolis. *Int. J. Mol. Sci.,* **20**(5): 1250.

De Almeida EB, Cardoso JC, De Lima AK, De Oliveira NL, De Pontes-Filho NT, Lima SO, Souza ICL and De Albuquerque-Júnior RLC (2013). The incorporation of Brazilian propolis into collagen-based dressing films improves dermal burn healing. *J. Ethnopharmacol.,* **147**(2): 419-425.

De Groot AC (2013). Propolis: A review of properties, applications, chemical composition, contact allergy, and other adverse effects. *Dermatitis,* **24**(6): 263-282.

Dhivya S, Padma VV and Santhini E (2015). Wound dressings a review. *Bio Medicine*, **5**(4): 22

Doll D, Luedi MM, Evers T, Kauf P and Matevossian E (2015). Recurrence-free survival, but not surgical therapy per se, determines 583 patients' long-term satisfaction following primary pilonidal sinus surgery. *Int. J. Colorectal Dis.,* **30**(5): 605-611.

Eyarefe OD, Ozota CA, Jarikre TA and Emikpe BO (2019). Pathological and immunohistochemical evaluation of wound healing potential of Nigerian bee propolis in albino rats. *Comp. Clin. Pathol.,* **28**(2): 455-466.

Fetterolf DE (2019). Estimating the economic value of emerging technologies in chronic wound therapy. *Int. Wound J.,* **16**(6): 1391-1397.

Goldberg SR and Diegelmann RF (2020). What makes wounds chronic? *Surg. Clin.,* **100**(4): 681-693.

Henshaw FR, Bolton T, Nube V, Hood A, Veldhoen D, Pfrunder L, McKew GL, Macleod C, McLennan SV and Twigg SM (2014). Topical application of the bee hive protectant propolis is well tolerated and improves human diabetic foot ulcer healing in a prospective feasibility study. *J. Diabetes Complications,* **28**(6): 850-857.

Jobir MD and Belay A (2020). Comparative study of different ethiopian propolis: *In vivo* wound healing, antioxidant, antibacterial, physicochemical-properties and mineral profiles. *J. Apither,* 7(2): 31-48.

Krupp T, Dos Santos BD, Gama LA, Silva JR, Arrais-Silva WW, De Souza NC, Américo MF and De Souza Souto PC (2019). Natural rubber-propolis membrane improves wound healing in second-degree burning model. *Int. J. Biol. Macromol.,* **131**: 980-988.

Martinotti S, Pellavio G, Laforenza U and Ranzato E (2019). Propolis induces AQP3 expression: A possible way of action in wound healing. Molecules, **24**(8): 1544.

McCallum IJ, King PM and Bruce J (2008). Healing by primary closure versus open healing after surgery for pilonidal sinus: Systematic review and meta-analysis. *Bmj,* **336**(7649): 868-871.

Meto A, Colombari B, Meto A, Boaretto G, Pinetti D, Marchetti L, Benvenuti S, Pellati F and Blasi E (2020). Propolis affects *Pseudomonas aeruginosa* growth, biofilm formation, eDNA release and phenazine production: Potential involvement of polyphenols. *Microorganisms,* **8**(2): 243.

Miguel MG and Antunes MD (2011). Is propolis safe as an alternative medicine? *J. Pharm. Bioallied Sci.,* **3**(4): 479.

Mills SJ, Hofma BR and Cowin AJ (2020). Pathophysiology of wound healing. *In*: Fitridge R. (eds) Mechanisms of Vascular Disease. Springer, Cham, pp.541-561

Mujica V, Orrego R, Fuentealba R, Leiva E and Zúñiga-Hernández J (2019). Propolis as an adjuvant in the healing of human diabetic foot wounds receiving care in the diagnostic and treatment centre from the regional hospital of Talca. *J. Diabetes Res.,* 2507578.

Olczyk P, Komosinska-Vassev K, Winsz-Szczotka K, Stojko J, Klimek K and Kozma EM (2013). Propolis induces chondroitin/dermatan sulphate and hyaluronic acid accumulation in the skin of burned wound. *Evid. Based Complement. Alternat. Med.,* **2013**: 290675.

Oryan A, Alemzadeh E and Moshiri A (2018). Potential role of propolis in wound healing: Biological properties and therapeutic activities. *Pharmacother.,* **98**: 469-483.

Przybyłek I. and Karpiński TM (2019). Antibacterial properties of propolis. *Molecules,* **24**(11): 2047.

Pushparaj M, Ranganathan R and Ganesan S (2019). Design and Development of Drug Delivery System for Chronic Wound Using Additive Manufacturing. In: Kumar L, Pandey P, Wimpenny D (eds) 3D Printing and Additive Manufacturing Technologies. Springer, Singapore. Pp.119-126

Russo A, Longo R and Vanella A (2002). Antioxidant activity of propolis: role of caffeic acid phenethyl ester and galangin. *Fitoterapia,* **73**: S21-S29.

Sahib A. H., Shafeeq M. A. A. and Mohsen S. M. (2020). The effectiveness of ethanolic extract of propolis on wound healing in albino rats. *Annals of Tropical Medicine and Health,* **23**: 543-554.

Sell SA, Wolfe PS, Spence AJ, Rodriguez IA, McCool JM, Petrella RL, Gark K, Ericksen JJ and Bowlin GL (2012). A preliminary study on the potential of manuka honey and platelet-rich plasma in wound healing. *Int. J. Biomater.,* 313781

Stauffer V, Luedi M, Kauf P, Schmid M, Diekmann M, Wieferich K, Schnüriger B and Doll D (2018). Common surgical procedures in pilonidal sinus disease: A meta-analysis, merged data analysis, and comprehensive study on recurrence. *Sci. Rep.,* **8**(1): 1-28

Wolska K, Gorska A, Antosik K and Lugowska K (2019). Immunomodulatory effects of propolis and its components on basic immune cell functions. *Indian J. Pharm. Sci.,* **81**(4): 575-588.

Wysocki A (2019). Primary open treatment of sacrococcygeal pilonidal disease. *Coloproctology,* **41**(2): 106-109.