DOI:10.34084/bshr.1289441 e-ISSN 2587-1641

Journal of Biotechnology and Strategic Health Research

Araştırma Makalesi /Research Article

http://dergipark.org.tr/tr/pub/bshr

Effect of Propolis on Wound Healing: A Clinical and Histomorphometric Study in Rats

Propolisin Yara İyileşmesindeki Etkisi: Sıçanlarda Klinik ve Histomorfometrik Bir Çalışma

Aysun Akpınar[1], Hakan Özdemir[2]

[1]Bursa Uludag University, Faculty of Dentistry, Department of Periodontology, Bursa, Türkiye

[2] Eskisehir Osmangazi University, Faculty of Dentistry, Department of Periodontology, Eskisehir, Türkiye

ORCID ID: Aysun Akpınar: https://orcid.org/0000-0002-6740-3598, Hakan Özdemir: https://orcid.org/0000-0001-9550-1999

***Sorumlu Yazar / Corresponding Author:** Hakan Özdemir , **e-posta / e-mail:** hozdemir52@hotmail.com

Geliş Tarihi / Received : 29-04-2023 **Kabul Tarihi / Accepted:** 31-05-2023 **Yayın Tarihi / Online Published:** 30-08-2023

Akpınar A., Özdemir H. Effect of propolis on wound healing: a clinical and histomorphometric study in rats. J Biotechnol and Strategic Health Res. 2023;7(2):81-88

Abstract

Aim Propolis is known to have antioxidant, antiinflammatory, antibacterial, antiviral, immunostimulant and local anesthetic effects. The aim of the present study was to investigate the clinical and histomorphometric effects of propolis on the healing of excisional palatal wounds in rats.

Material and Method Sixty male Wistar rats were used for the study. Six animals were sacrificed at beginning of the study as initial wound (0 day). The rats were divided into three groups: Propolis (P), Chlorhexidine (CHX) and Control (C). Subjects in all three groups were randomly selected to form nine subgroups of six rats each. Under anesthesia, circular excision wounds with a diameter of 3 mm were formed in the middle of the palate of the rats by punching. The mucoperiosteal part was removed with sharp dissection, and the area on the open bone surface was left to heal the secondary wound. Propolis was administered locally at a rate of 1 ml/day. The rats were sacrificed on days 7, 14, and 21, and pictures of the wound area were taken. Each photograph was transferred to a dedicated program to measure the defect area. Histological sections were taken and the presence of inflammatory cells, epithelialization, and degree of healing were assessed.

Results The average wound area between epithelial margins decreased significantly over time in all groups ($p<0.05$). compared to CHX and C groups, significant reduction of wound area was observed after seven, 14 and 21 days by using Propolis at (respectively 5.56±3.77, 3.70±1.76, 1.12±0.83). On day 21st day, the inflammatory cells were still observed in the Control group.

Conclusion The results of the study show that propolis has a positive effect on the healing of soft tissue by accelerating wound healing.

Keywords Chlorhexidine, oral wound healing, propolis

Özet

Amaç *Propolisin antioksidan, antiinflamatuar, antibakteriyel, antiviral, immunostimulant ve lokal anestezik etkileri olduğu bilinmektedir. Bu çalışmanın amacı, sıçanlarda eksizyonel damak yaralarının iyileşmesinde propolisin klinik ve histomorfometrik etkilerinin araştırılmasıdır.*

Gereç ve Yöntem *Çalışmada 60 adet erkek Wistar sıçan kullanıldı. Başlangıçta iyileşme referansı olarak (0 gün) altı hayvan sakrifiye edildi, Sıçanlar 3 gruba ayrıldı: Propolis (P), Klorheksidin (CHX) ve Kontrol (K). Tüm gruptaki denekler rastgele seçilerek altışar sıçandan oluşan dokuz alt grup oluşturuldu. Anestezi altına sıçanların damaklarının tam ortasında punch ile 3 mm çapında sirküler eksizyonel yara yüzeyleri oluşturuldu. Mukoperiosteal kısım keskin diseksiyonla uzaklaştırıldı ve açık kemik yüzeyindeki alan sekonder iyileşmeye bırakıldı. Propolis 1 ml/gün olacak şekilde gavaj yoluyla lokal olarak uygulandı. Sıçanlar yedinci, 14. ve 21. günlerde sakrifiye edildi ve yara bölgesinin fotoğrafları çekildi. Her bir fotoğraf özel bir programa aktarılarak defekt bölgesindeki yara alanı ölçüldü. Histolojik kesitler alınarak enflamatuar hücre varlığı, epitelizasyon ve iyileşme düzeylerine bakıldı.*

Bulgular *Epitelyal marjinler arasındaki ortalama yara alanı, tüm gruplarda zamanla önemli ölçüde azaldı ($p <0,05$). Propolis grubu, Chx ve K grubu ile karşılaştırıldığında yedi, 14 ve 21. günlerde (sırasıyla 5,56±3,77; 3,70±1,76; 1,12±0,83) belirgin bir yara alanı azalması gözlemlenmiştir 21. günde kontrol grubunda inflamatuar hücreler gözlenmeye devam etti.*

Sonuç *Çalışmanın bulguları ışığında propolisin yara iyileşmesini hızlandırarak yumuşak dokunun iyileşmesinde olumlu etkileri gözlenmiştir.*

Anahtar Kelimeler *Klorheksidin, yara iyileşmesi, propolis*

INTRODUCTION

Wound healing is characterised by the union of epithelial cells, endothelial cells, inflammatory cells, platelets, and fibroblasts and the performance of their normal functions in a specific order and sequence. It is well known that many people in the world suffer from chronic wounds.[1]

In the search for an agent that promotes oral wound healing and reduces postoperative complications, many agents have been investigated. Given the difficulty in postoperative plaque control after oral surgery, topical antimicrobial agents are recommended to improve wound healing by reducing plaque accumulation while reducing postoperative pain and swelling.[2,3]

Various plant extracts have been tested for their antimicrobial, analgesic, hemostatic, antibacterial, anti-inflammatory, antifungal, and antiviral effects.[4-7]

Propolis is a resinous product that honey bees collect from living plants and use to build their hives.[8-11] Several components of propolis, such as tannins, flavonoids, and essential oils, have been associated with pharmacological properties. These are thought to act on bacterial cell walls and their specific constituents, such as lipopolysaccharide and reduce the protein content of the oral biofilm.[4,8-10] Propolis extracts effectively inhibit the growth and attachment of Streptococcus mutans, Porphyromonas gingivalis, Prevotella intermedia, Fusobacterium sp, Capnocytophaga sp. and Eikenella sp.[11,12] In addition to the antimicrobial properties of propolis, anti-inflammatory, antipsoriatic and analgesic effects have also been noted.[13]

Jacob et al.[14] studied the effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts and tested their potential for wound healing. Propolis was reported to be an excellent candidate for the treatment of burns as it enhances proliferation, activation and growth capacity of skin cells. The therapeutic efficacy of propolis was verified by quantitative and qualitative analyses of the expression and degradation of collagen type I and III in the wound matrix, suggesting that propolis can create a favourable biochemical environment leading to re-epithelialization. The biological activity of propolis on wound healing and tissue regeneration may be related to its antimicrobial, anti-inflammatory and immunomodulatory properties investigated the effects of topical application of propolis on the healing and closure of diabetic wounds in a streptozotocin-induced type I diabetic mouse model, propolis was shown to accelerate wound closure by promoting TGF-β expression and its downstream signalling, increasing tip I collagen expression and deposition reducing matrix metalloproteinases, and decreasing inflammation.[15-17]

Publication have indicated that Propolis, a naturally occurring resinous substance collected by honey bees to protect the hive from fungal and bacterial infections, can improve tissue healing, especially after pathological injuries such as burns and periodontal disease.[15,16,18-22]

The aim of the present study was to investigate the clinical and histomorphometric effect of propolis on the healing of excisional palatal wounds in rats.

MATERIAL and METHODS

1. Animal and study protocol

The study protocol and experimental design were approved by the Animal Ethics Committee of the Faculty of Medicine, Cumhuriyet University (approval number: B.30.2.CUM.0.01.00.00-50/100). The study group consisted of 60 male Wistar rats that were 3 months old and weighed an average of 280 g.

Rats in each group were fed in different cages under the same conditions in a well-lit and well-ventilated room. All rats were fed ad libitum and maintained on a 12-h/12-h cycle at a temperature of 21 ± 1°C and humidity of 40–60%. The rats were acclimated to their living environment for 10 days prior to the study to avoid stress-induced disruption

of the experimental setup. The experimental phases of this study were performed in the animal laboratory of the Faculty of Medicine, Cumhuriyet University. Six animals were killed immediately and formed the initial group at time 0. The animals were randomly divided into three groups: Propolis, control and chlorhexidine

- (i) Baseline Control (B) group (n=6)
- (ii) Control (0.9% Saline solution.) (C) group (n=18)
- (iii) Chlorhexidine gluconate (0.05%) (CHX) group (n=18)
- (iv) Propolis (P) group (n=18)

Animals were killed by each group after 7, 14 and 21 days.

2. Formation of experimental palatal wound surface

After an adaptation period of 10 days, animals were anesthetized intraperitoneally with xylazine hydrochloride (Rompun; 10 mg/kg, Bayer Animal Health GmbH, Leverkusen, Germany) and ketamine hydrochloride (Ketalar; 40 mg/kg, Eczacibasi Ilac Sanayi, Istanbul, Turkey). A standardized circular wound outline was created on the anterior palate in the mucoperiosteum of the midline of the hard palate using a 3 mm diameter punch biopsy tool. The soft tissue was removed by sharp dissection to expose the underlying bone. Cotton gauze was placed on the wound until hemostasis was achieved. No drugs were administered throughout the experiment.

3. Preparation and administration of propolis extract

Propolis was produced by honey bees (Apis mellifera Linnaeus) in Trabzon, Turkey. Propolis was ground using an ultracentrifugal mill and 10 g of powder was dissolved in 100 ml of dimethyl sulfoxide (100% weight/ volume) by magnetic mixing for 24 hours at 37°C. Coarse particles were removed by filtration through 0.2-mM filters. The clear propolis preparation was diluted in sterile saline to achieve the desired concentrations. Propolis was administered locally at a rate of 1 ml/day.

4. Study process

Six animals were sacrificed immediately to maintain baseline values (BC group). The remaining 54 animals were randomly divided into three experimental groups. 0.5 ml of 0.09% saline (Polifarma İlaç Sanayi ve Ticaret AŞ, Tekirdağ, Türkiye), 0.05% Chlorhexidine Gluconate (Irrisept, Irrimax Corporation, Innovation Technologies, Inc., Lawrenceville, GA), or propolis was applied to each wound site with cotton pellets once daily for 1 minute. Six animals from each group were sacrificed at 7, 14, and 21 days postoperatively. The maxillae were dissected out, and the specimens were assessed photographically and compared with the histologic findings.

5. Photographic assessment

Specimens were photographed with a stereomicroscope (Stemi DV4, Carl Zeiss, Jena, Germany) (25X magnification). The surface of the wound was measured morphometrically using the software "Biowizard –Dwinter, version 3". Photographic evaluation was performed by a single examiner (H.O.) who did not known the identity of the specimens.

6. Histopathological assessment

Histological analysis was performed by a single inverstigator (F.G.) who was also blinded to the identity of the specimens. The specimens were fixed in 10% buffered neutral formalin for 48 hours. They were then dehydrated with alcohol and embedded in paraffin blocks. A microtome was used to make 5 μm serial sections perpendicular to the palatal midline at the largest diameter of the wound. The sections were stained with eosin and hematoxylin. The slides were examined under a light microscopy (Nikon Eclipse, E 600, Tokyo, Japan) for histological changes.

With regard to wound healing stages, specimes taken on the 7^{th}, 14^{th} and 21^{th} were evaluated for wound clearance, scaring re-epithelialization, and inflammatory of infiltration cells.

7. Statistical analysis

Data from the control and experimental groups were compared with each other and with baseline values. IBM Statistical Package for the Social Sciences Statistics for Windows, version 24 (IBM Corp; Armonk, NY, USA) was used for statistical analysis of the data. Mean, standard deviation, median, and frequency were used for descriptive statistics.

Two-way ANOVA or Oneway ANOVA with Tukey's multiple comparisons analysis and Student's t-test were applied. The difference between groups was considered significant when a value of $p< 0.05$.

RESULTS

During the experimental period, the animals did not lose weight, indicating that feeding behaviour was satisfactory in spite of the palatal wound. After surgical operation, photographs of the wound areas were taken with a light microscope (Nikon, DS-Fi1c, Tokyo, Japan), and the images were measured by an observer who was blind to the study groups.

Photographic Observation

On day 0, immediately postoperatively, the wound area was 12.12± 1.28 mm^2. The wound area measurements of all groups at the different time intervals are shown in Table 1, Figure 1. The mean area of circumscribed defects decreased significantly with time ($p<0.05$) in the experimental and control groups.

On the 7^{th}, 14^{th} and 21^{st} days, a statistically significant and faster healing was observed in the mean wound areas in the Propolis and Chx groups compared to the Control group.

On the other hand, in the chlorhexidine group, improvement was observed on the other days, but a significantly less improvement was observed only on the 7^{th} day compared to the propolis group.,

Histological Examination

When comparing the control group and the Chx group on the 7th day, the wound area where epithelization didn't ocur is clearly visible. When compared to the control group and the chlorhexidine group, scab started to form in the C. Spinosa group and it was observed that the inflammatory cells were infiltrated more intensely.

On day 14: Epithelial formation began to be observed in the control group, while more uniform epithelialization was observed in the experimental groups. In the experimental groups, the formation of collagen fibers by fibroblasts begins to migrate into this region in the subepithelial area, while the inflammatory cells are still present in the lower dermis compared to the control group.

Day 21: In the control group, the epithelium is complete and the dermis begins to form. The presence of infiltration of inflammatory cells in the deep region of the dermis compared to the experimental groups indigates that healing isn't yet complete. In the experimental groups, inflammatory cells in the deep dermis were reduced and wound healing was better than in the control group.

Table 1. Wound area measurement of groups (mm^2), mean and standart deviation (SD) (n=6)

Substance/Day	Baseline (means ± SD)	Control (means ± SD)	Chx (means ± SD)	Propolis (means ± SD)
0	12.12± 1.28			
7		10.91 ±0.61569*	9.25±0.96	5.56±3.77#
14		7.81 ±2.15*	4.81±0.62	3.70±1.76
21		4.05 ±3.72*	1.19±0.81	1.12±0.83

*different from control group on 7^{th},14^{th} and 21^{th} days
#different from Propolis group from Clx group on 7^{th} day

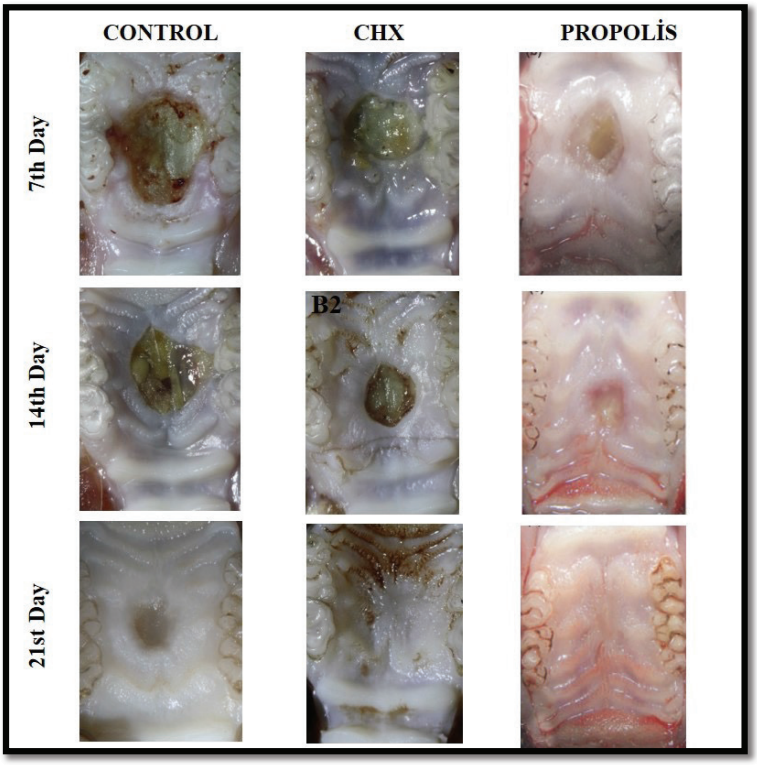

Figure 1. Clinical photographs of the wound area of all groups. C: Control group, Clx: Chlorhexidine group, P: Propolis group

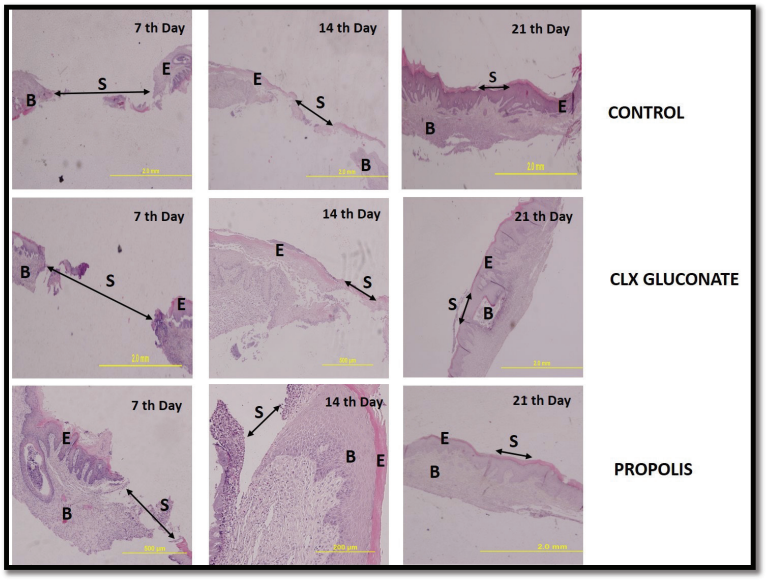

Figure 2. Histopathological sections of all groups (Hematoxylin and Eosin, S: Scarb, ( ) wound area, (E) epithelium, (B) connective tissue)

DISCUSSION

This work demonstrate that a single topical treatment with the plant bee resin propolis improves wound healing toward normal in a rat model of full-thickness cutaneous wound healing. The main endpoint of this study, epithelial closure, was significantly accelerated by propolis treatment of wounds in rats. Experiments were performed on rats, and propolis treatment was compared with saline treatment and conventional chlorhexidine gluconate treatment. The bacterial microflora in the oral cavity is very diverse, and these bacteria colonize the wounds.[23] Wounds on the palate are usually treated with antibacterial agents to prevent infections. In this study, we also used an antibacterial material, chlorhexidine gluconate, as a positive control. Propolis has also been previously reported to have antibacterial properties; therefore, this control allowed us to compare the efficacy of antibacterial treatment for wound healing. In the first two weeks of treatment, we observed the positive effects of antibacterial agents on oral wound healing in both propolis and chlorhexidine gluconate treatment, which promoted wound healing much more effectively than the control group. On the other hand, within the first two weeks, there was almost no difference between the group treated with propolis and the group treated with chlorhexidine gluconate, which shows the importance of the antibacterial properties for the initial phase of wound healing. Histological analysis showing granulation tissue with a constricted mucosal epithelial layer and complete repair and healing of the mucosal epithelium after propolis treatment also supports the critical effect of propolis on wound healing in palatal wounds.[24]

Propolis is considered to have antiseptic, antibacterial, antifungal, astringent, antispasmodic, anti-inflammatory, anesthetic, antioxidant, antifungal, anticancer, and immunomodulatory effects.[6,25] Some results confirm the therapeutic efficacy of propolis, namely, by quantitative and qualitative analyzes of the expression and degradation of type I collagen and III in the wound matrix, suggesting that propolis may have a favorable biochemical environment that supports re-epithelialization.[15]

The biological activities of propolis in wound healing and tissue regeneration might be related to its antimicrobial, anti-inflammatory and immunomodulatory properties.[26] Propolis shows immunostimulatory and immunomodulatory effects on macrophages in vitro, while in vivo it increases the ratio of CD4/CD8 T cells in mice. The results of this study showed that the application of propolis increased the rate of wound healing and re-epithelialization of diabetic wounds in rodents. It has also been suggested that propolis plays a different role in reducing neutrophil infiltration and normalizing macrophage influx into the wounded area.[27]

Wound healing and regeneration proceed through a finely tuned pattern of integrated phases, such as hemostasis, inflammation, cell proliferation, and tissue remodeling, involving a number of cellular and molecular processes.[16] This wound healing phenomenon includes migration and proliferation of epidermal cells and keratinocytes, adherence of fibroblasts, and contraction of the extracellular matrix (ECM). Propolis treatment stimulates a significant increase in ECM components in the initial phase of wound healing, followed by a decrease in ECM molecules. It is postulated that this biological effect of propolis is related to its ability to stimulate the expression of transforming growth factor-β (TGF-β), which is involved in the early stages of wound healing such as hemostasis and inflammation.[28,29]

Some works have studied the effect of propolis solutions in the treatment of animal wounds in clinical and experimental cases. The results showed that propolis is able to induce a good healing process, mainly by reducing the inflammatory response; therefore, the healing process was faster with propolis. The authors considered propolis suitable for wound treatment after the infection was eliminated.[1,30]

The healing properties of propolis may also be due to its

immunostimulant action. This property has been characterized in few clinical studies.[2,3] Propolis was administered, and cytokine secretion capacity was studied during and after treatment. Cytokine secretion capacity increased significantly and in a time-dependent manner during the treatment period. The authors concluded that propolis is able to induce immunoreactivity without side effects.[31]

The composition of propolis is complex and the samples from diferent areas are diferent from each other. Although drug substances that are prepared by using natural materials as starting materials are routinely used and are allowed to difer to a certain extent as batch-to-batch variations, it might be benefcial to use synthetic propolis preparations for future wound healing experiments as alternatives to these natural samples to achieve more chemically defned drug products.

CONCLUSION

The composition of propolis is complex and samples from different areas differ from each other. Although drugs prepared from natural materials are routinely used and may vary to some extent from batch to batch, it may be advantageous to use synthetic propolis preparations as an alternative to these natural samples for future wound healing trials to obtain chemically better defined drugs.

Ethical Approval

The study protocol and experimental design were approved by the Animal Ethics Committee of the Faculty of Medicine, Cumhuriyet University (approval number: B.30.2.CUM.0.01.00.00-50/100).

Peer-review

Externally and internally peer-reviewed.

Authorship Contributions

Concept: A.A., H.Ö., Design: A.A., H.Ö., Data Collection or Processing: A.A., H.Ö., Analysis or Interpretation: A.A., H.Ö., Literature Search: A.A., H.Ö., Writing: A.A., H.Ö.

Conflict of Interest

The authors declared no conflict of interest.

Funding

The study was self-funded by the authors.

References

1. Altan A, Aras MH, Damlar I, et al. The effect of Hypericum Perforatum on wound healing of oral mucosa in diabetic rats. Eur Oral Res. 2018; 52(3): 143-149.
2. Bakaeen GS, Strahan JD. Effects of a 1% chlorhexidine gel during the healing phase after inverse bevel mucogingival flap surgery. J Clin Periodontol. 1980; 7(1): 20-25.
3. Zambon JJ, Ciancio SG, Mather ML, et al. The effect of an antimicrobial mouthrinse on early healing of gingival flap surgery wounds. J Periodontol. 1989; 60(1): 31-34.
4. Feres M, Figueiredo LC, Barreto IM, et al. In vitro antimicrobial activity of plant extracts and propolis in saliva samples of healthy and periodontally-involved subjects. J Int Acad Periodontol. 2005; 7(3): 90-96.
5. Kalemba, D, Kunicka A. Antibacterial and antifungal properties of essential oils. Curr Med Chem, 2003; 10(10): 813-829.
6. Kujumgiev A, Tsvetkova I, Serkedjieva Yu, et al. Antibacterial, antifungal and antiviral activity of propolis of different geographic origin. J Ethnopharmacol.1999; 64(3): 235-240.
7. Banskota AH, Tezuka Y, KadotaS. Recent progress in pharmacological research of propolis. Phytother Res. 2001; 15(7): 561-571.
8. Lu LC, Chen YW, Chou CC. Antibacterial activity of propolis against Staphylococcus aureus. Int J Food Microbiol. 2005; 102(2): 213-220.
9. Grange JM, Davey RW. Antibacterial properties of propolis (bee glue). J R Soc Med. 1990; 83(3): 159-160.
10. Uzel A, Öncag Ö, Cogulu D, et al. Chemical compositions and antimicrobial activities of four different Anatolian propolis samples. Microbiol Res. 2005; 160(2): 189-195.
11. Duarte S., Koo H, Bowen WH, et al. Effect of a novel type of propolis and its chemical fractions on glucosyltransferases and on growth and adherence of mutans streptococci. Biol Pharm Bull. 2003; 26(4): 527-531.
12. Santos FA, Bastos E, Maia A, et al. Brazilian propolis: physicochemical properties, plant origin and antibacterial activity on periodontopathogens. Phytother Res. 2003; 17(3): 285-289.
13. Ledon N, Casaco A, Gonzalez R, et al. Antipsoriatic, anti-inflammatory, and analgesic effects of an extract of red propolis. Zhongguo Yao Li Xue Bao. 1997; 18(3): 274-276.
14. Jacob A, Parolia A, Pau A, et al. The effects of Malaysian propolis and Brazilian red propolis on connective tissue fibroblasts in the wound healing process. BMC Complement Altern Med. 2015; 15: 294.
15. Olczyk P, Wisowski G, Komosinska-Vassev G, et al. Propolis Modifies Collagen Types I and III Accumulation in the Matrix of Burnt Tissue. Evid Based Complement Alternat Med. 2013; 2013: 423809. doi.org/10.1155/2013/423809.
16. Martinotti S, Ranzato E. Propolis: a new frontier for wound healing? Burns & Trauma. 2015; 3: 9.
17. Hozzein WN, Badr G, Al Ghamdi AA, et al. Topical application of propolis enhances cutaneous wound healing by promoting TGF-beta/Smad-mediated collagen production in a streptozotocin-induced type I diabetic mouse model. Cell Physiol Biochem. 2015; 37(3): 940-954.
18. Gregory SR, Piccolo N, Piccolo MT, et al. Comparison of propolis skin cream to silver sulfadiazine: a naturopathic alternative to antibiotics in treatment of minor burns. J Altern Complement Med. 2002; 8(1): 77-83.
19. Ozturk, F., et al., The effects of acetylcholine and propolis extract on corneal epithelial wound healing in rats. Cornea, 1999. 18(4): p. 466-71.
20. Ozturk F, Kurt E, Inan UU, et al. The effect of propolis extract in experimental chemical corneal injury. Ophthalmic Res. 2000; 32(1): 13-18.
21. Bretz WA, Chiego DJ, Marcucci MC, et al. Preliminary report on the effects of propolis on wound healing in the dental pulp. Z Naturforsch C J Biosci. 1998; 53(11-12): 1045-1048.
22. Magro Filho O, de Carvalho AC. Application of propolis to dental sockets and skin wounds. J Nihon Univ Sch Dent. 1990; 32(1): 4-13.
23. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clinical Microbiology Reviews. 2001; 14(2): 244-269.
24. Van Rensburg CEJ, Van Straten A, Dekker J. An in vitro investigation of the antimicrobial activity of oxifulvic acid. Journal of Antimicrobial Chemotherapy. 2000; 46(5): 853-854.
25. Kuropatnicki AK, Szliszka E, Krol W. Historical aspects of propolis research in modern times. Evid Based Compl Alternative Med. 2013; 2013: 964149.
26. Castaldo SCF. Propolis, an old remedy used in modern medicine. Fitoterapia. 2002; 73(S1): 6. doi: 10.1016/S0367-326X(02)00185-5.
27. Toreti VC, Sato HH, Pastore GM, et al. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Compl Alternative Med. 2013; 2013: 697390.
28. Olczyk P, Komosinska-Vassev K, Wisowski G, et al. Propolis modulates fibronectin expression in the matrix of thermal injury. Biomed Res Int. 2014; 2014: 748101.
29. de Moura SA, Negri G, Salatino A, et al. Aqueous extract of brazilian green propolis: primary components, evaluation of inflammation and wound healing by using subcutaneous implanted sponges. Evid Based Compl Alternative Med. 2011; 2011: 748283.
30. Sutta J, Hanko J, Janda J, Tkac J. Experimental and clinical experiences in the treatment of wounds in domestic animals by local application of an alcoholic solution of propolis. Bratislava: Folia Veterinaria. 1974; 18: 143-147
31. Dimov V, Ivanovska N, Manolova N, et al. Influence on anti-infectious protection and macrophage function. Apidologie. 1991; 22: 155-162. doi: 10.1051/apido:19910208.