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Original Article

Wound Healing Potential of Forest Honey for Increasing TGF-β1 Protein Expression in Palatoplasty: *In-vivo* and *In-silico* Studies

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ABSTRACT

Background: With negative side effects of a lengthy healing time and high complication rate, cleft palate surgery is employed to treat cleft palate, which is a common congenital anomaly. Honey is believed to help accelerate wound healing post-treatment. However, its effect on the increase in the transforming growth factor (TGF)-β1 expression after palate surgery is unclear. **Objective:** This study investigates the effect of forest honey on TGF-β1 protein expression levels in the wound healing palate model. **Methods:** This study evaluates the TGF-β1 protein expression of a palatoplasty wound model using enzyme-linked immunosorbent assay. For this purpose, a punch biopsy model with 30 Sprague-Dawley rats is treated with forest honey (TG), Aloclair Gel (PC), and distilled water (NC) for 3 days. Analysis of the TGF-β1 expression on day 4 is performed by statistical one-way analysis of variance and *post hoc* least significance difference, with a 0.05 significant *P*-value. Online website software helped to predict the effect of honey components on the TGF-β1 expression. **Results:** Protein levels of treatment group (T), negative control (NC), and positive control (PC) exhibit mean levels of 16.13 ± 1.06883 ng/L, 7.36 ± 0.16543 ng/L, and 15.03 ± 0.34221 ng/L, respectively. The differential expression T group exhibits a 2.19-fold change in TGF-β1 relative to the NC group and a 1.07-fold change in the PC group (*P*-value of 0.01). TGF expression in the PC group increases in comparison to that in the NC group by 2.04-fold (*P*-value of 0.01). *In-silico* analysis revealed that genistein promotes macrophage proliferation and activation via the increase in the TGF-β1 expression. **Conclusion:** In summary, forest honey can boost the TGF protein expression via genistein to increase macrophage proliferation and activation.

KEYWORDS: *Flavonoid, honey bee, palatoplasty, punch biopsy, TGF-β1, wound healing*

BACKGROUND

Cleft palate occurs in 1 in 700 births worldwide.[1,2] Palatoplasty with reconstruction and gap closure with surrounding tissues is the definitive therapy for cleft palate (palate). Palatoplasty aims to separate the oral and nasal cavities and forms a watertight and airtight velopharyngeal valve to result in maxillofacial growth close to normal. VY pushback palatoplasty is frequently employed for this purpose,[3-5] which involves the compression of the adjacent tissues to seal the gap. However, this procedure leaves the bone exposed to environmental conditions

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that cause; therefore, the possibility of bacterial infection complications is increasing.[6,7]

Post-palatoplasty complications, especially exposed bones, can cause wound infections, open scars, and palate fistulas. Palatoplasty treats complications in two ways: dental prosthesis and surgery to seal the fistula in 6–12 months following palatoplasty.[8,9] Since ancient times, honey has been used as an alternative to accelerate wound healing, as well as to treat digestive tract and respiratory infections.[10] Several studies have reported that honey can accelerate wound healing through the homeostatic, inflammatory, proliferative, and remodeling phases.[11,12]

A type of honey with a high flavonoid content is obtained from the Pameungpeuk forest bee (*Apis dorsata*) due to low exposure to air pollution. This honey exhibits a high water content (24–26%), with a flavonoid content of 60–460 g in 100 g honey.[13,14] In addition, previous studies have reported that Pameungpeuk forest honey exhibits an inhibitor concentration (IC50) of 42.99 ppm to measure antioxidant activity in counteracting free radicals,[15,16] including the category of very strong in counteracting free radicals. In addition, the use of flavonoid-rich Rambutan honey can induce cytokines; as a result, it can respond to fibroblasts for wound healing and reduce free radicals—alondialdehyde (MDA).[17] The flavonoid content of honey can increase angiogenesis; thus, fibrosis and collagen formation are enhanced.[18-20] Flavonoids exhibit antibacterial, antioxidant, anti-inflammatory, and antidiabetic roles.[21,22]

Transforming growth factor-β (TGF-β) is a protein that plays an essential role in forming an extracellular matrix (ECM) as well as stimulating the chemotaxis of fibroblast cells.[23-26] The fibroblast process in the proliferative phase leads to granulation tissue formation, and the wound appears reddish. TGF-β has been identified to have three isoforms: one isoform is TGF-β1, which can affect the proliferation and differentiation of mesenchymal cells and increase ECM production during wound healing.[23] A high flavonoid content is thought to improve the wound healing ability via cell proliferation, especially in palatoplasty wound healing. However, the roles of Pameungpuk forest honey treatment to increase TGF-β1 protein expression in wound healing the punch biopsy palatoplasty model in the palate of Sprague-Dawley rats are not clear.

Materials and Methods

Research design

In this study, the *in-silico* approach was employed to determine the role of the active compounds present in honey in the mechanism of cell proliferation response. An *in-vivo* laboratory study with a post-test-only group design with 30 Sprague-Dawley rats palatoplasty model was conducted, in which 2–3-month-old Sprague-Dawley male rats were used, with a weight of 150–200 mg, under normal conditions. The normal definition is a state of calm, no injury, disability, or illness with a weight loss of 10% during acclimatization in the laboratory. Sprague-Dawley models were divided into three treatment groups: forest honey treatment group (T), Aloclair gel -treated positive control group[27-29] (PC), and distilled-water-treated negative control group (NC). The Ethical Commission approved all research procedures of the Faculty of Medicine, Jenderal Soedirman University, on July 22, 2020, with reference number 127/KEPK/VII/2020.

In-silico analysis: Interactions of secondary metabolites

Interaction prediction of the honey content with TGF-β1 expression was performed using web application software. Compounds in honey were searched by literature review from https://www.ncbi.nlm.here.gov/.[30] Canonical SMILES was employed to determine the bond structure using (http://www.pubchem.ncbi.nlm.here.gov/) active compounds of hones,[31] and the relationship interaction between the active compound of honey and TGF-β1 expression to learn signal pathways and mechanisms of biological function was analyzed using string (http://stitch.embl.of/).[32] Interaction relationship with protein signaling pathways based on KEGG pathways was predicted using http:///string.db.org/,[33] and the significance of the correlation was indicated by the significance value of $P < 0.05$.

Palatoplasty animal model

Sprague-Dawley rats were used as the animal model for palatoplasty, which were treated in cages and under experimental animal environments at temperatures of 16–27°C with 40–70% humidity and adequate lighting to avoid stress on rats. In terms of food, AD II feed (~20 g/head/day) was provided to Sprague-Dawley rats every morning to maintain healthy conditions of the animal model. Punch biopsy on the hard palate of animal models was conducted by an aseptic technique by smearing with povidone and anesthesia using 1–2 mg/kg ketamine via intravenous administration and 5–10 mg/kg ketamine via intramuscular

administration. Punch biopsy was conducted with a width of 3 mm using a seamless Premier Uni-Punch until the periosteal area was exposed, and the bone was visible. Monitoring was conducted for 5–10 min after the injury to determine ongoing bleeding. Markers of the palatoplasty model were observed until bleeding was not observed. As much as 30 mg of forest honey, PC, and NC were applied topically to the wound surface for 3 consecutive days in each group. On the 4th day, tissue samples were taken for the measurement of TGF-β1 protein expression levels.

Enzyme-linked immunosorbent assay

A sandwich enzyme-linked immunosorbent assay (ELISA) was employed for the analysis of TGF-β1 expression using rat transforming growth factor-beta BT by utilizing the Lab ELISA kit (Bioassay Technology, China), cat no. E0778Ra. At 37°C for 90 min, 0.1 mL of a dilution buffer was incubated, followed by the addition of 0.1 mL of a biotin-labeled antibody working solution and set at 37°C for 60 min. The standard solution was diluted to determine the absorbance at 450 nm using an automated ELISA plate reader. For research procedures, recommendations of the company were followed.

Statistical analysis

Statistical data were analyzed using SPSS version 22 software. Differences in the TGF-β1 expression of punch biopsy wounds were analyzed by one-way analysis of variance, followed by *post hoc* least significance difference analysis to observe a significant difference with a 95% confidence interval (CI) with $P < 0.05$, indicative of the significance value. Graphs and curves were rendered using Prism GraphPad version 9.0 for Windows (GraphPad Software, San Diego, CA, USA).

Results

Palatoplasty animal model

In this study, an animal model for palatoplasty using a 3-mm-diameter punch biopsy was developed. The findings indicated that punch biopsy is conducted until the palatal mucosa is split to the diameter of the tool and stained with blood. The palatoplasty model was successfully performed once palatal bleeding of experimental animals had ceased, and medication was administered [Figure 1].

Treatments of forest honey (T), Aloclair as the positive control (PC), and Aquadest as the negative control (NC) with a dose of 30 mg were given to each group, followed by covering all parts of the surface of the wound of the punch biopsy. Then, 200 mg of the damaged tissue was collected from each group for the investigation of the TGF expression on the 4th day.

Expression of TGF-β protein

Expression analysis using ELISA revealed the mean levels of TGF-β on forest honey-treated group (T), distilled water negative control group (NC), and Aloclair-gel-treated positive control group (PC) as 16.13 ± 1.06883, 7.36 ± 0.16543, and 15.03 ± 0.34221 ng/L, respectively. The average difference between groups was 1.1 between T and PC, 5.77 between T and NC, and 3.67 between PC and NC [Figure 2].

Statistical analysis revealed significant differences in TGF-β1 levels in the Experimental Animal Intervention Group (P-value <0.05) in Figure 3. Overexpression of TGF-β1 in the honey treatment group with a 2.19-fold change in comparison to the distilled water-treated group (NC) and 1.07 in comparison to the Aloclair-gel-treated group (PC) ($P < 0.01$) was noted. In addition, the TGF-β1 expression of the Aloclair-gel-treated

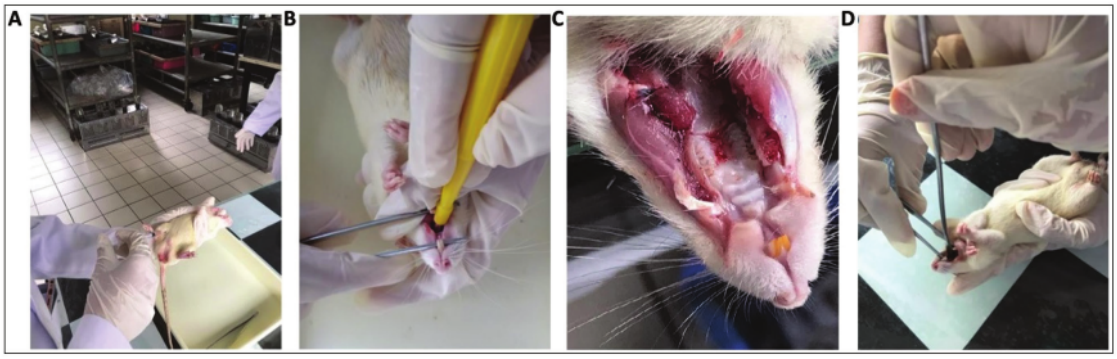

Figure 1: Punch biopsy method and honey treatment conducted for the palatoplasty animal model. (A) Inaction anesthesia administration. (B) Intervention of punch biopsy. (C) Procedure of punch biopsy. (D) Treatment with forest honey

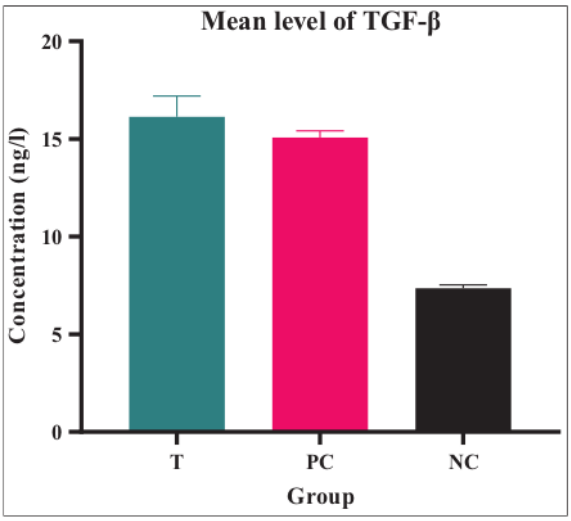

Figure 2: Mean levels of TGF-β in the Experimental Animal Intervention Group; negative control group (NC); 7.36 ± 0.16543 ng/L; Aloclair Gel positive control group (PC) 15.03 ± 0.34221 ng/L; and forest honey treatment group (T) 16.13 ± 1.06883 ng/L

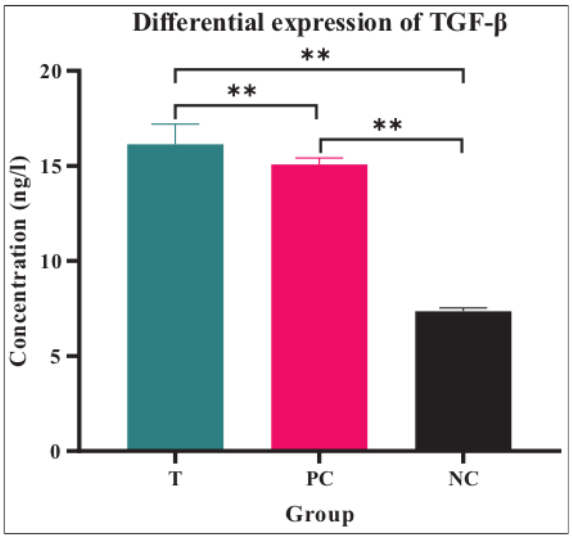

Figure 3: Significance of TGF-β1 levels in the Experimental Animal Intervention Group. PC exhibits an increase of 2.19 times compared with the negative control group (NC) and 1.07 times from Aloclair gel positive control group (PC). PC exhibits an increase of 2.04 times from NC (*$P < 0.05$ and **$P < 0.001$)

group (PC) increased in comparison to that of the distilled water-treated group (NC) 2.04 (*P*-value <0.01). Results of this study revealed that the 95% CI of the optical density values did not overlap for all sample groups. However, the increase in the number of samples can increase the precision of the statistical protein expression of each group.

Role of genistein in the TGF-β1 protein expression

In-silico analysis revealed that genistein is the key compound in forest honey that plays a direct role in TGF-β expression. Genistein directs the target analysis of several proteins such as CDK4, eMtor, CDKN1A, TRP53, SRC, ERBB2, PTK2, FOXO1, ESR1, CCND1, ESR2, and PPARG, affecting the signaling pathway of TGF-β1 expression; hence, the mechanism occurring in the cell changes. The analysis revealed that genistein affects the regulation of cell proliferation. In addition, changes in the TGF expression were affected by the interaction of receptors FOXO1 and SMAD3, as well as AKT1 and USP15, which are regulators of TGF-β1 receptor signaling pathways [Figure 4].

Discussion

The high incidence of abnormalities in the cleft palate is commonly treated by palatoplasty, which uses the punch biopsy method. However, potential complications caused by open wound infections, palate fistulas, alveolar arch deformities, and dental crowding worsen post-treatment problems. The complications increase when treatment to accelerate wound healing is insufficient. Honey is a natural ingredient known to speed up wound healing due to its high flavonoid content.[34] Quercetin, genistein, naringin, and apigenin are flavonoid compounds that play a role in increasing TGF-β levels.[35,36] Previous studies have reported that honey can accelerate burns via the acceleration of the inflammatory phase and stimulation of fibroblasts.[17,37,38]

In this study, the potential of forest honey to increase TGF-β1 levels in post-palatoplasty wounds was discovered. Expression increased with a 2.19-fold change compared to the NC and 1.07 compared to Aloclair (PC), with a significance value of $P < 0.05$. *In-silico* analysis revealed that quercetin in honey can regulate the mechanisms of TGF-β1 in several signaling pathways and affect keratosis stimulated by MMP-9. In addition, the high flavonoid content can increase the expression of TGF-β by increasing the capacity and activity of macrophages, thereby demonstrating potential to be an immunomodulator. In addition, flavonoids have been widely reported to operate as immunomodulators, enhancing PDGF, TGF-β, and VEGF via the secretion of cytokines on macrophage activity.[37,39]

Similarly, Rambutan honey used to expedite wound healing and prevent stress-induced fibroplasia via TGF-1 inhibits the synthesis of radicals—malondialdehyde (MDA)—in the oral mucosa[17] and angiogenesis in burn healing,[40] and it inhibits the effect of bacteria,[41,42] virus,[43] and fungi.[44] Increased macrophage activity triggers an earlier

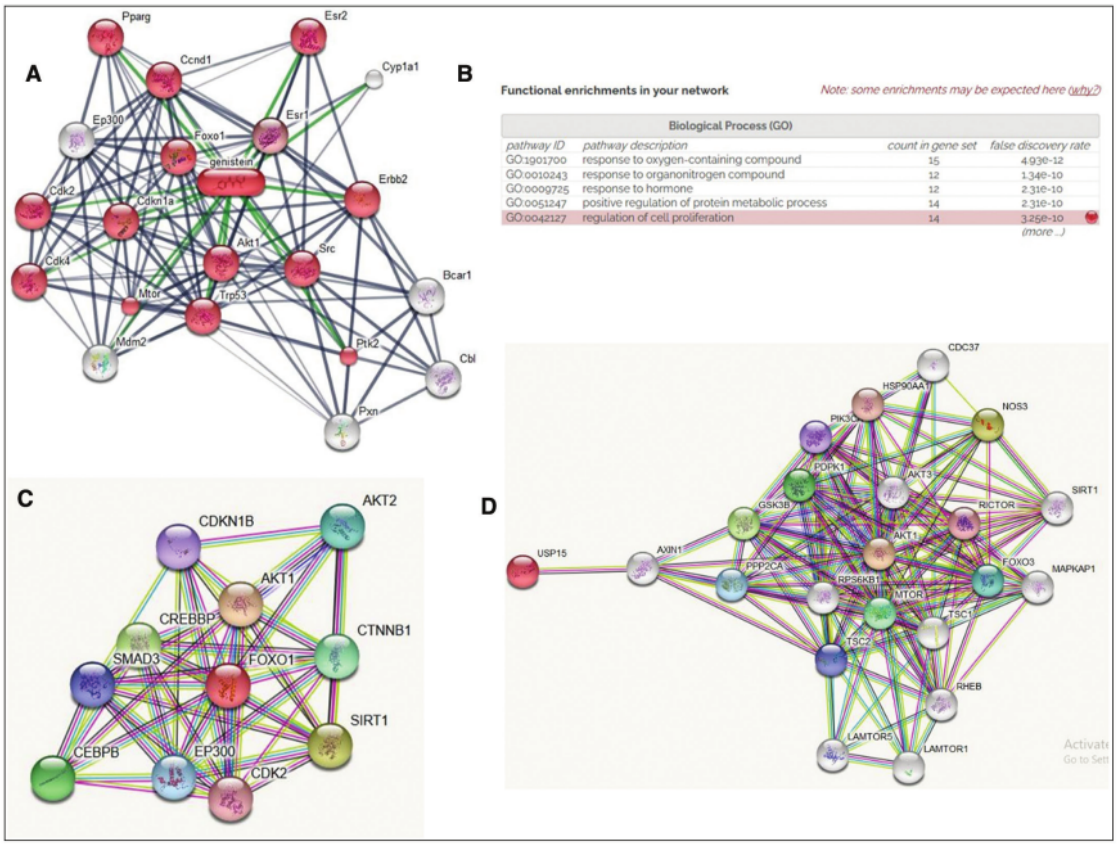

Figure 4: Mechanism prediction of genistein induces TGF-β1 protein expression. (A) Genistein protein target prediction. (B) Biological process gene influenced by genistein. (C) Regulation of the protein expression of the TGF-β1 protein receptor. (D) USP15 as a protein receptor of TGF-β1 targeted multiple proteins in proliferation

increase in blood vessels to accelerate the proliferative and inflammatory phases. Macrophages exchange on day 3 from M1 (proinflammatory) to M2 (anti-inflammatory), accelerating the proliferative phase, thus accelerating wound healing.[45] Anti-inflammatory work produces proinflammatory mediators to stimulate cells associated with inflammation such as lymphocytes, macrophages, and mast cells. In addition, quercetin (3,3′,4′,5,7-pentahydroxyflavone) is a flavonoid in honey that exhibits the same role for wound healing and anti-inflammatory via the induction of Smad3 (pSmad) for the enhancement of phosphorylation.[46,47] Quercetin increases the expression of TGF-β, which helps the fibroblast activity, ECM deposition, and granulation tissue formation. It also increases expression of TGF-β, which is secreted in wound tissues for the activation of macrophages and fibroblasts. TGF-β is required for the chemical attraction of monocytes, for stimulation of ECM production, and induction of myofibroblast formation in wound healing.[23,48,49] Genistein interacts with Akt1, and Foxo1 affects proliferation via the phosphorylation of Smad2 and Smad3 to increase TGF-β1 expression.[50-54]

In contrast, Aloclair gel (polyvinylpyrrolidone-sodium hyaluronate) is the standard treatment given after the oral surgical procedure against post-operative infections[29] derived from an aloe vera extract. The flavonoid content of aloe vera can induce TGF-β1 in modulating anti-inflammatory and immunomodulatory effects. The mannose-6 phosphate content of aloe vera can stimulate macrophages to produce TGF-β1.[55] However, the topical application of honey can render chemical debridement of dead cells, reduce pain, and stimulate granulation and epithelialization of wounds.[56] Therefore, high-dose administration can lead to the dehydration of granulation tissues, whereas low-dose administration does not affect wound healing.

In conclusion, the role of flavonoid content of honey via the induction of the increased expression of TGF-β protein was confirmed. Flavonoid compounds that enter the body interact with several proteins and

stimulate the immune system, such as AKT1, Foxo 1, and macrophages, thereby expanding proliferation by the phosphorylation process of Smad2 and Smad3 in the TGF-β signaling pathway.[57-61] Therefore, honey demonstrates the potential for use as an alternative or additional topical wound treatment after palatoplasty biopsy. It can render the chemical debridement of dead cells, reduce pain, and stimulate granulation and epithelialization of wounds. However, the drawback of this study was that it did not use the optimal dose variation of honey for palatoplasty wound healing by increasing the expression of TGF-β protein.

Conclusion

Forest honey can increase the expression of TGF-β protein of punch biopsy wounds in the palate of Sprague-Dawley rats due to its flavonoid content. Genistein can accelerate wound healing via the induction of the TGF-β signaling pathway mechanism to increase proliferation and macrophage activation.

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Conflicts of interest

All authors declared that there is no competing interest in this study.

References

1. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. Am J Med Genet C Semin Med Genet 2013;163C:246-58.
2. Dixon M, Marazita M, Beaty T, Murray J. Cleft lip and palate: Synthesizing genetic and environmental influences. Natl Inst Health 2011;12:167-78.
3. Woo AS. Velopharyngeal dysfunction. Semin Plast Surg 2012;26:170-7.
4. Reddy RR, Reddy SG, Banala B, Bronkhorst E, Kummer AW, Kuijpers-Jagtman AM, *et al*. Use of a modified Furlow Z-plasty as a secondary cleft palate repair procedure to reduce velopharyngeal insufficiency. Int J Oral Maxillofac Surg 2016;45:170-6.
5. Mercedes Acosta S, Guaschino Ginebra G, Mercedes Germán G, Monegro Peña K, Castillo PJ. VII Surgical operative for cleft lip and palate National Plastic Surgery Residency, Santo Domingo, Dominican Republic. Cir Plast Ibero-Latinoamericana 2020;46:489-94.
6. Guerrero CA. Cleft lip and palate surgery: 30 years follow-up. Ann Maxillofac Surg 2012;2:153-7.
7. Rossell-Perry P, Cotrina-Rabanal O, Barrenechea-Tarazona L, Vargas-Chanduvi R, Paredes-Aponte L, Romero-Narvaez C. Mucoperiosteal flap necrosis after primary palatoplasty in patients with cleft palate. Arch Plast Surg 2017;44:217-22.
8. Kamrava A, Mahmoud NN. Prevention and management of nonhealing perineal wounds. Clin Colon Rectal Surg 2013;26:106-11.
9. Akiba RT, Rodrigues FG, da Silva G. Management of complex perineal fistula disease. Clin Colon Rectal Surg 2016;29:92-100.
10. Eteraf-Oskouei T, Najafi M. Traditional and modern uses of natural honey in human diseases: A review. Iran J Basic Med Sci 2013;16:731-42.
11. Ibrahim NI, Wong SK, Mohamed IN, Mohamed N, Chin KY, Ima-Nirwana S, *et al*. Wound healing properties of selected natural products. Int J Environ Res Public Health 2018;15:2360.
12. Tottoli EM, Dorati R, Genta I, Chiesa E, Pisani S, Conti B. Skin wound healing process and new emerging technologies for skin wound care and regeneration. Pharmaceutics 2020;12:1-30.
13. Saputri DS, Putri YE. Aktivitas antioksidan madu hutan di beberapa kecamatan di kabupaten sumbawa besar. J Tambora 2017;2.
14. Nurlailatul WR. Pengaruh Konsentrasi Larutan Madu Lebah Hutan (Apis dorsata) Terhadap Hambatan Pertumbuhan Bakteri Porphyromonas gingivalis Dominan Gingivitis (Kajian in vitro). Publ Ilm 2016;14:1-49.
15. Aida A. Uji Aktivitas Antioksidan Madu Hutan Pameungpeuk dan Madu Ternak Djowo Mbah Sum. Universitas Islam Negeri Alauddin Makasar 2020:1-95.
16. Ustadi, Radiati LE, Thohari I. Komponen Bioaktif pada Madu Karet (Hevea brasiliensis) Madu Kaliandra (*Calliandra callothyrsus*) dan Madu Randu (*Ceiba pentandra*). J Ilmu Dan Teknol Has Ternak 2017;12:97-102.
17. Yuslianti ER, Bachtiar BM, Suniarti DF, Sutjiatmo AB, Mozef T. Effect of rambutan-honey and its flavonoid on TGF-β1 induce fibroplasia oral wound healing. Res J Med Plant 2016;10:435-42. https://doi.org/10.3923/rjmp.2016.435.442.
18. Rizzi ES, Dourado DM, Matias R, Muller JAI, Guilhermino JF, Guerrero ATG, *et al*. Wound-healing potential of *Sebastiania hispida* (mart.) Pax (Euphorbiaceae) ointment compared to low power laser in rats. Braz J Biol 2017;77:480-9.
19. Oryan A, Alemzadeh E, Moshiri A. Potential role of propolis in wound healing: Biological properties and therapeutic activities. Biomed Pharmacother 2018;98:469-83.
20. Almas AI, Purnawati RD, Istiadi H, Susilaningsih N. The effect of honey in second degree burn healing on Wistar rats (overview of angiogenesis and the number of fibroblasts). J Med Heal 2020;11:27-32.
21. Panche AN, Diwan AD, Chandra SR. Flavonoids: An overview. J Nutr Sci 2016;5:e47.
22. Ginwala R, Bhavsar R, Chigbu DGI, Jain P, Khan ZK. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants 2019;8:35.
23. Frangogiannis N. Transforming growth factor-β in tissue fibrosis. J Exp Med 2020;217:e20190103.
24. Epstein FH, Border WA, Noble NA. Transforming growth factor β in tissue fibrosis. N Engl J Med 1994;331:1286-92.
25. Minutti CM, Modak RV, Macdonald F, Li F, Smyth DJ, Dorward DA, *et al*. A macrophage-pericyte axis directs tissue restoration via amphiregulin-induced transforming growth factor beta activation. Immunity 2019;50:645-54.e6.
26. Pakyari M, Farrokhi A, Maharlooei MK, Ghahary A. Critical role of transforming growth factor beta in different phases of wound healing. Adv Wound Care (New Rochelle) 2013;2:215-24.

27. Kartiningtyas AT, Prayitno P, Lastianny SP. Pengaruh aplikasi gel ekstrak kulit citrus sinensis terhadap epitelisasi pada penyembuhan luka gingiva tikus sprague dawley. Maj Kedokt Gigi Indones 2015;1:86.
28. Nofikasari I, Rufaida A, Aqmarina CD, Failasofia F, Fauzia AR, Handajani J. Efek aplikasi topikal gel ekstrak pandan wangi terhadap penyembuhan luka gingiva. Maj Kedokt Gigi Indones 2017;2:53.
29. López-Jornet P, Camacho-Alonso F, Martinez-Canovas A. Clinical evaluation of polyvinylpyrrolidone sodium hyaluronate gel and 0.2% chlorhexidine gel for pain after oral mucosa biopsy: A preliminary study. J Oral Maxillofac Surg 2010;68:2159-63.
30. National Library of Medicine. NCBI PubMed Overview. Natl Cent Biotechnol Inf 2021. https://www.ncbi.nlm.here.gov/. [Last accessed on 2021 Jul 20].
31. Information NC for B. PubChem Database. NCBI. 2021. Https://PubchemNcbiNlmNihGov/Compound/. http://www.pubchem.ncbi.nlm.here.gov/. [Last accessed on 2021 Jul 26].
32. STITCH Consortium. STITCH—Protein compound interaction 2021. http://stitch.embl.of/. [Last accessed on 2021 Jul 29].
33. Szklarczyk D, Gable AL, Lyon D, Junge A, Wyder S, Huerta-Cepas J, *et al.* String v11: Protein–protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res 2019;47:D607-13.
34. Mandal MD, Mandal S. Honey: Its medicinal property and antibacterial activity. Asian Pac J Trop Biomed 2011;1:154-60.
35. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and their antidiabetic effects: Cellular mechanisms and effects to improve blood sugar levels. Biomolecules 2019;9:430.
36. Fang JY, Lin CH, Huang TH, Chuang SY. *In vivo* rodent models of type 2 diabetes and their usefulness for evaluating flavonoid bioactivity. Nutrients 2019;11:530.
37. Shah A, Amini-Nik S. The role of phytochemicals in the inflammatory phase of wound healing. Int J Mol Sci 2017;18:1068.
38. Yaghoobi R, Kazerouni A, Kazerouni O. Evidence for clinical use of honey in wound healing as an anti-bacterial, anti-inflammatory anti-oxidant and anti-viral agent: A review. Jundishapur J Nat Pharm Prod 2013;8:100-4.
39. Yahfoufi N, Alsadi N, Jambi M, Matar C. The immunomodulatory and anti-inflammatory role of polyphenols. Nutrients 2018;10:1618.
40. Samiee-rad F, Hashemi SM, Zangivand AA, Soleimanitadi K. Effect of acacia honey-impregnated placenta membrane on pain and burn wound repair. Comp Clin Path 2018;27:1457-63.
41. Al-Waili NS, Haq A. Effect of honey on antibody production against thymus-dependent and thymus-independent antigens in primary and secondary immune responses. J Med Food 2004;7:491-4.
42. Emsen IM. A different and safe method of split thickness skin graft fixation: Medical honey application. Burns 2007;33:782-7.
43. Tallett S, MacKenzie C, Middleton P, Kerzner B, Hamilton R. Clinical, laboratory, and epidemiologic features of a viral gastroenteritis in infants and children. Pediatrics 1977;60:217-22.
44. Kumar KS, Bhowmik D. Medicinal uses and health benefits of honey: An overview. J Chem Pharm Res 2010;2:385-95.
45. Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The role of macrophages in acute and chronic wound healing and interventions to promote pro-wound healing phenotypes. Front Physiol 2018;9:419.
46. Ahmed S, Bradshaw A-D, Gera S, Dewan M, Xu R. The TGF-β/Smad4 signaling pathway in pancreatic carcinogenesis and its clinical significance. J Clin Med 2017;6:5.
47. Phan TT, Lim IJ, Chan SY, Tan EK, Lee ST, Longaker MT. Suppression of transforming growth factor beta/Smad signaling in keloid-derived fibroblasts by quercetin: Implications for the treatment of excessive scars. J Trauma 2004;57:1032-7.
48. Li B, Wang JH. Fibroblasts and myofibroblasts in wound healing: Force generation and measurement. J Tissue Viability 2011;20:108-20.
49. Darby IA, Laverdet B, Bonté F, Desmoulière A. Fibroblasts and myofibroblasts in wound healing. Clin Cosmet Investig Dermatol 2014;7:301-11.
50. Hu HH, Chen DQ, Wang YN, Feng YL, Cao G, Vaziri ND, *et al.* New insights into TGF-β/Smad signaling in tissue fibrosis. Chem Biol Interact 2018;292:76-83.
51. Derakhshani A, Silvestris N, Hemmat N, Asadzadeh Z, Shadbad MA, Nourbakhsh NS, *et al.* Targeting TGF-β-mediated SMAD signaling pathway via novel recombinant cytotoxin II: A potent protein from naja naja oxiana venom in melanoma. Molecules 2020;25:5148.
52. Walton KL, Johnson KE, Harrison CA. Targeting Tgf-β mediated Smad signaling for the prevention of fibrosis. Front Pharmacol 2017;8:461.
53. Zhang YE. Non-Smad signaling pathways of the TGF-β family. Cold Spring Harb Perspect Biol 2017;9:a022129.
54. Zhang YE. Non-Smad pathways in TGF-beta signaling. Cell Res 2009;19:128-39.
55. Chasanah N, Bashori A, Krismariono A. Ekspresi Tg Fβ1 setelah pemberian ekstrak gel aloe vera pada soket. J Biosains Pascasarj 2018;20:1-9.
56. Medhi B, Puri A, Upadhyay S, Kaman L. Topical application of honey in the treatment of wound healing: A meta-analysis. JK Sci 2008;10:166-9.
57. Piersma B, Bank RA, Boersema M. Signaling in fibrosis: TGF-β, WNT, and YAP/TAZ converge. Front Med (Lausanne) 2015;2:59.
58. Kiyono K, Suzuki HI, Matsuyama H, Morishita Y, Komuro A, Kano MR, *et al.* Autophagy is activated by TGF-β and potentiates TGF-β-mediated growth inhibition in human hepatocellular carcinoma cells. Cancer Res 2009;69:8844-52.
59. Vezzali R, Weise SC, Hellbach N, Machado V, Heidrich S, Vogel T. The FOXG1/FOXO/SMAD network balances proliferation and differentiation of cortical progenitors and activates Kcnh3 expression in mature neurons. Oncotarget 2016;7:37436-55.
60. Perumal N, Perumal M, Halagowder D, Sivasithamparam N. Morin attenuates diethylnitrosamine-induced rat liver fibrosis and hepatic stellate cell activation by co-ordinated regulation of Hippo/Yap and TGF-β1/Smad signaling. Biochimie 2017;140:10-9.
61. Noguchi S, Saito A, Nagase T. YAP/TAZ signaling as a molecular link between fibrosis and cancer. Int J Mol Sci 2018;19:3674.

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