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Effect of Propolis and Liquid Smoke Nanogel on TGF-β and Macrophage Activity in Rattus Norvegicus with Traumatic Ulcer

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ABSTRACT

Traumatic ulcers are a disorder of the oral mucosa, the incidence of which reaches 83.6% in both men and women. Chronic traumatic ulcers have malignant potential if not properly managed. Propolis is a natural ingredient derived from the resin collected by honeybees, which is widely used in alternative medicine because of its content. Liquid smoke is a compound resulting from the condensation of a hot reactor containing various chemical compounds that act as antioxidants, antiseptics, antibacterials, and as preservatives. This research aims to evaluate whether a nanogel combining propolis extract and liquid smoke can more effectively enhance the healing of chronic traumatic ulcers compared to propolis alone. This study involved 36 male rats aged 2-3 months, divided into three groups: a control group, a group given only propolis, and a group given a combination of propolis and liquid smoke, with treatments administered twice daily for 7 days. The expression of TGF-β cells and macrophages in the healing process of traumatic oral ulcers in rats showed significant differences between the administration of nanogels with a combination of propolis extract and liquid smoke and the administration of propolis extract nanogels. The average number of TGF-β cells and macrophages in the experimental group treated with the combined nanogel of propolis extract and liquid smoke increased significantly on the third day ($P < 0.05$), more rapidly than in the other groups. Propolis extract and liquid smoke combination nanogel accelerate the healing process of chronic traumatic ulcers to prevent malignancy.

1. INTRODUCTION

Traumatic ulcers are one of the most common disorders of the oral mucosa. The incidence of traumatic ulcers is as high as 83.6% in both men and women [1]. The prevalence of traumatic ulcers in one area of Indonesia, Minahasa, and Manado, reached 93.3% and 90.01%, respectively, which is relatively high compared to other oral lesions. Indonesians often refer to ulcers as canker sores. Canker sores can cause pain, difficulty speaking, and even difficulty swallowing, affecting nutritional intake and the quality of life of those affected [2].

Traumatic ulcers can be treated with topical anti-inflammatory steroids, one of which is the gold standard, triamcinolone acetonide 0.1%. This drug acts as an anti-inflammatory agent in the healing process of traumatic ulcers, but when used on the oral mucosa, it has several side effects, such as irritation, itching, swelling, dryness, redness, and burning in the treated area. In addition, long-term use can lead to immune system resistance and epithelial cell atrophy. Based on the above description, it is necessary to look for alternative natural ingredients that have fewer side effects and are anti-inflammatory.

Triamcinolone acetonide is a synthetic glucocorticoid widely used for anti-inflammatory and immunosuppressive properties. It is particularly effective in treating various oral conditions, including traumatic ulcers, oral lichen planus, and other inflammatory lesions, which have functions by binding to glucocorticoid receptors, leading to the modulation of gene expression that reduces inflammation and promotes healing [3]. In the last five years, in the context of oral traumatic ulcers, triamcinolone acetonide has often been administered topically as a paste or through intralesional injections to alleviate pain and accelerate the healing process [4, 5].

Common adverse effects associated with triamcinolone acetonide include local skin necrosis, ulceration, and systemic effects such as Cushing's syndrome [6, 7]. These side effects underscore the importance of exploring alternative treatments, especially for patients who may be at risk for adverse reactions. For example, the use of autologous platelet-rich plasma (PRP) has been shown to be effective in managing erosive lichen planus and oral submucous fibrosis, presenting a promising

alternative to traditional corticosteroid therapies [8].

Propolis, also known as bee glue, is a natural ingredient derived from the resin collected by honeybees from various plants, and is widely used in traditional medicine. Propolis is a natural, non-toxic resin with antiviral, antifungal, antimicrobial, anticancer, and anti-inflammatory properties due to the presence of flavonoids, phenolic acids, and Caffeic Acid Phenethyl Ester (CAPE), which represents 50% of the total composition. This has led to propolis receiving attention in both the dental and medical fields [9, 10]. Topical administration of propolis can speed up the healing process of traumatic ulcers by increasing the growth of macrophages and transforming growth factor-beta (TGF-β), which supports the migration of immune cells to injured areas, the formation of extracellular matrix components such as collagen, fibronectin, and Vascular Endothelial Growth Factor (VEGF) for the repair of injured tissue [11].

Liquid smoke is a compound resulting from simultaneous condensation through pyrolysis techniques from the hot reactor and condensation in the cooling system. Liquid smoke contains various chemical compounds such as alcohol, aldehydes, ketones, organic acids, phenols, carbonyls, tar, and water, which act as antioxidants, antiseptics, antibacterials, and as preservatives [12]. Phenol and acetic acid are compounds that play a role in inhibiting and preventing fat oxidation by stabilizing free radicals and increasing blood flow so that they can help the wound-healing process in traumatic ulcers [13].

The use of nanotechnology in the manufacture of medicinal preparations causes the active substance content in propolis extract and liquid smoke to dissolve more easily because of its very small size, thus allowing the content to go to a more specific area [14]. The development of nanostructured lipid carriers for propolis has been reported to improve the antioxidant activity of its components, further supporting its use in wound healing and oral ulcer treatment [15]. The application of nanotechnology in formulating liquid smoke can enhance its stability and bioavailability, making it more effective in medicinal applications. By utilizing nanoparticles to encapsulate liquid smoke, researchers can improve its delivery to specific sites, such as inflamed tissues or ulcers, thereby enhancing its therapeutic potential [16]. The combination of propolis and liquid smoke in nanocarrier systems could provide a synergistic effect, leveraging the unique properties of both substances to address oral ulcers more effectively.

The selection of gel form as a medicinal preparation is based on the numerous benefits that gels offer, including cooling and moisturizing properties, ease of use, and the ability to penetrate the skin. These attributes can help to reduce pain and accelerate the healing process around wounds [17]. This research aims to evaluate whether a nanogel combining propolis extract and liquid smoke can more effectively enhance the healing of chronic traumatic ulcers compared to propolis alone.

2. MATERIALS AND METHODS

The type of research used is a true experimental design in vivo laboratory with a research randomized post-test only control group design. The research was conducted at the Oral Biology Laboratory of the Faculty of Dentistry Universitas Brawijaya and the Physiology and Anatomical Pathology Laboratory of the Faculty of Medicine Universitas Brawijaya.

2.1 Research population and sample

The samples used were 36 male Wistar white rats (Rattus Norvegicus) obtained from the Malang Murine Farm. The rats were 2-3 months old, weighed about 250-350 grams, and were in healthy condition. They were kept at the Oral Biology Laboratory, Faculty of Dentistry, Brawijaya University, Malang. The experimental animals were divided into 3 groups: KT with topical drug administration of triamcinolone acetonide 0.1%, KP with the administration of propolis extract nanogel, and KA with administration of a nanogel combination of propolis extract and liquid smoke. Each dose of triamcinolone was 0.1%, propolis was 5%, and a combination of propolis extract nanogel and liquid smoke 5% in a 3:2 ratio was administered twice daily. The selection of these doses on the basis of research has resulted in a composition that has proved to be quite optimal and stable [14, 18].

2.2 Research design

The production of nanogel from a combination of propolis extract and liquid smoke begins with the extraction process of propolis and liquid smoke solution with 70% ethanol solvent. The maceration method was chosen because it does not require heating at high temperatures, so the chemical compounds contained in the material are not damaged [18]. Ethanol was chosen as the solvent in the extraction process because ethanol has polar properties, so this solvent is often used in the flavonoid identification process [19]. Ethanol with a concentration of 70% was chosen because, based on research by Muli and Maingi [20], this concentration works to dissolve the most active ingredients and can produce products with the greatest antioxidant activity compared to other concentrations. The preparation of nanogels was then carried out using the sonication method, and the preparation of nanogels was done using the gelling agent Carbopol 940. Preparation of nanogels by mixing Carbopol 940 as a gelling agent, glycerin, propyleneglycol, methylparaben, triethanolamine, and distilled water and then mixing using a stirrer. The propolis used was obtained from PT. Kembang Joyo Sriwijaya, Karangploso, Malang, East Java. The liquid smoke used was produced by PT. Lubna Company, Semarang, Indonesia.

The experimental animals were anaesthetised with an intramuscular injection, and then traumatic ulcer wounds were created by thermal induction using a 3 mm diameter cement stopper, 100°C in 40 seconds. After the creation of a traumatic ulcer, each experimental animal was treated twice daily according to the treatment group by swabbing the labial mucosa of the experimental animal with a cotton swab. The rats were then decapitated using the cervical dislocation technique on the necks of the rats on days 3, 5, and 7 according to group allocation. The labial mucosal tissues of the rats were harvested by excision to obtain injury induced mucosal tissues [21].

2.3 Observation of macrophage cells and TGF-β expression

Macrophage expression assay using the hematoxylin and eosin (HE) staining method. Macrophages stained with HE appear brown, so they are easy to see at (400x) using a microscope and image raster software to obtain calculation

results, and the results are averaged and then compared between one group and another.

TGF-β expression was determined by the immunohistochemical staining method using TGF-β or anti-TGF-β monoclonal antibodies in 36 mucosal tissue samples. Dripping SA-HRP (Streptavidin Conjugated with Horseradish Peroxidase) and incubated for 40 min. In histological preparations, these cells are shown as brownish spots. TGF-β expression was observed using a digital microscope with high magnification (400x). The examination and calculation of TGF-β cell expression were conducted by observing cells that expressed TGF-β with the appearance of brown colour, using a microscope and imageraster software. Slides were then photographed in five different fields of view, and the results were averaged and then compared between one group and another.

2.4 Data analysis

Data analysis was performed using normality and homogeneity tests from SPSS 27 for Mac. The one-way ANOVA hypothesis test is performed if the data are normally distributed. If the data are not normally distributed, the Kruskal-Wallis test is used, followed by the Mann-Whitney test.

3. RESULTS

The expression of macrophages and TGF-β cells in the treatment group with topical application of nanogel combined with propolis extract and liquid smoke showed a greater increase compared to the treatment group with topical application of propolis nanogel and the control group. The average results of TGF-β cell and macrophage expression with topical application of a gel combination of propolis extract and liquid smoke are shown in the following graphic (Figure 1 and Figure 2).

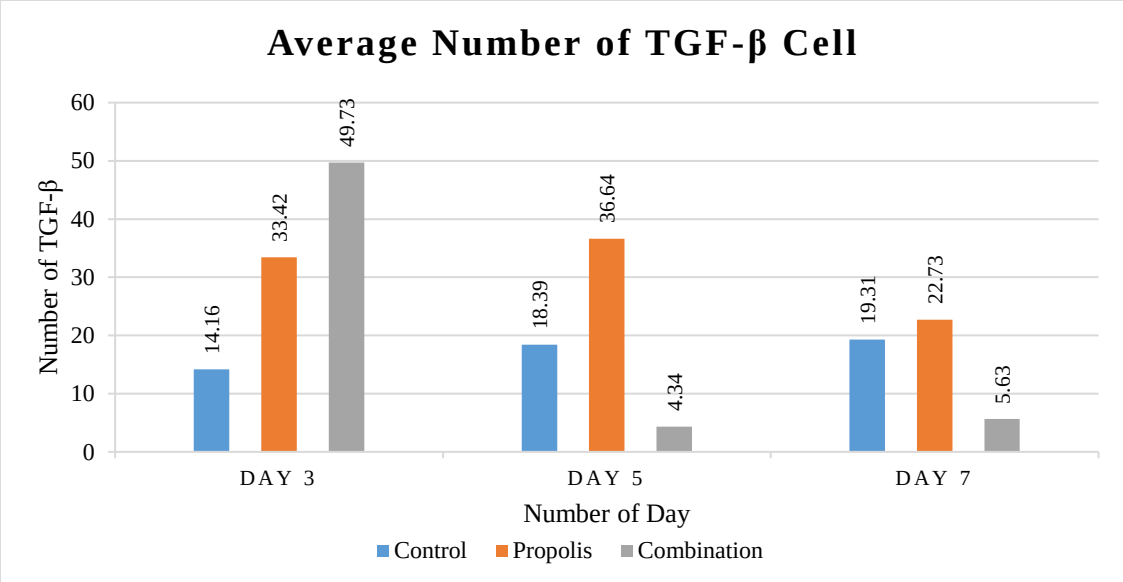

Figure 1. Comparison of TGF-β levels on days 3, 5 and 7

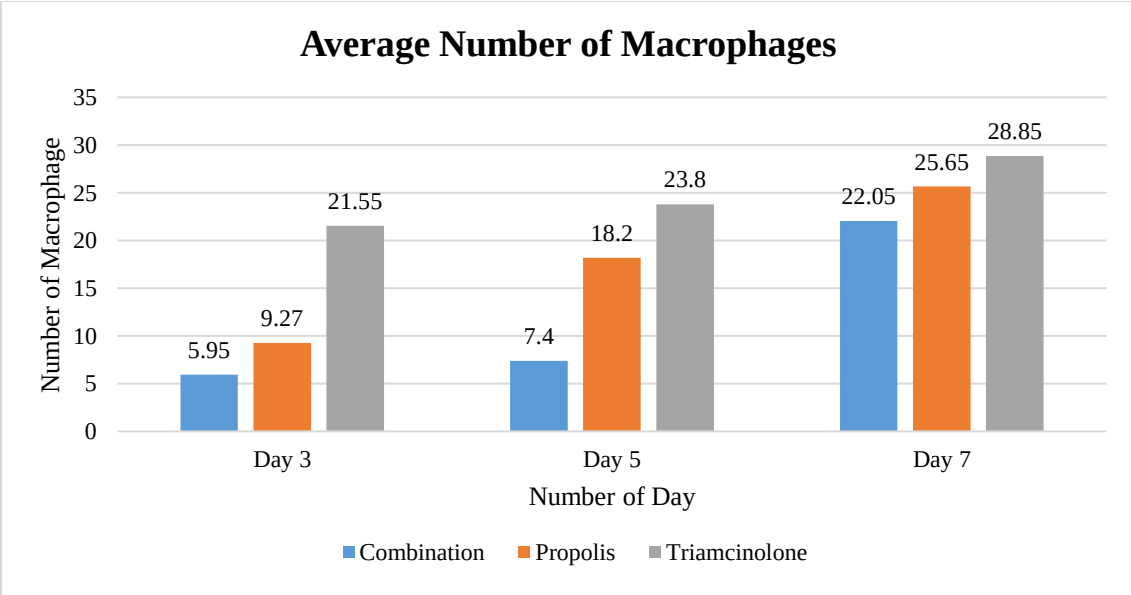

Figure 2. Comparison of macrophage levels on days 3, 5 and 7

Based on Figure 1, observations on the 3rd day showed that the combination group (KA) had an average total TGF-β is highest, namely 49.73; the control group (KT) had an average number of TGF-β; the lowest is 14.16, and the propolis group (KP) is between the two. Observation on the 5th day of the average amount of TGF-β; the highest was the propolis group with 36.64, then the control group with 18.39, and the lowest was the combination group with 4.34. Observation on the 7th day of the average amount of TGF-β: the highest was the propolis group with 22.73, then the control group with 19.31, and the lowest with the combination group with 5.63 (Figures 3, 4, 5).

The Kruskal-Wallis test results obtained showed that there were significant differences (P<0.05) in TGF-β expression of each group on days 3, 5, and 7 with the value of P=0.002 (day 3), P=0.007 (day 5) and P=0.001 (day 7). The number of macrophages in the rats treated with propolis and liquid smoke was significantly different from that in the control group, with a significant increase in the propolis and liquid smoke treated groups (Figure 2). After treatment with propolis and liquid smoke for three days (5.95 ± 3.99) and five days (7.40 ± 5.49). The number of macrophages following five days of treatment with propolis and liquid smoke was significantly higher than after three days (P = 0.014) (Figure 2).

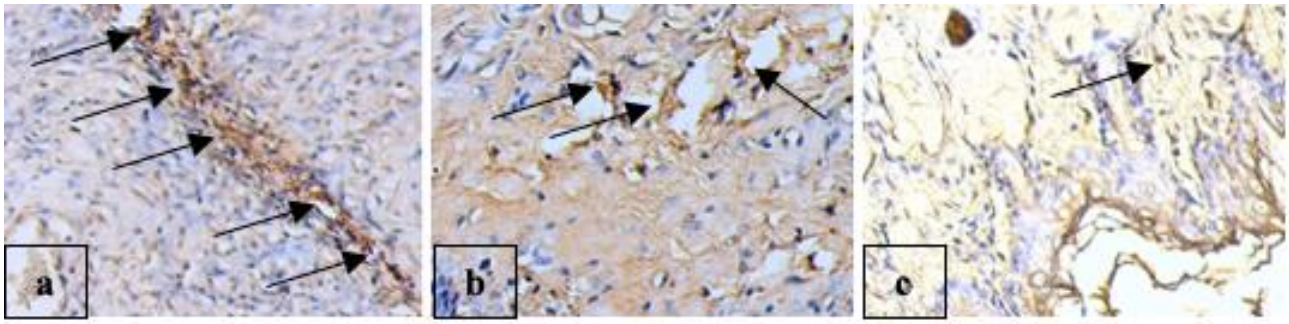

Figure 3. Comparison of histology images on day 3 immunohistochemistry staining at 400x magnification showing TGF-β expression: (a) KA3 group, (b) KP3 group, (c) KT3 group
β: Cell growth code.

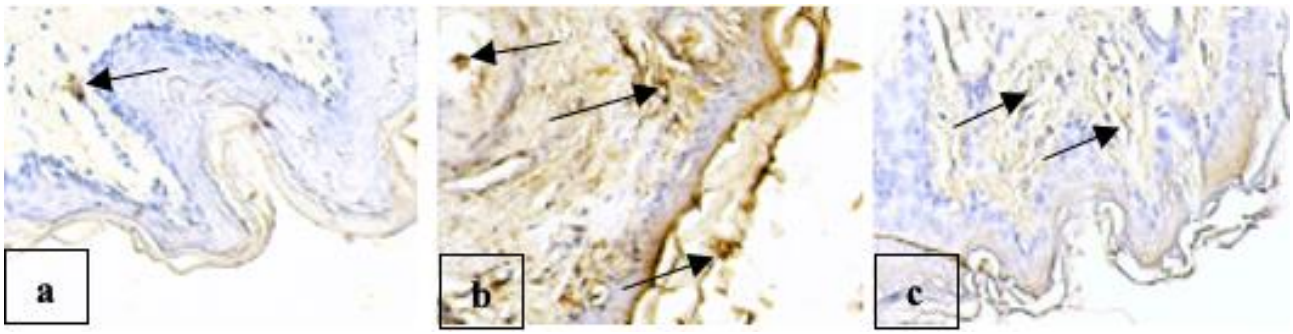

Figure 4. Comparison of histology image on day 5 immunohistochemistry staining at 400x magnification showing TGF-β expression: (a) KA5 group, (b) KP5 group, (c) KT5 group

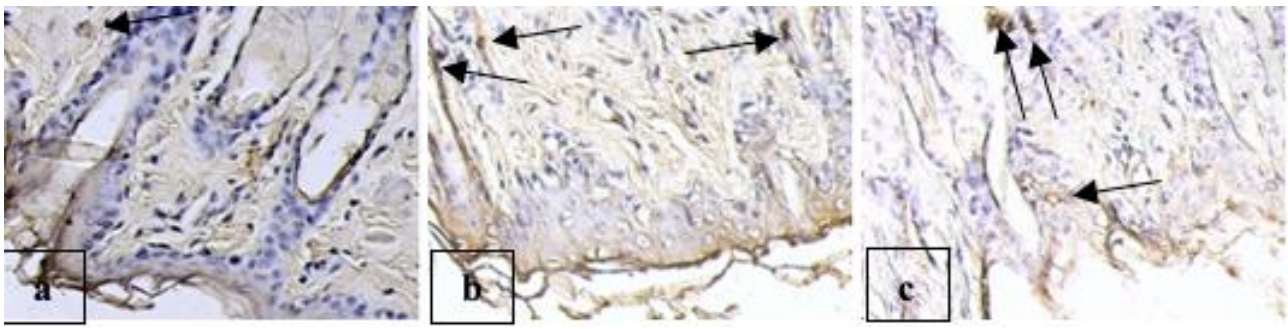

Figure 5. Comparison of histology image on day 7 immunohistochemistry staining at 400x magnification showing TGF-β expression: (a) KA7 group, (b) KP7 group, (c) KT7 group

The Pearson test results showed that the expression of macrophages and TGF-β cells differed significantly (P=0.004 and P<0.05, respectively). Thus, it was concluded that there was a positive correlation between increased macrophage cell expression and TGF-β cell expression. In the fibrosis process, macrophages secrete fibroblast growth factor, platelet-derived growth factor (PDGF), and TGF-β (transforming growth factor), so the amount of TGF-β in this study also increased in line with the increase in the number of macrophage cells. The nanogel application was performed twice a day to observe the activity of the active ingredients contained in the propolis extract and liquid smoke [14].

Based on the results of the study, TGF-β tends to decrease because in the healing process, after passing the peak period,

TGF-β will decrease to continue the healing process, while macrophages have a tendency to increase because macrophages function to excrete hugeamounts of pro-inflammatory cytokines include TGF-β.

4. DISCUSSION

Based on the research results, the treatment group given the nanogel combination of propolis extract and liquid smoke experienced an increase in the average number of macrophages and TGF-β cells were highest compared to the propolis extract nanogel group alone and the control group. This is due to the active ingredient content of propolis and liquid smoke, which can accelerate the wound healing process when combined in the right dose. These results are also in line with research by Ernawati and Puspa [11] on propolis's content in treating traumatic ulcers and research by Arundina et al. [2] on liquid smoke, which can accelerate wound healing. This research shows that topical application of liquid smoke and propolis increased the infiltration of macrophages, lymphocytes, IL-6, and TGF-β expression and fibroblasts number in traumatic ulcers. Therefore, it accelerated the healing of traumatic ulcers.

In the nanogel group with the combination of propolis extract and liquid smoke, the increase in the number of macrophages and TGF-β cells was highest on day 3 and then decreased on days 5 and 7. This shows that the combination nanogel group experienced a peak inflammatory phase on day 3. The inflammatory phase occurs in the first 72 hours after injury and can last for approximately 2-4 weeks, along with other wound healing phases [22-24]. This process also occurs simultaneously with angiogenesis, granulation formation, epithelialization, and wound retraction. The large increase in TGF-β cells is caused by phagocytic cells, namely macrophages, which have peak concentrations in wounds during the inflammatory phase. Macrophages store and produce large amounts of growth factors such as TGF-β, FGF, and EGF to regulate inflammatory responses, stimulate angiogenesis, and increase granulation tissue formation [25]. Increased TGF-β in ulcer conditions can potentially accelerate the healing process into the next phase, the proliferative phase. Thus, after reaching peak concentration and ensuring that all bacteria and excess debris in the wound area have been cleared, the number of macrophages and TGF-β decrease on days 5 and 7 to continue the process of re-forming vascular channels, granulation tissue, and re-epithelialization of the wound surface [24].

The propolis extracts nanogel group experienced an increase in the number of macrophages and TGF-β cells on days 3 and 5, then a decrease on days 7. On day 3, the propolis extract nanogel group was in the inflammatory phase. However, phagocytic cell activity was still high, so they experienced an increase in the number of macrophages and TGF-β cells on day 5. Meanwhile, the control group showed increased expression of macrophages and TGF-β from day 3 to day 7 (Figure 1). This indicates that the inflammatory phase lasted longer in the control group than in the treatment group.

Considering that this research was conducted on the 3rd to 7th day after induction of a traumatic ulcer, i.e., the time when the inflammatory and proliferative phase occurs. Generally, the amounts of macrophages and TGF-β are highly expressed; a decrease in the average number of macrophages and TGF-β cells in the treatment group showed that the inflammatory and proliferative phase had passed. This is consistent with research by Gilbert that an increase in macrophages and TGF-β in wound conditions results in an acceleration of the wound healing process to the next stage [26]. In addition, the results of the macrophage TGF-β computational analysis are also supported by the clinical appearance of lesions from the combination nanogel group, which began to become weak on day 5, and lesions from the propolis extract nanogel group, which began to become weak on day 7.

Propolis extract contains various active ingredients such as flavonoids, the most abundant of which is 50%, phenols, tannins, alkaloids, and CAPE. The content of active ingredients stimulates the migration of macrophages for the production of cytokines and growth factors such as IL-1, IL-4, IL-8, TGF-β and EGF, which functions for the induction of fibroblasts in the production of ECM as well as the induction of fibroblast proliferation and migration [27]. Growth factors produced by macrophages contribute to several cellular processes, namely inflammation, migration, and proliferation [28]. Liquid smoke contains active ingredients, one of which is phenol. According to research by Arundina et al. [2], phenol increases the number of macrophage cells, lymphocytes, fibroblasts, and TGF-β by inhibiting the production of pro-inflammatory cytokines.

The combination of Propolis Extract and Liquid Smoke is expected to produce a synergistic effect, enhancing the activity of their active ingredients to increase TGF-β levels. This increase in TGF-β is anticipated to boost cellular activity in the wound healing process. This is in line with research by Valluru et al. [29] that TGF-β induces angiogenesis, induces the expression of adhesion molecules as a chemoattractant, and triggers pro-inflammatory molecules for leukocyte and fibroblast migration. These cells have a strong anti-inflammatory effect and are said to be a potent fibrogenic substance that can increase collagen in the ulcer healing process [30]. So, in this case, increased expression of macrophages and TGF-β may help speed up the healing of traumatic ulcers. In addition, the compound is being produced in the form of nanoparticles in gel form in the hope that they will penetrate more easily into specific areas. This is similar to research by Elkhateeb et al. [31] that nano preparations make the active ingredient content more soluble due to their small particle size, and research by Kurniawan and Dwiaprinia [17] on the advantages of gel medicinal preparations, namely that they are cooling and easily penetrate the skin.

The results of this study indicate that the application of the nanogel combination of propolis extract and liquid smoke to Wistar rats induced by traumatic ulcers by applying it twice a day can accelerate the inflammatory and proliferative phases, as seen by the increase in the number of macrophages and TGF-β cells in the oral mucosa of experimental rat with traumatic ulcers. The clinical implications of propolis and liquid smoke in the treatment of traumatic ulcers are significant. This combination of drugs has been demonstrated to have a dual effect in the management of wounds, both aiding infection control and supporting the healing process. The maintenance of a moist wound environment is essential for effective healing; the use of this drug, therefore, facilitates this process. This drug's benefits in facilitating healing are well-documented, and the practical advantages of this treatment are evident. However, the limitations of this study must be acknowledged: specifically, the minimum, optimum dose, and toxic dose have not yet been determined. It was therefore concluded that the nanogel combination of propolis extract

and liquid smoke affected increasing the number of macrophages and TGF-β cell expression in the healing process of traumatic oral mucosal ulcers in rats. This research hypothesis can, therefore, be accepted. It is recommended that further research be undertaken to determine the minimum, optimal, and toxic doses in this research area.

5. CONCLUSIONS

The study shows that the use of nanogel with propolis extract and liquid smoke, propolis extract nanogel alone, and a standard topical medication (triamcinolone acetonide 0.1%) resulted in significant changes in macrophage numbers and TGF-β cell expression during oral mucosal ulcer healing in rats in each group on days 3, 5 and 7 with the value of P=0.002 (day 3), P=0.007 (day 5) and P=0.001 (day 7). Compared to the other treatments, the nanogel containing liquid smoke and propolis extract showed significant differences (P<0.05 TGF-β expression on days 3, 5, and 7). On day 3, both nanogel treatments showed an initial increase in immune cells, followed by different patterns on subsequent days. These findings suggest that propolis extract and liquid smoke-containing nanogel may be an effective way to accelerate the healing of traumatic oral mucosal ulcers.

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REFERENCES

[1] DeLong, L., Burkhart, N.W. (2020). General and Oral Pathology for the Dental Hygienist. Massachusetts: Jones & Bartlett Learning.

[2] Arundina, I., Diyatri, I., Kusumaningsih, T., Surboyo, M.D.C., Monica, E., Afanda, N.M. (2021). The role of rice hull liquid smoke in the traumatic ulcer healing. European Journal of Dentistry, 15(1): 33-38. https://doi.org/10.1055/s-0040-1714445

[3] Sebaiy, M.M., El-Adl, S.M., Baraka, M.M., Mohram, M.S., Elkady, Y.M. (2021). Stability indicating HPLC method for the simultaneous estimation of triamcinolone acetonide and benzyl alcohol in pure form and Epirelefan® vial. American Journal of Medical Chemistry, 123: 1-6.

[4] Hassan, T., Shahzad, M.K., Hanif, N., Anwar, M., Ambreen, A., Waheed, M.U. (2021). Effectiveness of aloe vera mouthwash in comparison with triamcinolone acetonide 0.1% in patients with OLP at a tertiary care hospital. Pakistan Journal of Medical & Health Sciences, 15(11): 3138-3140. https://doi.org/10.53350/pjmhs2115113138

[5] Riyaz, S.M.A. (2023). Efficacy of clobetasol propionate (0.05%), amlexanox (5%), and triamcinolone acetonide (0.1%) in the treatment of oral lichen planus. Journal of Pharmacy and Bioallied Sciences, 16(Suppl 1): S736-S737.

[6] Song, H., Tan, J., Fu, Q., Huang, L., Ao, M. (2018). Comparative efficacy of intralesional triamcinolone acetonide injection during early and static stages of pathological scarring. Journal of Cosmetic Dermatology, 18(3): 874-878. https://doi.org/10.1111/jocd.12690

[7] Saki, N., Mokhtari, R., Nozari, F. (2019). Comparing the efficacy of intralesional triamcinolone acetonide with verapamil in treatment of keloids: A randomized controlled trial. Dermatology Practical & Conceptual, 9(1): 4-9. https://doi.org/10.5826/dpc.0901a02

[8] Sharma, S., Kamarthi, N., Goel, S., Malik, S., Gupta, S., Sharma, A. (2023). Comparative evaluation of autologous platelet-rich plasma and triamcinolone acetonide injection in the management of erosive lichen planus and oral submucous fibrosis: A clinical study. International Journal of Basic and Clinical Pharmacology, 12(3): 439-445. https://doi.org/10.18203/2319-2003.ijbcp20231125

[9] Pelegrini, B.B., Becker, A.A., Ferreira, C.A., Machado, G.R., Gauer, M.A., Mazarin, S.R., Dembogurski, D.S., Kaneshima, A.M., Da Silva, D.B., Becker, T.C.A. (2024). Antineoplastic activity evaluation of Brazilian brown propolis and artepillin C in colorectal area of Wistar rats. Asian Pacific Journal of Cancer Prevention, 25(2): 563-573. https://doi.org/10.31557/APJCP.2024.25.2.563

[10] Khurshid, Z., Naseem, M., Zafar, M.S., Najeeb, S., Zohaib, S. (2017). Propolis: A natural biomaterial for dental and oral healthcare. Journal of Dental Research, Dental Clinics, Dental Prospects, 11(4): 265-274. https://doi.org/10.15171/joddd.2017.046

[11] Ernawati, D.S., Puspa, A. (2018). Expression of vascular endothelial growth factor and matrix metalloproteinase-9 in Apis mellifera Lawang propolis extract gel-treated traumatic ulcers in diabetic rats. Veterinary World, 11(3): 304-309. https://doi.org/10.14202/vetworld.2018.304-309

[12] Ridho, R., Sabiq Irwan H, M., Malis, E., Mislan. (2021). Utilization of liquid smoke for odor control at the final disposal site Kalibaru, Banyuwangi. GANDRUNG: Jurnal Pengabdian Kepada Masyarakat, 2(1): 149-157. https://doi.org/10.36526/gandrung.v2i1.1224

[13] Sari, L.M., Mubarak, Z., Sari, D.K. (2023). Evaluation of clinical, histology, TNF-α, and collagen expressions on oral ulcer in rats after treatment with areca nut and chrysanthemum oral gel. F1000Research, 10: 623. https://doi.org/10.12688/f1000research.54887.6

[14] Cahyati, M., Susilo, A., Nurjannah, Pranowo, D., Hermanto, F.E., Primandasari, E.P. (2024). Virtual screening of antiviral properties of bioactive compounds from liquid smoke against 2A protease of Coxsackievirus-A16. AIP Conference Proceedings, 3055: 040003. https://doi.org/10.1063/5.0193867

[15] Suhandi, C., Wilar, G., Lesmana, R., Zulhendri, F., Suharyani, I., Hasan, N., Wathoni, N. (2023). Propolis-based nanostructured lipid carriers for α-mangostin delivery: Formulation, characterization, and in vitro antioxidant activity evaluation. Molecules, 28(16): 6057. https://doi.org/10.3390/molecules28166057

[16] Mahmad, A., Chua, L.S., Noh, T.U., Siew, C.K., Seow, L.J. (2023). Harnessing the potential of Heterotrigona itama propolis: An overview of antimicrobial and antioxidant properties for nanotechnology–based delivery systems. Biocatalysis and Agricultural Biotechnology, 54: 102946. https://doi.org/10.1016/j.bcab.2023.102946

[17] Kurniawan, M.F., Dwiaprinia, R. (2021). Formulation

and antibacterial activity of Citrus limon peel essential oil and Eucalyptus globulus essential oil combination as gel hand sanitizer with the simplex lattice design method. Ad-Dawaa' Journal of Pharmaceutical Sciences, 4(2): 71-86.

[18] Susilo, A., Pranowo, D., Cahyati, M., Nurjannah, Prihanto, A.A., Masyithoh, D., Pangesti, I.F., Ihwanudin, A.Y. (2024). Physicochemical quality of transparent solid soap with the addition of forest honey (Apis dorsata) as an environmentally friendly sanitizer. BIO Web of Conferences, 88: 00011. https://doi.org/10.1051/bioconf/20248800011

[19] Woźniak, M., Mrówczyńska, L., Kwaśniewska-Sip, P., Waśkiewicz, A., Nowak, P., Ratajczak, I. (2020). Effect of the solvent on propolis phenolic profile and its antifungal, antioxidant, and in vitro cytoprotective activity in human erythrocytes under oxidative stress. Molecules, 25(18): 4266. https://doi.org/10.3390/molecules25184266

[20] Muli, E.M., Maingi, J.M. (2007). Antibacterial activity of Apis mellifera L. propolis collected in three regions of Kenya. Journal of Venomous Animals and Toxins Including Tropical Diseases, 13(3): 655-663. https://doi.org/10.1590/S1678-91992007000300008

[21] Mardiyantoro, F., Prasetyaningrum, N., Cahyati, M., Abidin, Z.Z., Nakamura, N. (2021). Potential effect of Djambal catfish (Pangasius djambal) gelatin as biomaterial product on healing socket after tooth extraction in rats. Indian Journal of Forensic Medicine & Toxicology, 15(2): 2379-2387. https://doi.org/10.37506/ijfmt.v15i2.14728

[22] Puspasari, A., Harijanti, K., Soebadi, B., Hendarti, H.T., Radithia, D., Ernawati, D.S. (2018). Effects of topical application of propolis extract on fibroblast growth factor-2 and fibroblast expression in the traumatic ulcers of diabetic Rattus norvegicus. Journal of Oral and Maxillofacial Pathology, 22(1): 54-58. https://doi.org/10.4103/jomfp.JOMFP_82_17

[23] Abbasi, A. J., Mohammadi, F., Bayat, M., Gema, S. M., Ghadirian, H., Seifi, H., Bayat, H., Bahrami, N. (2018). Applications of propolis in dentistry: A review. Ethiopian Journal of Health Sciences, 28(4). https://doi.org/10.4314/ejhs.v28i4.16

[24] Ellis, S., Lin, E.J., Tartar, D. (2018). Immunology of wound healing. Current Dermatology Reports, 7: 350-358. https://doi.org/10.1007/s13671-018-0234-9

[25] Theoret, C. (2016). Physiology of wound healing. In Equine Wound Management. https://doi.org/10.1002/9781118999219.ch1

[26] Gilbert, R.W., Vickaryous, M.K., Viloria-Petit, A.M. (2016). Signalling by transforming growth factor beta isoforms in wound healing and tissue regeneration. Journal of Developmental Biology, 4(2): 21. https://doi.org/10.3390/jdb4020021

[27] Chen, S., Saeed, A.F., Liu, Q., Jiang, Q., Xu, H., Xiao, G.G., Rao, L., Duo, Y.H. (2023). Macrophages in immunoregulation and therapeutics. Signal Transduction and Targeted Therapy, 8(1): 207. https://doi.org/10.1038/s41392-023-01452-1

[28] Corliss, B.A., Azimi, M.S., Munson, J.M., Peirce, S.M., Murfee, W.L. (2016). Macrophages: An inflammatory link between angiogenesis and lymphangiogenesis. Microcirculation, 23(2): 95-121. https://doi.org/10.1111/micc.12259

[29] Valluru, M., Staton, C.A., Reed, M.W., Brown, N.J. (2011). Transforming growth factor-β and endoglin signaling orchestrate wound healing. Frontiers in Physiology, 2: 89. https://doi.org/10.3389/fphys.2011.00089

[30] Trinh, X.T., Long, N.V., Anh, L.T.V., Nga, P.T., Giang, N.N., Chien, P.N., Nam, S.Y., Heo, C.Y. (2022). A comprehensive review of natural compounds for wound healing: Targeting bioactivity perspective. International Journal of Molecular Sciences, 23(17): 9573. https://doi.org/10.3390/ijms23179573

[31] Elkhateeb, O.M., Badawy, M.E., Noreldin, A.E., Abou-Ahmed, H.M., El-Kammar, M.H., Elkhenany, H.A. (2022). Comparative evaluation of propolis nanostructured lipid carriers and its crude extract for antioxidants, antimicrobial activity, and skin regeneration potential. BMC Complementary Medicine and Therapies, 22(1): 256. https://doi.org/10.1186/s12906-022-03737-4