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Effect of Propolis Trigona Sp on Expression of TNF-α in Superficial Dermal Burns through In Vivo Test

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Abstract: Burn is caused by exposure to high-temperature substances such as hot, solid liquids or gases such as smoke, steam, engines, stoves, radiators and objects that emit heat energy. In severe burns, there is an excessive neutrophil inflammatory response that triggers SIRS, where there is an excessive increase in pro-inflammatory mediators such as TNF-α and IL-6. Propolis contains a number of compounds, such as flavonoids, CAPE, phenol compounds, arginine, ferulic acid and albumin which play a good role in healing burns. This study aimed to find out the effect of propolis on TNF-α expression through in-vivo testing in cases of superficial dermal burns. This was an experimental and laboratory study to assess the bioactive compounds contained in *Trigona Sp propolis*. Descriptive analysis was performed on the bioactive composition of Trigona Sp propolis and experimental effectiveness of propolis on TNF-α expression in superficial dermal burns. The study was conducted at the laboratory of experimental animals *"Alike Quality System"*, Manado. The results showed that the group treated with propolis had smaller burn areas compared to the groups treated with 1% silver sulfadiazine or with 0.9% NaCl. Granulation had been formed throughout all wounds, however, macroscopically, burns with propolis treatment showed reddish wound appearance while treated wounds with 1% silver sulfadiazine and 0.9% NaCl were darker in color and had thicker crust formation. The one-way ANOVA test showed no significant difference between propolis and 1% silver sulfadiazine on TNF-α expression in dermal superficial burns (p=0.666) meanwhile the effect of propolis compared with NaCl 0.9% on TNF-α expression in dermal superficial burns, showed significant differences (p=0.006 and p=0.040). In conclusion, administration of propolis can reduce the expression of TNF-α in superficial dermal burn.
Keywords: propolis; *Trigona sp*; burns; *in vivo* study; TNF-α

INTRODUCTION

A burn is a type of injury caused by exposure to high-temperature substances such as hot, solid liquids or gases such as smoke, steam, engines, stoves, radiators and objects that emit heat energy.[1] World Health Organization estimates that 11 million burns occur each year worldwide, of which 180,000 cases are fatal. Most cases occur in low- and middle-income countries and nearly two-thirds occur in Africa and Southeast Asia. In addition, child mortality rates from burns are seven times higher in low- and middle-income countries than in high-income countries. Nonfatal burns are a major cause of morbidity, including prolonged hospital stay and disability that often stigmatize and reject patients with burns.[2]

In Indonesia, research conducted by Cipto Mangunkusumo Hospital in 2011–2012 found 303 cases of burns, where men were more than women with a ratio of 2.26: 1. The average age of patients was 25.7 years (15–54 years) with burn area between 20–50%. The etiology of burns in adults is fire (53.1%), while in children it is hot water (52%). Another study conducted at Dr. Soedarso Hospital, Pontianak, during the period 2017–2020 showed that most burn cases occurred in men (81.5%), with a male-female ratio of 4.4:1. With the most cases caused by electrical burns (34.3% of cases), 77% of cases were grade II burns with the average length of hospital stay was 16.15 days and the mortality rate was around 7.4%.[3] Injuries from burns induce global changes throughout the immune system resulting in suppressed immune function and vulnerability to infection. This immunopathological response can contribute to the development of systemic inflammatory response syndrome (SIRS) and multiple organ failure.[4]

The inflammatory phase of burn begins within 24 hours after the injury and lasts for weeks to months depending on the severity of the injury, wherein neutrophils and macrophages release cytokines and chemokines. These inflammatory mediators include IL-1, IL-6, IL-8 and tumor necrosis factor (TNF), as well as growth factors including tumor growth factor (TGF) β, insulin growth factor (IGF) and vascular endothelial growth factor (VEGF).[5] In severe burns, there is an excessive neutrophil inflammatory response that triggers SIRS, where there is an excessive increase in pro-inflammatory mediators such as TNFα and IL-6.[6]

Trigona sp bees are bees that do not have stings. Morphologically, *Trigona sp* is the same as other bees, namely members of the group *Insecta* and family *Apidae*. *Trigona sp* bees live in groups in tropical and subtropical regions, where the main products produced are honey, pollen, propolis and wax.[7] Propolis is a resinous substance collected by bees as a physical and biochemical protection material for the hive. The biological activity of propolis includes antibacterial, antiviral, antifungal, anti-parasitic, antioxidant and anti-inflammatory. Recent scientific research shows that the therapeutic properties of propolis are due to the content of secondary metabolites of plants such as phenolics and terpenoids.[8] Propolis contains a number of compounds, such as flavonoids, caffeic acid phenethyl ester (CAPE), phenol compounds, arginine, ferulic acid and albumin which play a good role in healing burns.[9] This research is expected to obtain the anti-inflammatory potential of propolis through ex-vivo testing in burn cases.

METHODS

This study was conducted at the laboratory of Experimental Animals *"Alike Quality System"*, Manado. This was an experimental and laboratory study to assess the bioactive compounds contained in *Trigona Sp* propolis, in Gowa, South Sulawesi. This study used white rat test animals of the *Rattus Norvegicus* strain, 27 males. The inclusion criteria consisted of white rats of the *Rattus Novergicus* strain, male, healthy, weighing about 180-300 gr, aged about 3-4 months, normal behavior and activity, no visible anatomical complaints, no dull hair, loss, and active movement. The exclusion criterion was rat that was sick and died during the treatment period. Descriptive analysis of the bioactive composition of *Trigona Sp* propolis and experimental effectiveness of propolis on inflammatory response in the form of TNF-α expression levels in superficial dermal burn injury was performed. The results of the study were presented in the form of tables.

Maceration was done by cutting 200 g of propolis into sizes which were then put into a 500 ml bottle and soak with methanol to a volume of 250 ml. The bottle was shaken every two hours for 2-3 minutes for two days. The methanol filtrate was filtered and separated. The maserat solution was concentrated by inserting it into the evaporating rotary flask, the waterbath temperature was set to 60^0C, and rotary speed 55 rpm. The evaporation process was carried out until all the methanol in the maserat was evaporated. The viscous extract was transferred into a container, then the volume of the randemen was measured.

In the flavonoids test, a sample of 2 mL was inserted into a test tube and 1 mL of water was added, then 0.5 g of mg powder was added to the reaction tube and 10 drops of concentrated HCl and the changes were observed. Positive flavonoids if orange clear foam was formed.

Acclimatization of test animals before treatment included temperature, humidity, light, sound, nutrition and hygiene. The selection of strains, sex, weight and age had to be exactly 3-4 months old with a body weight of 180-300 g, healthy condition, normal activity and aggressiveness, no body defects, rectal temperature 37.5^{0C}, feed consumption per day 5 g/100 g BW, drinking water consumption per day 8-11 ml/100 g BW, urine excretion per day 5.5 ml/100 g BW. Test animals were placed in clean individual cages with chaff bedding, laboratory room temperature was maintained at 25^0C. During the acclimatization process a number of qualified test animals were selected as above to be used as research subjects.

In burn induction, the rat's back was shaved until part of the skin was visible, and an injection of local anesthesia was given to the skin of the mouse's back. Stainless steel plate with a size of 1x1 cm was heated using boiling water (100°C) for 5 minutes, then was attached to the cleaned skin for 5 minutes. The injured skin would appear in the shape of a white box.

For treatment of burns, a total of 27 male rats were grouped into three groups, namely group I, treated with propolis extract, group II, treated with Burnazin ointment, and group III, treated with physiological NaCl. Each rat in each group that had been induced by burns on the skin of its back was placed in an individual cage. The burn was treated by applying the test material then was covered with sterile gauze fixed with a plaster. The burn treatment was performed on rats for 7-10 days and the wound healing process was observed in each rat in each treatment group. During the treatment, rats had to be kept clean by changing the husks regularly every day and were given standard feed and drinking moderately.

After the burn treatment period for seven days, the rats would undergo a tissue termination process. The rats were given intra-peritonial injections of ketamine injection 0.1 ml anesthetic drug. After the drug worked optimally, then 70% alcohol was applied to the wound area, and the wound area was cut using a sterile scalpel and scissors until under the skin then the tissue immediately was soaked in neutral formalin buffer 10% for 1x24 hours before proceeding to the next process. Rats that had undergone tissue termination were immediately treated by applying wound ointment and covered with bandages, until the wound was completely closed and healed. *S*kin samples were taken and paraffin block preparations were made and then continued on the TNF–α immunohistochemical examination test.

RESULTS

The test animals used were male white rats of *the Rattus Norvegicus* strain aged 3-4 months. The average body weight of white rats during acclimatization for each group of test animals was 234.4 g in the test group with propolis, 226.7 g in the test group with silver sulfadiazine 1%, and 228.9 g for the test group with NaCl 0.9% (Figure 1).

The macroscopic picture of the results of burn treatment can be assessed through several factors, namely the area of the burn and granulation of the wound tissue. From the results of the burn treatment study, treatment with propolis showed a smaller burn area compared to treatment with silver sulfadiazine 1% or with NaCl 0.9%. The measurement of burn area after day 7 treatment showed that the burn area in treatment with propolis was 0.87 cm, silver sulfadiazine 1% 1.49 cm and with NaCl 0.9% 1.61 cm (Figure 2).

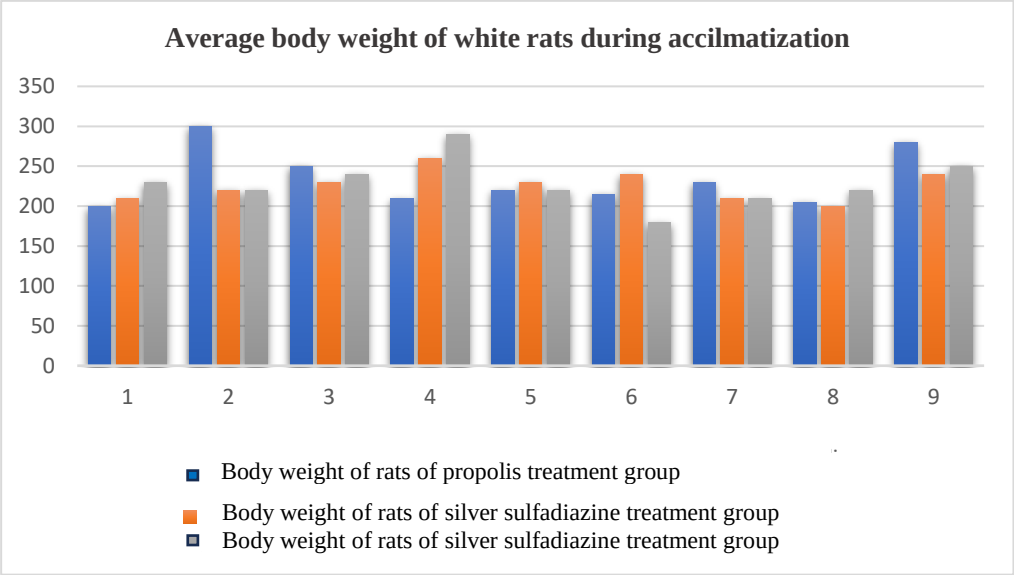

Figure 1. Average body weight of white rats during acclimatization

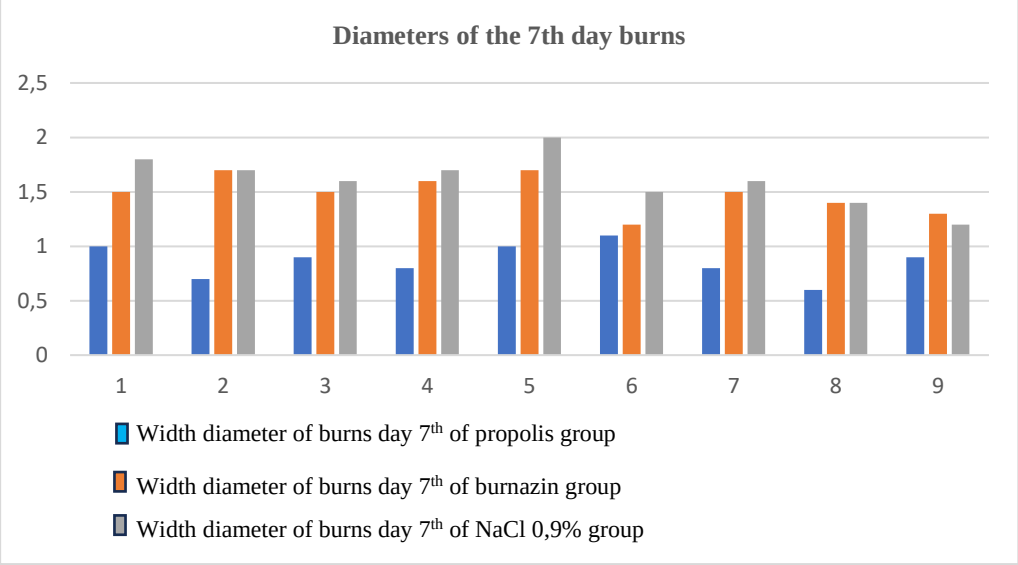

Figure 2. Diameters of the 7th-day burns

While the process of tissue granulation formation in the wound with propolis treatment, silver sulfadiazine and NaCl 0.9% showed that granulation tissue was formed throughout the wound. Macroscopically, it can be seen that the treatment of burns with methanol extract of propolis produced wounds that looked reddish-white with the formation of thin crusts. Wound treatment with silver sulfadiazine 1% and NaCl 0.9% resulted in darker-looking lesions with thickened crusts (Figure 3). Results of qualitative examination of flavonoids in propolis methanol extract *Trigona sp*. showed positive results. In the results of TNF-α immunohistochemical examination, positive results were shown with brownish cells (Figure 4).

In the TNF-α immunohistochemical staining test, burn samples with propolis methanol extract treatment showed grading scores of 0 (0–10% stained cells) and scores of 1 (11–25% stained cells). While TNF-α immunohistochemical staining of burn samples with 1% silver sulfadiazine treatment showed the highest grading at a score of 1 (11–25% stained cells) and a score of 2 (26 –50% stained cells). For TNF-α immunohistochemical staining, burn samples with 0.9% NaCl treatment showed the highest grading at a score of 3 (51–100% stained cells). Figure 5 showed the TNF-α immunohistochemical staining grading.

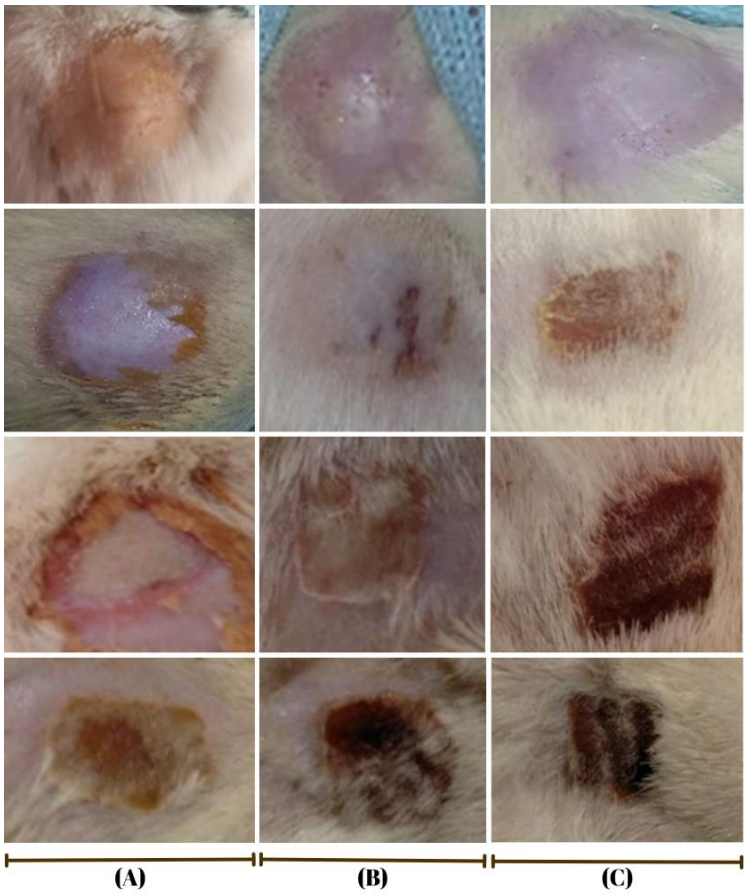

Figure 3. Macroscopic skin of rats after dermal superficial burn injury on days 1, 3, 5 and 7. A, burn treatment with propolis; B, burn treatment with silver sulfadiazine 1%; C, burn treatment with NaCl 0.9%

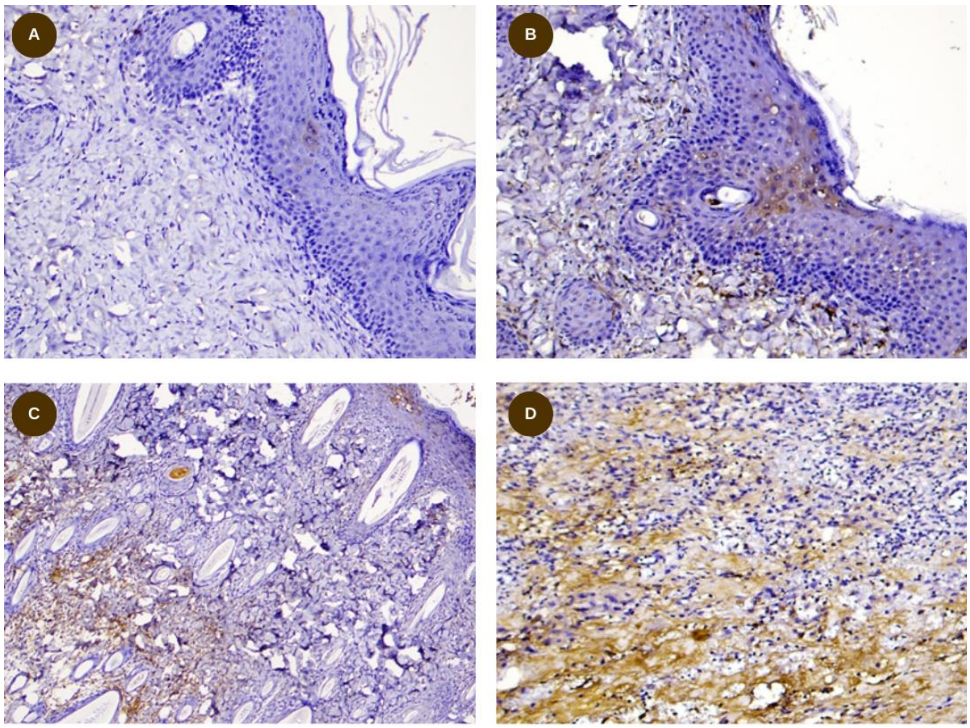

Figure 4. Immunohistochemistry examination of TNF-α. A, 0-10% of colored cells, score 0; B, 11-25% of cells were colored, score 1; C, 26-50% of cells were colored, score 2; D, 51-100% of cells were colored, score 3

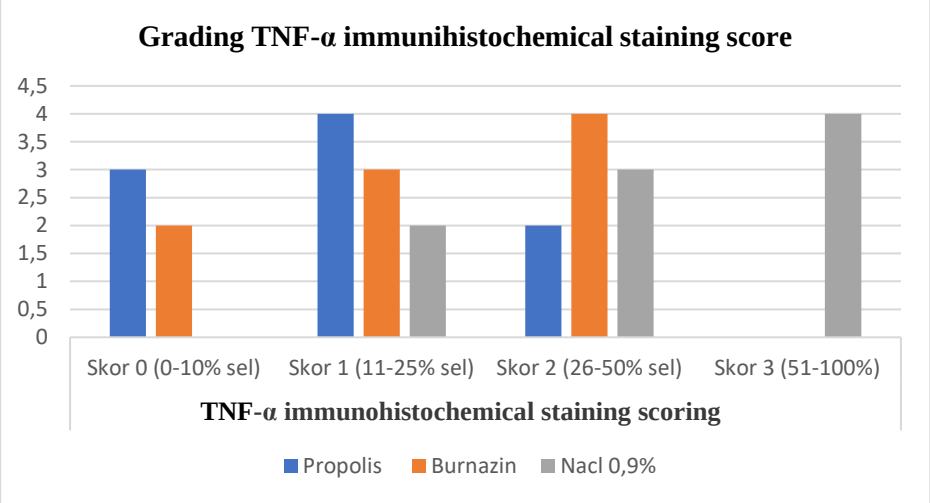

Figure 5. The width diameters of the 7th-day burns

Table 1 showed the one-way ANOVA test indicating no significant difference between propolis and 1% silver sulfadiazine on TNF-α expression in dermal superficial burns, with a sig. of 0.666 ($p>0.05$). While the effect of propolis compared with NaCl 0.9% on TNF-α expression in dermal superficial burns, showed significant differences with sig. 0.006 and 0.040 ($p<0.05$).

Table 1. Statistical test of the effect of different administration of methanol propolis extract on expression TNF- α on dermal superficial burn injury using the Tukey HSD

	Treatment	Mean difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower bound	Upper bound
Propolis	1% Silver sulfadiazine	-0.33333	0.38490	0.666	-1.2945	0.6279
	0.9% NaCl	-1.33333*	0.38490	0.006	-2.2945	-0.3721
Burnazin	Propolis	0.33333	0.38490	0.666	-0.6279	1.2945
	0.9% NaCl	-1.00000*	0.38490	0.040	-1.9612	-0.0388
NaCl	Propolis	1.33333*	0.38490	0.006	0.3721	2.2945
	1% Silver sulfadiazine	1.00000*	0.38490	0.040	0.0388	1.9612

*. *The mean difference is significant at the 0.05 level.*

DISCUSSION

This study used white rats from the *Rattus Norvegicus strain*, 27 male types. Before use, test animals must go through the acclimatization stage. First, the animal cage must meet the requirements of temperature, humidity, light, sound, nutrition and hygiene. The age must be exactly 3-4 months old with a body weight of 180-300 g. In addition, the condition of the test animals was healthy, normal activity and aggressiveness, no body defects, rectal temperature 37.5°C, feed consumption per day 5 g/100 g BW, drinking water consumption per day 8-11 ml/100 gr BB and urine excretion per day 5.5 ml/100 g BW. Test animals were placed in clean individual cages with chaff bedding, laboratory room temperature was maintained at 25^0C. During the acclimatization process a number of qualified test animals were selected to be used as research subjects.

In this study, phytochemical compounds contained in propolis methanol extract *Trigona sp* showed positive result of flavonoid phytochemical tests. This result is in line with the important compounds in propolis including flavonoids, phenylpropenoids, cinnamic acid, and glycerides.

Caffeic acid phenefthyl ester (CAPE) is also a component of some varieties of propolis. In addition, propolis also contains some essential oils, terpenes and sesquiterpens, beeswax, naphthalene, stilbene derivatives, and other components such as vitamins, proteins, amino acids, steroid β, alcohol, and sugar. These aromatic compounds are responsible for the anti-bacterial, anti-fungal, antiviral, anti-inflammatory and anti-cancer properties of propolis.[7,8]

In this study, dermal superficial burn injury were performed on the experimental animals, then divided into three treatment groups consisting of wounds smeared with propolis extract, wounds smeared with silver sulfadiazine 1% (positive control) and untreated wounds (negative control). Furthermore, the wounds were treated daily with methanol extracts of propolis, silver sulfadiazine 1%, or Nacl 0.9% for 7 days. Then macroscopic observations were made on the wound on day 7 covering the area of burns, granulation tissue and crusts formed. Skin burns were excised for tissue sampling, then paraffin block preparations were made for TNF-α immunohistochemical examination.

The average body weight of white rats on acclimatized mass for each group of test animals was 234.4 g for the test group with propolis, 226.7 g for the test group with silver sulfadiazine 1% and 228.9 g for the test group with NaCl 0.9%. Body weight, age and stage of animal development used in research were characteristics that could affect the results of research.[10,11]

The macroscopic picture of the results of dermal superficial burn treatment with propolis, silver sulfadiazine 1% and NaCl 0.9% could be seen from the area of burns and granulation in wound bed. Treatment with propolis showed a smaller burn area compared to treatment with silver sulfadiazine 1% or with NaCl 0.9%, namely the burn area in treatment with propolis 0.87 cm, silver sulfadiazine 1% 1.49 cm, and with NaCl 0.9% 1.61 cm. The acceleration of burn healing is thought to be due to the presence of secondary metabolite compounds, namely flavonoids, phenolics, tannins, and saponins in propolis extract. Suriawanto et al[12] examined the effect of stingless bee propoli extract on the healing of burns of white rats (*Rattus norvegicus*), showing the acceleration of wound healing was equivalent to the positive control (Bioplasenton). The flavonoid content in propolis was considered to reduce the activity of NF-κB which would inactivate TNF-α so that it would reduce the inflammatory process. When the inflammatory process was reduced, wound healing would occur faster.

In this study, it was found that the formation of granulated tissue in the wounds with propolis, silver sulfadiazine, and NaCl 0.9% treatment had been formed throughout the wounds. However, macroscopically burns with propolis treatment showed reddish wound appearance. While treated wounds with silver sulfadiazine 1% and NaCl 0.9% were darker in color and had thicker crust formation. This showed that both propolis and 1% silver sulfadiazine were able to increase granulation in the wounds. Wound repair and regeneration take place through a well-regulated, integrated phase pattern, such as hemostasis, inflammation, cell proliferation and tissue remodeling that all involve a number of cellular and molecular processes. These phenomena include migration and proliferation of epidermal cells as well as keratinocytes, fibroblasts and extracellular matrix (ECM) contraction. Propolis treatment stimulates a significant increase in the ECM component during the initial phase of wound repair.[13] Research showed that propolis increased wound healing rates and diabetic wound reepithelialization in rodents. It has also proposed another role for propolis in reducing neutrophil infiltration and normalizing the entry of macrophages into injured areas.[14]

The results of the one-way ANOVA test showed no significant difference between propolis and 1% silver sulfadiazine on TNF-α expression in dermal superficial burns, with a sig. of 0.666 ($p>0.05$). Meanwhile the effect of propolis compared to NaCl 0.9% on TNF-α expression in dermis superficial burns showed significant differences with sig. of 0.006 ($p<0.05$). This means that propolis has the same effect as silver sulfadiazine (positive control) to suppress TNF-α expression and will decrease tissue inflammation.

Giving propolis can suppress the expression of TNF-α, so it will reduce inflammation. Propolis has a strong anti-inflammatory activity that can reduce the expression of the iNOS gene,

a cytokine mediated by NF-κB activation and immune response in T cells. Moreover, propolis has anti-inflammatory substances to inhibit and downregulate TLR4, MyD88, IRAK4, TRIF, NLRP, and pro-inflammatory cytokines such as IL-1β, IL-6, IFN-γ and TNF-α. The flavonoid content in propolis is considered to reduce the activity of NF-κB which will inactivate TNF-α, so that it will reduce the inflammatory process. This is in accordance with several previous studies, inter alia Bae et al[15] that examined the effects of the type of chrysin propolis that had the ability to reduce the expression of pro-inflammatory cytokine genes, such as TNF-α, IL-1β, IL-4 and IL-6 in mast cells through the Nf-ƙβ and caspase-1 mechanism pathways.

Another study by Dewi et al[16] showed that propolis has the ability to suppress pro-inflammatory cytokines in anthrax cells injected into the skin of test animals. Further explained in this study, the mechanism of action of propolis to fight TNF-α was by inhibiting NF-κB activation and neutrophil migration, thereby causing a decrease in TNF-α production which would slow down the release of pro-inflammatory cytokines and prevent the progression of the inflammatory process.

CONCLUSION

Administration of propolis can reduce the expression of TNF-α in dermal superficial burns. Further research are needed to explore the bioactive compounds in *Trigona sp. propolis* which may play a role in suppressing TNF-α expression and decreasing inflammatory processes as well as determination of the dose effectiveness and toxicity of *Trigona sp propolis.*

Conflict of interest

The authors affirm no conflict of interest in this srudy.

REFERENCES

1. GBD 2019 Diseases and Injuries Collaborators. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. Lancet. 2020;396(10258):1204-22. Doi: 10.1016/S0140-6736(20)30925-9
2. World Health Organization. Burns [Internet]. 2016 [cited 2023 Sep 24]. Available from: https://www.who.int/news-room/fact-sheets/detail/burns
3. Setiawan D, Haryono W. Epidemiology and Characteristics of Burn Patients in Dr . Soedarso General Hospital during 2017 – 2020 : Retrospective Study. B-Clinical Sciences. 2021;9(B):711–4. Doi: https://doi.org/10.3889/oamjms.2021.6544
4. Zulhendri F, Lesmana R, Tandean S, Christoper A, Chandrasekaran K, Irsyam I, et al. Recent update on the anti-inflammatory activities of propolis. Molecules. 2022;27(23):1–61. Doi: 10.3390/ molecules27238473
5. Ulandari KS, Yoga PCP, Lestari MW, Agustini NNM. Effectiveness of propolis extract in healing grade II burns. 2021;1(2):80–90. Doi: 10.23887/gm.v1i2.39185
6. Strudwick XL, Cowin AJ. The role of the inflammatory response in burn injury. In: Kartal SP, Bayramgürler D, editors. Hot Topics in Burn Injuries. Intechopen, 2017. Doi: 10.5772/intechopen.71330. Available from: https://www.intechopen.com/chapters/57431
7. Erwan, Wiryawan IKG, Syamsuhaidi, Purnamasari DK, Sumiati, Muhisinin M. Antibacterial and antioxidant activitiy of honey trigona sp in North Lombok District. Ternak Tropika. 2022;23(1):18–28. Doi https://doi.org/10.21776/ub.jtapro.2022.023.01.3
8. Zulhendri F, Lesmana R, Tandean S, Christoper A, Chandrasekaran K, Irsyam I, et al. Recent update on the anti-inflammatory activities of propolis. Molecules. 2022;27(23):1–61. Doi: 10.3390/ molecules27238473
9. Ulandari KS, Yoga PCP, Lestari MW, Agustini NNM. Effectiveness of propolis extract in healing grade II burns. Ganesha Medicina Journal. 2021;1(2):80–90. Doi: 10.23887/gm.v1i2.39185
10. Kilkenny C, Parsons N, Kadyszewski E, Festing MFW, Cuthill IC, Fry D, et al. Survey of the quality of experimental design, statistical analysis and reporting of research using animals. PLoS ONE. 2009;4(11):1-11. Doi: https://doi.org/10.1371/journal.pone.0007824
11. Jackson SJ, Andrews N, Ball D, Bellantuono I, Gray J, Hachoumi L, et al. Does age matter? The impact

of rodent age on study outcomes. 2017;51(2):160-9. Doi: 10.1177/0023677216653984
12. Suriawanto N, Setyawati E, Narwan. The effect of stingless bee propolis extract on the healing of burns of white rats (Rattus norvegicus). Jurnal Bioteknologi & Biosains Indonesia. 2021;8(1):68–76. Doi: https://doi.org/10.29122/jbbi.v8i1.4585
13. Martinotti S, Ranzato E. Propolis: a new frontier for wound healing? Burn Trauma. 2015;3(9):1–7. Doi: http://dx.doi.org/10.1186/s41038-015-0010-z
14. Toreti VC, Sato HH, Pastore GM, Park YK. Recent progress of propolis for its biological and chemical compositions and its botanical origin. Evid Based Complement Alternat Med. 2013;2013:1-13. Doi: 10.1155/2013/697390
15. Bae Y, Lee S, Kim SH. Chrysin suppresses mast cell-mediated allergic inflammation: Involvement of calcium, caspase-1 and nuclear factor- κ B. Toxicol Appl Pharmacol. 2011;254(1):56–64. Doi: http://dx.doi.org/10.1016/j.taap.2011.04.008
16. Dewi RTK, Redhono D, Susanto A, Prasetyo DH, Nurhayatun E, Nurwati I, et al. Indonesian propolis inhibit proinflammatory cytokines, apoptosis and oxidative stress in anthrax animal Model. Indones Biomed J. 2022;14(3):309–15. Doi:10.18585/inabj.v14i3.1873