

Potential Use Beecomb Gel (Trigona Sp) in the Healing Process of Periodontitis (Wistar Experimental Animal Research)

Rismawati
Poltekkes Kemenkes Semarang

Supriyana
Poltekkes Kemenkes Semarang

Endah Aryati Eko Ningtyas
Poltekkes Kemenkes Semarang

Abstract:- Periodontitis is an advanced disease of untreated gingivitis which results in the destruction of the periodontal tissue which includes the gingiva, alveolar bone and cementum which is characterized by pocket formation. Periodontitis can be treated by providing therapy with antibiotics and anti-inflammatory drugs. Honeycomb (Trigona Sp) contains flavonoids, phenolic acids and their esters, which act as anti-inflammatory agents. The purpose of this study was to prove that honeycomb gel (Trigona Sp) as an alternative material has an effect on changes in clinical conditions in periodontitis in Wistar rats. This type of research is true experiment and design using posttest only with control group design. Consisting of the 10% beehive gel (trigona sp) intervention group, the positive control group was 10% povidone iodine and distilled water. Observations were made for 7 days. The variables studied were gingival inflammation using index gingiva and periodontal pocket. This study showed that there were inflammatory changes in Wistar rats with periodontitis, based on the results of the unpaired test the honeycomb gel ($p=0.001$), povidone iodine ($p=0.002$) and distilled water ($p=0.003$) groups had significant differences. As for changes in periodontal pockets, based on the results of the unpaired test in the honeycomb gel group ($p=0.009$), povidone iodine ($p=0.001$) and distilled water ($p=0.001$), there was a significant difference in each treatment group.

Keywords:- Honeycomb Gel (Trigona Sp), Inflammation, Periodontitis, Wistar Rats.

I. INTRODUCTION

Dental and mouth problems that many people experience in general include cavities, gingivitis, and tartar. According to WHO statistical data, 98% of people in the world have a history of inflammatory diseases of the oral cavity, most of which are inflammatory periodontal diseases and are the most common oral diseases after caries.¹RISKESDAS 2018 data shows the percentage of periodontitis cases in Indonesia is 74.1%.²Dental plaque is the culprit.

The main cause of dental and oral diseases such as gingivitis (inflammation of the gums) and periodontitis (inflammation of the supporting tissues of the teeth).³Plaque is a soft deposit that is clinically yellow-gray in color attached to the surface of the teeth and hard surfaces in the oral cavity consisting of 70-80% microorganisms and an intercellular matrix consisting of organic and inorganic materials derived from saliva, gingival crevicular fluid and bacterial product. Porphyromonas gingivalis bacteria were found in 85.75% of subgingival plaque which is the beginning of periodontitis.⁴

Gingivitis that is not treated will develop into periodontitis, to prevent periodontitis can be done by maintaining oral hygiene by preventing the formation of dental plaque buildup by brushing teeth, and removing calculus. Periodontitis can be treated by providing mechanical therapy such as scaling and root planing which is supported by the administration of antibiotics and anti-inflammatory drugs.⁵

Anti-inflammatory agents must meet several requirements, including not being toxic to the human body but lethal to microorganisms, having a broad antibacterial spectrum that can kill microbes, low resistance, namely the lack of ability of bacteria to become resistant to bacterial agents and have substantivity. The drug that is often used to prevent inflammation is povidone iodine and is also commonly used as a wound medicine. However, for long-term use it can cause side effects such as allergic reactions. Honeycomb (Trigona Sp) contains flavonoids which act as anti-inflammatory agents.⁶

Particles contained in beehives are propolis which act as pharmacological are flavanoids, phenolic acids and their esters, these parts effectively affect microorganisms, parasites and infections. Honeycomb (Trigona Sp) has the ability as a mitigation exercise, immunomodulator, and antitumor and can be used in treating infections.⁷Apart from minimizing the side effects of using drugs that contain chemicals, the use of herbal ingredients is also due to the use of ingredients from nature because they are easier to obtain and from an economic point of view they are also more economical so that they can be used by all groups of people.

The gel provides fast drug release and is highly biocompatible with a lower risk of inflammation and side effects and is easy to use, besides that the use of gel dermatologically has several beneficial properties such as non-greasy, easily removed, easily spreadable, thixotropic, emollient, non- coloring, and soluble or water soluble and compatible with some excipients.^{8,9}

Honeycomb gel with a concentration of 10% has antibacterial ability so it is effective in inhibiting the formation of biofilms which are the places for the development of porphyromonas gingivalis bacteria, because it has a bacteriostatic effect by inhibiting the synthesis of macromolecules of bacterial cells and causing damage to the bacterial cell membrane due to its lipophilic nature.¹⁰Wistar rats are widely used as laboratory animals because they have advantages such as a relatively short life cycle, large number of offspring per birth, high variation in traits, easy to handle, and production properties and characteristics of wistar rats

which are often used as objects of clinical research because of their different anatomical and physiological structures. has similarities with the structure of human anatomy and physiology.¹¹Because of that the researchers used honeycomb gel using a concentration of 10% in wistar rats.

II. RESEARCH METHOD

This research uses true experimental research, namely real research by controlling all external variables that affect the course of the research. The design of this study uses a posttest only with control group design. Sampling was carried out using Simple Random Sampling.¹² Subjects in this study were divided into two groups, namely the treatment group and the control group. The treatment group will be given honeycomb gel (trigona sp) while the positive control group will be given povidone iodine and the negative control group will be given distilled water.

III. RESULTS AND DISCUSSION

Table 1. Treatment tests on days 1, 3 and 7 of the 10% honeycomb gel group, the povidone iodine group and the distilled water group (Gingival Index)

Treatment	Day	Mann withney	kruskall-wallis	
		Sig.	Sig	Sig
Honeycomb gel (Trigona Sp) 10%	1 and 3	0.009	0.001	0.570
	3 and 7	0.018		
	1 and 7	0.008		
Povidone Iodine 10%	1 and 3	0.002	0.002	
	3 and 7	0.003		
	1 and 7	0.008		
Aqudest	1 and 3	0.002	0.003	
	3 and 7	0.003		
	1 and 7	0.001		

Based on the Mann-Withney unpaired test, inflammation was measured using the Gingival Index in rats with periodontitis which could be observed to have a p value <0.05 so it can be concluded that there was a significant difference between days 1 and 3, days 3 and 7 and days 1 and 7 in each of the three different treatment groups.

For the Kruskal-Wallis test to compare the three variables or groups in each treatment day it has a p value <0.05 so that there is also a significant difference between treatment days in the honeycomb gel (trigona sp) group, povidone iodine treatment days and in aquades treatment days. Meanwhile, to compare the 3 treatments, the value of p> 0.05 was obtained so that there was a change but there was no significant difference from the treatment of the three groups.

Table 2. Treatment tests on days 1, 3 and 7 of the 10% honeycomb gel group, the povidone iodine group and the distilled water group (periodontal pocket)

Treatment	Day To-	Mann-Withney	Kruskall-Wallis	
		Sig.	Sig.	Sig
Honeycomb gel (Trigona Sp) 10%	1 and 3	0.001	0.009	0.379
	3 and 7	0.008		
	1 and 7	0.006		
Povidone Iodine 10%	1 and 3	0.004	0.001	
	3 and 7	0.002		
	1 and 7	0.008		
Aqudest	1 and 3	0.001	0.001	
	3 and 7	0.001		
	1 and 7	0.007		

Based on the unpaired Mann Withney test, periodontal pockets measured using a periodontal probe in rats with periodontitis obtained a p value <0.05 so that there was a significant difference between treatment days 1 and 3, on days 3 and 7 and on days 1 and 7 on each -each three different treatments.

In the unpaired Kruskal-Wallis test in each of the 3 groups, a p value <0.05 was obtained so that on each treatment day in the honeycomb gel (trigona sp) group, on the povidone iodine treatment day and on the aquades treatment day there was a significant difference. Meanwhile, to compare all 3 treatment groups, a p> 0.05 value was obtained so that there was a change but there was no significant difference between the three groups.

The active ingredient contained in the trigona sp beehive is propolis. Propolis consists of ingredients namely flavonoids, phenols, aromatic compounds, amino acids, minerals, vitamins A, E and B complex. Nearly 50% of propolis is a flavonoid compound consisting of acetin, quercetin, naringenin and galangin. Based on the components of these compounds, honeycomb extract can be used as an anti-inflammatory. The mechanism of action of honeycomb extract (Trigona Sp) as an anti-inflammatory is by reducing the number of inflammatory mediators, namely macrophages.¹³ Another content of propolis, namely apigenin and tt-farnesol, causes a decrease in the amount of polysaccharides in the biofilm of microorganisms. Apigenin and tt-farnesol will affect one of the polysaccharides in the biofilm which functions for attachment to the cell surface, thereby stopping the formation of biofilms.¹⁴

➤ *Effectiveness of honeycomb gel (Trigona Sp) to reduce gingival inflammation in periodontitis*

Based on a paired test to see changes in the condition of gingival inflammation every day using the Mann-Withney test showed that 10% honeycomb gel (trigona sp) obtained values on days 1 and 3 (p=0.009), on days 3 and 7 (p=0.018) and on days 1 and 7 (p=0.008), the value (p<0.05) which means that the administration of 10% beehive gel (trigona sp) had a significant difference and was effective in reducing gingival inflammation in Wistar rats with periodontitis. Then, the Kruskal-Wallis test was carried out to compare each treatment group. 10% honeycomb gel (trigona sp) had a value (p=0.001), the positive control group in the form of povidone iodine had a value (p=0.002) and the negative control group in the form of distilled water had a value (p=0.003) where the value (p<0.0).

Honeycomb gel (trigona Sp) as an alternative material that is safe, non-toxic and effective. The active ingredient contained in beehives (trigona sp) is propolis which acts as a pharmacological agent, namely flavanoids, phenolic acids and their esters, these parts effectively affect microorganisms, parasites and infections. Reaction as an anti-inflammatory and antioxidant from honeycomb (trigona sp) through inhibition of NF kβ activation and downregulation of pro-inflammatory enzymes, cyclooxygenase-2 by reducing inflammatory mediators produced through the arachidonic acid pathway, reducing swelling of blood vessels in connective tissue,

encouraging the migration of various cells including fibroblasts in the wound area.¹⁵ Honeycomb (Trigona Sp) has the ability as a mitigation, immunomodulatory, and antitumor exercise and can be used in treating infections, the mechanism of propolis' antimicrobial activity is based on inhibiting the motility and activity of different bacterial enzymes and weakening the stability of the cytoplasmic membrane.¹⁶

In line with this, a study conducted by Nila Kusuma also showed that the content of beehives in the form of propolis is effective in reducing inflammation levels thereby curing mild gingivitis, other ingredients of propolis, especially B-complex vitamins, will help absorb minerals the body needs such as iron, zinc, copper, chrome silizium, vanadium, manganese, and sixteen essential amino acids needed for cell regeneration.¹⁷

➤ *Effectiveness of honeycomb gel (Trigona Sp) on changes in periodontal pocket depth in periodontitis*

Based on the mann-withney unpaired test to see changes in the state of the periodontal pocket using 10% honeycomb gel (trigona sp) obtained values on days 1 and 3 (p=0.001), days 3 and 7 (p=0.008), days 1 and 7 (0.006). The value (p<0.05) indicated that there was a significant difference and the administration of honeycomb gel (trigona sp) was effective in changing the periodontal pocket in a better direction.

Then an unpaired Kruskal-Wallis test was performed to compare the effect of the three treatments on changes in the depth of the periodontal pocket. Honeycomb gel (trigona sp) had a value (p=0.009), the povidone iodine and distilled water groups had the same value (p=0.001). Value (p <0.05) so that this means that the three treatments have an influence on changes in the periodontal pocket of Wistar rats who experience periodontitis.

The decrease in pocket depth can be caused by the work of the honeycomb content in the form of propolis which directly eliminates the microbes in the periodontal pocket. So that one of the key factors in periodontal therapy is to first eliminate the causative factor, namely periodontopathogenic bacteria. In accordance with the results of studies from various researchers who stated that the main characteristic of propolis is its ability as an antimicrobial agent.¹⁸ This is in line with research conducted by Hetty Noveilga. The results of this study show that propolis gel can inhibit the growth of the Porhpyromonas gingivalis bacteria. The content of flavonoids in propolis has antibacterial activity through inhibiting the function of bacterial DNA so that the ability of bacterial replication and translation is inhibited. The biological activity of the flavonoid compounds in propolis against bacteria is carried out by damaging the cytoplasmic membrane of the bacteria.¹⁹

The changes that occur in the transition from a normal gingival sulcus to a periodontal pocket are related to differences in the number of bacterial cells in dental plaque. The periodontal ligament connects the tooth to the jawbone and also supports the tooth in its socket and absorbs the loads applied to the tooth. Periodontal disease causes loss of

attachment of the periodontal tissues and cementum exposed to the oral environment. Periodontal tissue damage was significantly mediated by MMP levels. TIMP regulates MMP by inhibiting proteinase enzymes and blocking MMP activation autocatalytically.²⁰In line with another study, a study by AE Zaenal Hasan, the results showed that there was antibacterial activity of honeycomb extract (*trigona* sp) containing propolis on the growth of *Salmonella* sp. The group of compounds identified in the honeycomb are flavonoids and tannins, indicating that these compounds are responsible for the antibacterial activity.²¹

IV. CONCLUSIONS AND SUGGESTIONS

Administration of 10% beehive gel (*trigona* sp) proved to be effective and had an effect on changes in clinical conditions in periodontitis in Wistar rats, with changes in gingival inflammation and changes in the depth of the periodontal pocket towards the healing process.

- Administration of honeycomb gel (*trigona* sp) 10% proved effective against inflammatory changes in periodontitis conditions in Wistar rats. The mechanism of action of honeycomb gel (*trigona* sp) reduces inflammatory mediators, namely macrophages.
- Administration of honeycomb gel (*trigona* sp) 10% proved to be effective and had an effect on changes in periodontal pocket depth in periodontitis conditions in Wistar rats. The transition from a normal gingival sulcus to a periodontal pocket is related to differences in the number of bacterial cells.

Suggestions for future research are to conduct a histopathophysiological test to find out more about the pathophysiology related to healing and indicators of inflammation, and it is expected to carry out an organoleptic test on honeycomb gel (*trigona* sp) which includes taste test, color test, odor test, texture test, test pH and viscosity test.

REFERENCES

- [1]. WHO. Oral health : achieving better oral health as part of the universal health coverage and non-communicable disease agendas towards 2030. Who [Internet]. 2020;(December):1–6. Available from: <https://www.who.int/news-room/fact-sheets/detail/oral-health>
- [2]. Republic of Indonesia Ministry of Health. Basic Health Research Results 2018. Indonesian Ministry of Health. 2018;53(9):1689–99.
- [3]. A'yun Q, Fatmasari D, Hendrartini J. Pediatric Caries Predictor Software Based on Child Factors, Mother's Behavior, and UKGS. *Maj. Dentistry Indonesia*. 2015;1(1):68.
- [4]. Dwipriastuti D, Putranto RR, ANGKAI W. Differences in the Effectiveness of 0.2% Chlorhexidine Gluconate with Green Tea. *ODONTO Dent J*. 2017;4:50–4.
- [5]. Tamara A, Oktiani BW, Taufiqurrahman I. Effect of kelulut propolis flavonoid extract (*G.thoracica*) on the number of neutrophil cells in periodontitis (in vivo study on male wistar rats (*rattus norvegicus*). *Dentine*. 2019;III(1):10–6.
- [6]. Wachidah, Nurlailatul R. Effect of Forest Bee (*Apis dorsata*) Honey Solution Concentration on Growth Inhibition of *Porphyromonas gingivalis* Dominant Gingivitis (in vitro study). 2016.
- [7]. Murtikasari M. The Effect of 10% Propolis Gel and Near Infrared Phototherapy on Post Curettage Wound Healing (Study on sparague dawley rats in terms of collagen fiber density). Semarang: Sultan Agung University; 2021. 6 p.m.
- [8]. Novitasari AIM, Indraswary R, Pratiwi R. Effect of Application of 10% Duck Eggshell Membrane Extract Gel on Density of Collagen Fibers in Gingival Wound Healing Process. *ODONTO Dent J*. 2017;4(1):13.
- [9]. Jadhav MS, Jadhav MS, Agrawal YS, Goyal SM. A Review Article On Topical Gel For The Treatment Of Skin Fungal Infection. 2019;06(05):10117–29.
- [10]. Kurniawan H, Widyastuti W, Hutapea ME. The effectiveness of the combination of *Moringa oleifera* extract and propolis on *Porphyromonas gingivalis* biofilms compared to 0.7% tetracycline. *Dent J (Dentistry Magazine)*. 2021;54(2):63.
- [11]. Nugroho RA. Recognizing Mice as Laboratory Animals. Samarinda: Mulawarman University Press; 2018.
- [12]. Notoatmojo S. Health Research Methodology Third Edition. Jakarta: Rineka Pustaka; 2005.
- [13]. Saidah M, Oktiani BW, Taufiqurrahman I. The Effect Of Kelulut Propolis Flavonoids (*Trigona* spp) Extract On Macrophage Cell Number In Periodontitis (In Vivo Study In Male Wistar Rate (*Rattus norvegicus*) Gingiva). *Dentino J Dentist*. 2020;5(1):28.
- [14]. Asdar, Cindrakori HN. Inhibitory Power of Propolis Gel from South Sulawesi on the Growth of *Porphyromonas Gingivalis* Bacteria. *J B-Dent*. 2015;2(2):101–9.
- [15]. Aidina LN. The Effect of Gel Administration on Fibroblast Cell Count in Male Wistar Rats Induced by Periodontitis. 2019.
- [16]. Okińczyc P, Paluch E, Franiczek R, Widelski J, Wojtanowski KK, Mroczek T, et al. Antimicrobial activity of *Apis mellifera* L. and *Trigona* sp. propolis from Nepal and its phytochemical analysis. *Biomed Pharmacother*. 2020;129(June).
- [17]. Kasuma N. Propolis Toothpaste as The Initial Therapy of Mild Gingivitis. *J Science Farm Clinic [Internet]*. 2016;1(1):89–94. Available from: <http://jsfkonline.org/index.php/jsfk/article/view/16>
- [18]. Asdar. Effects of Application of Trigona Propolis Gel as Adjunctive Therapy in Periodontal Pockets Review of Clinical Effects d Matrix and Metalloproteinase-8 Levels. Makassar Hasanuddin University; 2017.
- [19]. Cindrakori HN. The Effectiveness of Propolis Trigona sp Extract on the Growth of *Porphyromonas Bacteriagingivalis*. Hasanuddin University. 2017;
- [20]. Abdurrohman MMS, Putranto RR. Metronidazole Gel Effect on Rats With Bacteria-Induced Periodontitis. *ODONTO Dent J*. 2020;7(1):48.
- [21]. Hasan A, Artika I, Fatoni A, Haryanto B. Antibacterial Activity Of Propolis Trigona Spp. From Bukittinggi West Sumatra Against *Salmonella* Sp. 2019;55–9.