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Anti –Inflammatory Activity of Propolis *Trigona sp*. Water Extract from North Lombok with Red Blood Cell Membrane Stability Method

Ani Fatin Humaira[1], Siti Rahmatul Aini[2,*], Raisya Hasina[2]
[1]Departement of Pharmacy, Faculty of Medicine and Health Science, University of Mataram, Indonesia.
[2]Departement of Pharmacist Professional Education, Faculty of Medicine and Health Science, University of Mataram, Indonesia.

Corresponding author*
sitira@unram.ac.id

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Abstract

Propolis is a bee product that can be used as an anti-inflammatory. Cultivation of propolis-producing bees is also carried out in North Lombok. However, propolis has not been utilized optimally by the people of North Lombok. Apart from that, testing of North Lombok propolis is still limited to the chemical content and antioxidant activity of propolis extracted with ethanol solvent. Therefore, this study aims to determine the activity and effective concentration of North Lombok propolis water extract as an anti-inflammatory using the red blood cell membrane stability method. Groups include control groups such as positive control (diclofenac sodium), negative control (distilled water), and test groups (propolis water extract concentrations of 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, 27.5%, and 30%). In The first step human blood was centrifuged of human blood, and then part of the red blood cells (sediment) was taken and saline was added to obtain a red blood cell suspension. Next, mixing the test solution was carried out in the order of 0.5 ml of red blood cell suspension, then 0.5 ml of extract was added (0.1% w/v Na-diclofenac solution in the positive control, distilled water in the negative control), 1 mL of buffer solution and 2 mL of hyposaline solution, then homogenized. Each group was incubated at 37°C for 30 minutes, then centrifuged for 10 minutes at 3000 rpm. The supernatant was taken, and the absorbance was read with a UV-Vis spectrophotometer at 560 nm. Next, the percentage value of red blood cell hemolysis protection was calculated. The data obtained were tested statistically using One-way ANOVA and post-hoc (LSD) tests SPSS version 29. The results showed that propolis water extract concentrations were 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, 27.5%, and 30% have anti-inflammatory activity because they can increase the stability of the red blood cell membrane with a percentage of hemolysis protection of respectively 57.92%, 59.99%, 60.99%, 61.99%, 64.31%, 69.59%, 75.07%, 79.77% and 84.45%. Propolis water extract concentrations of 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, and 25% had anti-inflammatory effects that were not significantly different from the positive control ($p>0.05$). The 27.5 % and 30% concentrations had a higher percent hemolysis protection value than the positive control ($p<0.05$).

Keywords: anti-inflammatory; propolis; percent hemolysis protection.

INTRODUCTION

Propolis is a bee product that can be used as an anti-inflammatory. People consume propolis to maintain body stamina and relieve sore throats and canker sores (Siregar et al., 2011). Propolis is also used as a mouthwash to cure gingivitis and periapical abscesses (Suranto, 2010). There are many other empirical uses of propolis, such as healing burns, aches, typhus, and diabetes. So far, various propolis products are available on the market, including raw propolis, liquid, powder (capsules), spray, and others (Siregar et al., 2011).

Cultivation of honey bees Trigona sp. is also carried out in North Lombok. Products produced by Trigona sp. include honey, propolis, and bee bread. However, the only bee product utilized optimally by the people of North Lombok is honey, while propolis and bee bread have not been utilized optimally. Djajasaputra (2010) stated that *Trigona sp* bees produce more propolis than honey yearly (Djajasaputra, 2010). Propolis also has a high economic value, reaching IDR 400,000.00/kg, and honey is worth IDR 250,000.00/litre (Askary et al., 2022). Research related to North Lombok propolis is still limited to its chemical content and antioxidant activity (Zahra et al., 2021). Carreño et al. (2017) showed that the compounds in Sonoran propolis extract protect against inflammation caused by oxidative stress (Carreño et al., 2017). Next, it is necessary to test the anti-inflammatory activity to increase the selling power of North Lombok propolis.

Based on WHO data (2018) shows that 73% of deaths are caused by non-communicable diseases involving inflammation, including cardiovascular disease (heart and blood vessels) 35%, cancer 12%, chronic respiratory disease (neurodegenerative) 6%, diabetes mellitus 6 %, and 15% are caused by other non-communicable diseases (Ministry of Health, 2019). Pharmacologically, inflammation can be treated using anti-inflammatory

steroids (AIS) and non-steroidal anti-inflammatory (NSAID) drugs. However, both classes of drugs have side effects such as peptic ulcers, decreased immunity, osteoporosis, hypertension, congestive heart failure, and oedema. Apart from synthetic drugs, the use of traditional medicines as an alternative treatment in society is increasing. Natural medicines are considered easy to obtain, safer, and have fewer side effects than conventional medicines.

Therefore, this study tested the anti-inflammatory activity of propolis water extract from North Lombok with the red blood cell membrane stability method. The red blood cell membrane stability method is used because it is an analogue of the lysosomal membrane, which plays a role in the inflammatory process. Thus, it can represent the mechanism of action of CAPE (Caffeic Acid Phenethyl Ester) compounds and flavonoids as anti-inflammatory agents, namely inhibiting the formation of inflammatory mediators by COX-2 due to damage to the lysosomal membrane. (Kumar et al., 2020).

MATERIALS AND METHODS

Tools and Materials

The tools used are laboratory glassware, stir sticks, test tube racks, spatulas, analytical balances, centrifuges, UV-Vis spectrophotometers, EDTA tubes, hot plates, and thermometers.

The ingredients used in this study were distilled water, propolis, the human's venous blood, disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), sodium dihydrogen phosphate monohydrate ($NaH_2PO_4.H_2O$), infusion fluid (0.9% NaCl), and diclofenac sodium.

Procedures

Preparation of phosphate buffer solution (pH 7.4)

13.35 grams of $Na_2HPO_4.2H_2O$ was dissolved in distilled water to 500 mL (0.15 M). 2.070 grams of $NaH_2PO_4.H_2O$ was dissolved in distilled water to 100 mL (0.15 M). At room temperature, 405 mL of $Na_2HPO_4.2H_2O$ solution (0.15 M) was mixed with 95 mL of $NaH_2PO_4.H_2O$ solution (0.15 M).

Preparation of 0.25% (w/v) hyposaline solution

27.7 mL of isosaline solution was diluted using a phosphate buffer pH 7.4 to a volume of 100 mL.

Preparation of red blood cell suspension 10% (v/v)

1 mL of red blood cells was added to isosaline solution until the volume was 10 mL, and then homogenized.

Membrane stability test by hypotonicity induction

The mixing of the test solution was carried out in the order of 0.5 ml of red blood cell suspension, then 0.5 ml of extract (Na-diclofenac solution 0.1% w/v in the positive control, aquates in the negative control), 1 mL of buffer solution and 2 mL of hyposaline solution, then the mixture was homogenized. Next, the solution mixture was incubated for 30 minutes at 370C, then centrifuged for 10 minutes at 3000 rpm. The absorbance of the supernatant was measured at a wavelength of 560 nm.

From the positive control, negative control and each test sample absorbance values, the percentage (%) of red blood cell hemolysis protection was calculated using the following formula (Umeti et al., 2019):

Percentage of hemolysis protection =

$$100 - [(\frac{A2}{A1}) \times 100]$$

Where:

A1 : Absorbance of negative control solution

A2 : Absorbance of the test solution/positive control

Data analysis

The percentage value of red blood cell hemolysis protection was then analyzed using the SPSS version 29 application to determine differences in the ability of each group to protect red blood cell hemolysis. Next, the data was analyzed using the One-way ANOVA and post-hoc tests (LSD).

RESULTS AND DISCUSSION

Propolis water extract was tested for anti-inflammatory activity in vitro to protect red blood cell hemolysis. Red blood cells are used because their membrane is similar to the lysosomal membrane, influencing the inflammatory process. In the process of inflammation, the stability of the lysosomal membrane limits the inflammatory response by preventing the release of enzymes from the lysosomes, which is caused by neutrophil activation. The release of enzymes from lysosomes can cause disorders such as inflammation of tissues and extracellular fluids (Kumar et al., 2020). Therefore, the stability of the lysosomal membrane against these disorders can be seen from the stability of the red blood cell membrane. The mechanism of red blood cell stability can be seen from its ability to protect hemolysis, which can cause the release of haemoglobin from red blood cells when given hypotonic stress (Hillman et al., 2011).

In this study, the anti-inflammatory activity of propolis water extract can be seen from its ability to maintain red blood cell stability, namely the ability to prevent or protect the hemolysis of red blood cells given a 0.1% hypotonic solution. Hemolysis of red blood cells is proportional to the amount of haemoglobin, which is read at 560 nm using UV-vis spectrophotometry in the form of absorbance values. The higher the absorbance, the higher the hemolysis of red blood cells and the anti-inflammatory activity of a test sample. The percentage

value of red blood cell hemolysis protection describes anti-inflammatory activity. The percentage value of red blood cell hemolysis protection obtained can be seen in Table 1.

Table 1. Percentage of Red Blood Cell Hemolysis Protection.

Group treatment		% Hemolysis Protection			Mean % Hemolysis Protection ± SD
		1	2	3	
Control	Negative	0	0	0	0 ± 0,00
	Positive	62,73	66,47	69,32	66,17 ± 3,30
Extract	10%	49,71	65,65	58,41	57,92 ± 7,98
	12.5%	52,40	67,55	60,02	59,99 ± 7,57
	15%	53,30	68,32	61,36	60,99 ± 7,52
	17.5%	53,60	68,53	63,84	61,99 ± 7,64
	20%	57,35	70,12	65,45	64,31 ± 6,46
	22.5%	64,73	75,13	68,91	69,59 ± 5,23
	25%	75,15	76,18	73,88	75,07 ± 1,15
	27.5%	79,58	79,19	80,53	79,77 ± 0,69
	30%	85,61	84,55	83,19	84,45 ± 1,21

Discussion

Table 1 shows the results of the anti-inflammatory activity test of propolis North Lombok using the human red blood cell membrane stability method. These results show that all variations in concentration (10%, 12.5%, 15%, 17.5%, 20%, 22.5%, 25%, 27.5% and 30%) of propolis North Lombok influence the stability of red blood cell membranes. By administering propolis water extract to the red blood cell suspension (SSDM), the red blood cell membrane was more stable compared to administering distilled water to the negative control, so it can be said that propolis North Lombok was able to protect red blood cell hemolysis when given a 0.1% hypotonic solution. 30% propolis North Lombok has the highest percent (%) hemolysis protection value, namely 84.45% ± 1.21%, so it is the maximum concentration based on the % hemolysis protection value. This value is higher than the percent hemolysis protection of diclofenac sodium as a positive control. These results were in line with Mendes-Encinas et al. (2023) (Mendez-Encinas et al., 2023).

The percentage protection from hemolysis of red blood cells in all treatments was then carried out with statistical analysis using SPSS version 29. Therefore, parametric tests were carried out, namely One-way ANOVA and post-hoc (LSD) tests. The results of the One-way ANOVA test were that all treatments were significantly different ($p<0.05$). The differences between test groups can be seen in the post-hoc data (LSD); the concentrations of propolis North Lombok 10%, 12.5%, 15%, 17.5%, 20%, 22.5% and 25% were not significantly different or the same as the positive control ($p> 0.05$). In comparison, propolis North Lombok concentrations of 27.5% and 30% were significantly different from the positive control ($p<0.05$). From these results, it can be confirmed that propolis North Lombok concentrations of 10%, 12.5%, 15%, 17.5%, 20%, 22.5% and 25% have the same anti-inflammatory activity as diclofenac sodium.

The anti-inflammatory activity of propolis is influenced by the compounds contained in it. The compounds that act as anti-inflammatory agents in propolis are Caffeic Acid Phenetyl Ester (CAPE) and flavonoids. Several studies explain that the mechanism of propolis (CAPE and flavonoids) as an anti-inflammatory is by inhibiting the enzymes LOX (lipooxygenase) and COX (cyclooxygenase) during the inflammatory process. Lipooxygenase is the main enzyme in neutrophils, which will later produce leukotrine compounds, while cyclooxygenase produces prostaglandins, which will become mediators in inflammatory reactions. Inhibition of the lipoxygenase and cyclooxygenase pathways will reduce vasodilation of blood vessels, and blood flow will be reduced so that migration of leukocytes (PMN) to areas of inflammation also decreases (Bankova et al., 2014). Research related to propolis as an anti-inflammatory was also carried out in vivo on carrageenan-induced white mice; it was found that the 95% ethanol extract of Mayan Propolis showed 9% inhibition of oedema in the feet and ears by 22% during the first 2 hours at a dose of 50 mg/kg (Xool-Tamayo et al., 2020).

CONCLUSIONS

Based on the results of in vitro anti-inflammatory activity tests, it can be concluded that:

- Propolis water extract has anti-inflammatory activity because it can increase the stability of red blood cell membranes.
- The concentrations of propolis water extract that effective as an anti-inflammatory are 10%, 12.5%, 15%, 17.5%, 20%, 22.5%, and 25%.

Competing Interests: The authors claim that there are no conflicts of interests.

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