



Original article

In-vitro antioxidant, in-vivo anti-inflammatory, and acute toxicity study of Indonesian propolis capsule from *Tetragonula sapiens*



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ABSTRACT

Propolis is widely used as traditional medicine since ancient times. It was necessary to conduct the pre-clinical study because of its relevant curative properties. This study aimed to investigate in-vitro antioxidant, standardize quality parameters, study acute toxicity, and determine in-vivo anti-inflammatory. Three spectrophotometric methods were used to determine antioxidant activity. The standardization includes physical, chemical, and microbiological evaluation. Furthermore, an acute toxicity test was conducted using 20 female Sprague Dawley (SD) strain rats divided into 4 groups with different dose of propolis. The in vivo anti-inflammatory test was carried out using the carrageenan induction method on rats' soles. A total of 36 female SD rats were classified into 6 groups as follows, Group normal, negative control, diclofenac sodium, and three propolis groups (72; 144; and 288 mg/kg BW). The results demonstrated the IC₅₀ values of the DPPH and ABTS scavenging activity 9.694 ppm and 2.213 ppm, respectively. The FRAP reducing power was 189.05 mg AaE/g. The physical appearance of propolis capsule was vegi-caps as white – white, size 0, with light brown granule. Moreover, the content weight was 418.88 mg with a disintegration time of 7 min 53 s, while the water, flavonoid, and polyphenol contents were 9.07%, 1.59%, and 98.0821 mg GAE/g respectively. The content of heavy metal and microbial contamination were not detected. The acute toxicity results showed LD₅₀ ≥ 5 g/kg BW, no toxicity symptoms, and no abnormalities in all rats. The anti-inflammatory inhibition percentage for groups III, IV, V, and VI was 11.86%, 6.53%, 7.81%, and 6.63% respectively, while the anti-inflammatory drugs effectiveness percentage compared to positive controls were 55.00%, 65.83%, and 55.83% respectively. Based on these results, it can be concluded that propolis capsules fulfilled the standardization requirements, and it is likely to be non-toxic, and effective as antioxidant and anti-inflammatory.

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1. Introduction

Propolis is a complex resin mixture collected by honey bees from various gums from plants, which is enriched by saliva and enzyme secretions and used for the construction and protection of beehives (Hasan et al., 2014). The composition of propolis from various types of bees varies depending on the type of plant resin available and the substances synthesized and secreted by the bees.

Propolis is widely used as traditional medicine since ancient times used to treat many diseases. The biological activity of

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propolis includes its antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory, and anticancer properties, among others (Al Faris et al., 2020; Alqarni et al., 2019; Diva et al., 2019; Farida et al., 2019; Flamandita et al., 2020; Iqbal et al., 2019; Pratami et al., 2020a, 2018; Sahlan et al., 2021a, 2021b; Soekanto et al., 2019). Propolis has various benefits, but its therapeutic applications are limited because of the variability of its chemical composition and various weaknesses, such as low solubility in water. Its taste is bitter, and it has a strong smell. Microencapsulated propolis enhances the physical characteristic of propolis (Pratami et al., 2020b). Propolis contains >108 active compounds, some of which are antioxidants from phenolic compounds, such as polyphenol and flavonoids (Segueni et al., 2016). Flavonoids can capture free radicals and inhibit lipid peroxidation (Banjarnahor and Artanti, 2014; Trembl and Šmejkal, 2016), and the biological activity of propolis is often associated with the presence of flavonoids. Several types of flavonoids are known to have various effects on health (Valenzuela-Barra et al., 2015; Wang et al., 2016). Therefore, flavonoid content can be used as a parameter or index to evaluate the quality of the propolis used. Phytochemical analysis of extracts of 40 active compounds in propolis from different regions of origin revealed the presence of general constituents such as phenols, tannins, and flavonoids (Bankova et al., 2018). These antioxidant compounds can be used to neutralize free radicals. In the previous research, the chemical component identified Indonesian propolis by UPLC-TOF-MS using the MSE mode were (-)-Sesamin, Curcumin, 8-epiHelenalin, and Kushenol F (Pratami et al., 2018).

In this study, the propolis extracts were dried using a spray-drying technique to preserve its medicinal properties. The propolis extract had been tested as an anti-inflammatory by reducing the TNF-Alpha and INOS concentration in vitro (Sahlan et al., 2021b). Whereas, in vivo showed the ability to reduce inflammation in Sprague Dawley induced by carrageenan (Sahlan et al., 2019a). Furthermore, propolis was reported to have an immunomodulatory effect (Afif et al., 2021; Al-Hariri, 2019). Besides, it increases the phagocyte index, and also significantly increase the NO and the IgG antibody production (Kalsum et al., 2017). Caffeic acid phenethyl ester (CAPE), as one of the important propolis's components, has been reported to play a role in reducing RAC signaling protein activity in human cells, hence, it is useful for inhibiting or eliminating pulmonary fibrosis (Bachevski et al., 2020).

The product's activity as an anti-inflammatory can be utilized with in case an increase the levels of proinflammatory cytokines (IL-6, IL-10, and TNF- α) (Alqarni et al., 2019; Sahlan et al., 2021b). The use of anti-inflammatory agents can be an alternative therapy to avoid an increase in cytokine levels (cytokine storm) for the treatment of certain diseases like COVID-19. The compounds can effectively provide a stronger immune system and suppress inflammation symptoms. Whereas, those found in Indonesian propolis can potentially become standardized herbal medicines (SHM) for anti-inflammatory. However, in the development of this medicine, it is necessary to conduct preclinical studies and standardize the parameter quality according to traditional medicine requirements. Quality test parameters include organoleptic, water content, disintegration/disintegration time, dissolution, and weight uniformity. Meanwhile, tests were conducted for safety standards, microbial contamination, heavy metals, and medicinal chemicals. Identification and the determination of active ingredients levels were carried out to maintain the quality by testing the total phenolic and flavonoid content. Moreover, in support of the SHM data, acute toxicity and preclinical tests of anti-inflammatory activity were conducted in experimental animals.

2. Materials and methods

2.1. Sample

Propolis capsule powder was sourced from the bee *Tetragonula sapiens* in South Sulawesi, Indonesia. Also, Spray Drying Extract (SDE) powder was obtained from propolis ethanol extract which was microencapsulated through maltodextrin and gum arabic using a spray drying device produced by the Phytochemindo Reksa Company (Bogor, West Java, Indonesia). Meanwhile, Batch Number T100920 refers to the procedure carried out by Pratami et al. (2020) (Pratami et al., 2020b).

2.2. Chemical

1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ), sodium acetate trihydrate, Fe (III) chloride hexahydrate (FeCl₃), gallic acid, sodium carbonate (Na₂CO₃), Folin-Ciocalteu, methanol, ethanol, ascorbic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), quercetin, aluminum chloride (AlCl₃), and potassium acetate (CH₃COOK) were purchased from Sigma-Aldrich (Merck KGaA, Missouri, United States). Sodium agar, potato dextrose agar, soybean casein digest broth, macconkey broth, rappaport vasiladis salmonella enrichment broth, xylose lysine deoxycholate agar, salmonella enrichment broth, salmonella shigella agar, imvic media, and sulfid indole motility, triple sugar iron agar (TSIA) to determined the microbial contamination were purchased from QLab Laboratory, Faculty of Pharmacy, Pancasila University (Jakarta, Indonesia) Rat feed, refillable drinking water, 10% BNF, ketamine or xylazine (Sedative), 10% PBS solution, hematoxylin eosin dye, diclofenac sodium, carrageenan 1%, Na CMC 0.5%, and Aqua were purchased from Biofarmaka Laboratory, IPB University (Bogor, West Java, Indonesia).

2.3. Tool

The tools were animal cage, micropipette, microcentrifuge, test animal stomach sonde, 1.5 mL disposable plastic tube, water bath, vortex, centrifuge, 5 mL test tube, microcentrifuge tube, and 20 μ L, 100 μ L, 1000 μ L of Eppendorf pipettes. Moreover, yellow and blue Eppendorf tips, hematoanalyzer, injection syringe (one med plus needle), pletismometer, weight scale, sonde (probe needle), stopwatch, mortar pestle, and Karl-Fischer (870 KF TITRINO Plus Methorm) were used. Others are, disintegration tester BJ-2, analytical balance (AND type HR-120), petri dish (IWAKI), ose needle (USBECK), incubator (memmert), autoclave (Hirayama), Laminar Air Flow (LAF), Atomic Absorption Spectrophotometer (Shimadzu AA-7000), and UV Vis Spectrophotometer.

2.4. Antioxidant activity

Three simple spectrophotometric methods: (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging activity assay (DPPH), [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] free radical scavenging activity assay ABTS) and Ferric ion Reducing Antioxidant Power assay (FRAP) for determination of antioxidant activity were carried out. The antioxidant activity determination using method described by Djamil et al. (2021) with slight modification (Djamil et al., 2021).

Antioxidant activity test used DPPH free radicals methods by making 125 μ M DPPH stock in methnaol, propolis and ascorbic acid preparation then the procedure of propolis and ascorbic acid with DPPH. The measurement of inhibition used a spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the sample is determined by the amount of DPPH radical uptake

resistance through the calculation of the percentage of DPPH absorption inhibition with the following formula Equation (1):

$$\%DPPH = \left[\frac{A_{\text{blanko}} - A_{\text{sample}}}{A_{\text{blanko}}} \right] \times 100\% \quad (1)$$

The antioxidant activity was determined using the ABTS method by dissolving the propolis sample into ethanol p.a. and diluting it to obtain the appropriate concentration for analysis. A total of 1 mL of ABTS solution was mixed with each sample solution, homogenized, and incubated for 6 min. The absorbance was measured at 670.5 nm. The inhibition concentration (IC₅₀) value is the concentration of antioxidants (ppm) capable of inhibiting 50% of free radical activity. The percentage of inhibition was calculated using the standard calibration curve at various concentrations with the following formula Equation (2):

$$\%ABTS = \left[\frac{A_{\text{blanko}} - A_{\text{sample}}}{A_{\text{blanko}}} \right] \times 100\% \quad (2)$$

The antioxidant activity was determined using the FRAP method by dissolving 3 mg of propolis powder with ethanol absolute and then increasing the volume to 10.0 mL to create a mother liquor. The solution was prepared at concentrations of 150, 100, and 50 mg/L by taking 100 µL of each concentration and adding 300 µL of distilled water and FRAP solution to a volume of 3.0 mL in each solution. Subsequently, each solution was supplemented with 10.0 mL of ethanol absolute. The solution was allowed to stand for 25 min at room temperature. Absorption was measured using a UV-Vis spectrophotometer at 593 nm. Measurements were carried out three times. Ascorbic acid was used as a standard, and FRAP activity was expressed as mg AAE/g of sample; increasing absorbance of the sample indicated greater ferric reducing antioxidant power.

2.5. Standardization of *Tetragonula sapiens* propolis capsule

Product standardization was in accordance with the Regulation of National Food and Drug Agency of Republic Indonesia Number 32, 2019 concerning the Safety and Quality Requirements of Traditional Medicines (BPOM RI, 2019). The parameters tested were organoleptic, water content, disintegration time, weight uniformity, determination of total flavonoid and phenolic contents, as well as heavy metal (Pb, Cd, Hg, As) and microbial contamination (ALT, AKK, *E. coli*, *Salmonella* sp, *Shigella* sp).

2.5.1. Organoleptic test

The examination included color, smell, and taste.

2.5.2. Water content test

This was examined by titration method using Karl Fischer reagent (iodine and sulfur dioxide). Approximately 3–5 mg of powder (B1) was weighed, and kept in the tool, while the leftover (B2) was reweighed. Furthermore, both B1 and B2 data were entered and titrated for approximately 3–5 min. The water content which can be automatically read by the tool had conditions < 10.0% (BPOM RI, 2019).

2.5.3. Disintegration time test

The disintegration time test was conducted by placing six capsules in a basket, subsequently raising and lowering them regularly (30 times per minute). A water medium with a temperature of 37 ± 2 °C was used. Observation of capsules was carried out, as they were all crushed without the shell part. Except when stated otherwise, the time required to crush all six capsules should not exceed 15 min (BPOM RI, 2019).

2.5.4. Weight uniformity test

This test was conducted with twenty capsules being weighed individually. The contents were removed while the entire shell was weighed, subsequently, the contents weight and the average weight of each were calculated. The difference in the percentage weight of each content to the average weight should not exceed ±7.5% or ±15% for every 2 capsules (BPOM RI, 2019).

2.6. Determination of total flavonoid and polyphenol levels

The total flavonoid levels were calculated based on the standard calibration curve for quercetin comparison using the method from the Indonesian Herbal Pharmacopoei (Depkes, 2008). The sample was diluted with a 5% v/v solution of glacial acetic acid in methanol P to reach a suitable concentration for analysis. Also, the absorbance was measured at 427 nm using UV-Vis spectrophotometry. The total flavonoid content as a comparison flavonoid can be calculated with equation (3):

$$\% = \frac{C_p(A_u - A_{bu})}{(A_p - A_{bp})} \times 1,25 \times \frac{100}{\text{Sample Weight}} \quad (3)$$

% = Total flavonoid levels were calculated as comparison flavonoids as shown in the monograph

C_p = Concentration of comparison solution

A_u = Absorption of the test solution with an aluminum chloride solution

A_{bu} = Absorption of test solution without aluminum chloride solution

A_p = Absorption of comparison solution with an aluminum chloride solution

A_{bp} = Absorption of comparison solution without aluminum chloride solution

1,25 = Constant factor

Total polyphenol levels were calculated using the method from the Indonesian Herbal Pharmacopoeia based on the standard calibration curve for gallic acid comparison (Depkes, 2008). The sample was diluted with ethanol to achieve a concentration suitable for analysis. 0.5 mL sample solution was added with 0.4 mL of *Folin Ciocalteu* reagent, homogenized, and allowed to stand for 8 min, subsequently, 4 mL of 7.5% sodium carbonate was added and made up to 10 mL of distilled water. It was incubated for 85 min at room temperature.

The absorbance was measured by UV-Vis spectrophotometry at 758.5 nm. The total polyphenol content of the sample was calculated to obtain results that were declared equivalence to gallic acid, with the value of mg Galic Acid Equivalent/gram extract (mg GAE/g). Phenolic content was determined using equation (4) below:

$$\text{Polyphenol Level} = \frac{\text{extract concentration}}{\text{initial concentration}} \times FP \quad (4)$$

2.7. Heavy metal contamination test

The heavy metal content in the capsule preparations was analyzed according to the USP 42 method (The United States Pharmacopoeia Commission Inc., 2019) using Atomic Absorption Spectrophotometer (Shimadzu AA-7000) and an Inductively Coupled Plasma-Optical Emission Spectrometry ICP-OES (iCAP 7600) instrument. The determination of the heavy metals examined included the levels of Pb (Lead), Cd (Cadmium), As (Arsenic), Hg (Mercury) with the condition <10 mg/kg, 0.3 mg/kg, 5 mg/kg, 0.5 mg/kg respectively.

2.8. Microbial contamination test

Microbiological tests were conducted according to the USP 42 (The United States Pharmacopoeia Commission Inc., 2019) method, such as the Total Plate Count, Yeast and Mold Plate Count, *Escherichia coli*, Enterobacteriaceae parameters with the condition less than 10^5 CFU/g, 10^3 CFU/g, 10^5 CFU/g, 10^3 CFU/g respectively, and does not contain pathogenic bacteria of *Salmonella sp.*, *Clostridium sp.*, *Shigella sp.*

2.9. Selection and preparation of test animals

The test animals used were female white rats (Sprague Dawley) aged 8–12 weeks with a weight of 200–250 g. The healthy animals were obtained from the National Food and Drug Agency of Republic Indonesia and acclimatized in the experimental room for at least 5 days. Furthermore, they were grouped randomly with weight variations below 20% of the average body weight.

2.10. Feed and cage condition

The animals were kept in groups inside plastic boxes measuring $40 \times 32 \times 15$ cm lined with bedding made of wood shavings (2–3 animals/box). The room temperature was maintained at approximately $22^\circ \pm 3^\circ\text{C}$, with a relative humidity of 60–70%, as well as 12 h each for light and darkness. The boxes were cleaned 2 times per week. Meanwhile, standard feed from Indofeed and drinking water (refilled gallon bottle water) were provided indefinitely (ad libitum).

2.11. Ethical approval

Ethical approval was granted by the Tropical Biopharmaceutical Study Center, Institute for Research and Community Service (LPPM), IPB University (No. 007–2021 KEH TROP BRC). The anti-inflammatory research was conducted at the Experimental Animal Cage Unit lasted for 15 days (14 days of acclimatization and 1 testing day). The duration of the acute toxicity test was 20 days (5 days of acclimatization, 1 day of testing, and 14 days after taking a single dose of blood).

2.12. Acute toxicity test

The toxicity test was carried out in accordance with the 2014 Regulation of National Food and Drug Agency of Republic Indonesia Number 7, concerning Guidelines for In Vivo Non-Clinical Toxicity examination (BPOM RI, 2014). A total of 20 female SD strain rats were divided into 4 groups. The test animals were fasted for ± 16 h, weighed, and given the test preparation orally (feeding) with a gastric probe. The doses tested include 50, 300, 2000, and 5000 mg/kg body weight. Furthermore, observations were made for 24 h and continued for 14 days on, (a). Death and symptoms of toxicity, behavior, skin, fur, mucous membranes, eyes, respiration, circulation, nervous system, somatomotor activity, tremor, convulsions, salivation, diarrhea, lethargy, sleep, and coma, (b). Bodyweight before and during weekly treatment, (c). Pathological examination, such as macroscopic changes of organs necropsied at the end of the experiment.

2.13. In-vivo anti-inflammatory test

Non-clinical anti-inflammatory pharmacodynamic tests were conducted through the carrageenan induction method on rats' soles in accordance with the Guidelines for Non-clinical Pharmacodynamic Tests of Traditional Medicines on National Food and Drug Agency of Republic Indonesia (BPOM RI, 2021). A total of 36 female

Sprague Dawley (SD) strain rats were divided into 6 treatment groups, namely Group I (normal control), II (negative control), III (diclofenac Na 13.5 mg/kg BW), IV (propolis 72 mg/kg BW), V (propolis 144 mg/kg BW), VI (propolis 288 mg/kg body weight). Meanwhile, the route of administration was orally using a gastric probe for test preparations, and intraplantar injection for inflammation induction. The animals were fasted for ± 8 –10 h before administering the solution and measuring the foot volume (V0). Furthermore, the samples were weighed and given the test preparation orally, they were also induced on the left foot by 0.05 mL–0.1 mL intraplantar 0.5–2% carrageenan solution and on the right as normal control. The increase in foot volume was measured with the instrument. The rat's foot was marked as the immersion limit on the plethysmometer. Meanwhile, the edema was measured every 30 min for the first 3 h and every hour for the second 3 h after carrageenan (Vt) injection.

2.14. Statistical analysis

The data was obtained as a volume curve on the foot's edema. This volume was the difference between the edema before and after induction, as carried out by Equation (5)

$$Ve = Vt - Vo \quad (5)$$

With: Ve = Edema volume

Vt = The volume of the test animal's foot after carrageenan induction

Vo = Initial volume of test animal foot before carrageenan induction.

Moreover, the area under the curve (AUC) was determined after calculating the edema volume using Equation (6), where t is the time interval of the measurement in an hour.

$$AUC = \frac{Ve_n + Ve_{(n-1)}}{2} \times (t_n - t_{(n-1)}) \quad (6)$$

The percentage of inflammatory inhibition was calculated using the AUC value with Equation (7).

$$\text{Inflammatory inhibition}(\%) = 1 - \left(\frac{AUCt}{AUCnc} \right) \times 100\% \quad (7)$$

Description :

AUCt = the AUC treatment

AUCnc = the AUC negative control

The data obtained were analyzed by Analysis of Variance (ANOVA), while statistical data processing was carried out using the F test at the level of $\alpha = 5\%$. The test is continued with the BNT when there is a significant difference in the results (Small Significant Difference). In addition, the SPSS program was used to analyze the data.

3. Results

3.1. Antioxidant activity

This study determined the antioxidant activity of propolis using three simple spectrophotometric methods: the DPPH free radical scavenging activity assay, ABTS free radical scavenging activity assay, and FRAP. The antioxidant activity results are presented in Table 1.

The antioxidant activity of the phenolic compounds was tested by DPPH, ABTS, and FRAP methods. The IC₅₀ results for propolis indicate that propolis belongs in the category of very strong antioxidants.

Table 1
Antioxidant activity of propolis.

Antioxidant activity	Value
DPPH scavenging activity	IC ₅₀ 9.694 ppm
ABTS scavenging activity	IC ₅₀ 2.213 ppm
FRAP reducing power	189.05 mg AaE/g

3.2. Results of physical quality evaluation

3.2.1. Organoleptic test

The appearance color of capsule vegicaps was white – white, with size 0, light brown granule, and characteristic odor or taste, while the propolis powder mesh was size 40 by 95.45%. The propolis SDE powder and capsule are shown in Fig. 1.

3.2.2. Disintegration time

The average disintegration time of the capsule was 7 min 53 s. The result has passed the requirement of the Regulation of the National Food and Drug Agency of Republic Indonesia, Number 32, 2019 concerning the safety and quality requirements of traditional medicine, the disintegration time for a good capsule is 30 min at 37 °C (BPOM RI, 2019).

3.2.3. Water content

The test was conducted to determine the water content in propolis capsules using a Karl Fisher apparatus with volumetric titration. According to the results, 9.07% water content was obtained, which showed that it fulfilled the requirements by Regulation of the Food and Drug Supervisory Agency Number 32 of 2019 as well as the safety and quality of traditional medicines (BPOM RI, 2019).

3.2.4. Weight of content

The range of weight content was 400.00 mg + 5% (380.00 mg–420.00 mg), while the average weight content was 418.88 mg.



Fig. 1. Propolis SDE powder and capsule.

3.3. Determination of total flavonoid and polyphenol levels

The determination results of the total flavonoid and polyphenol content from the propolis microencapsulated powder were shown in Table 2

3.4. Heavy metal contamination

The value of heavy metal determination was below the minimum limit as Pb (Lead), Cd (Cadmium), As (Arsenic), and Hg (Mercury) with conditions <10 mg/kg, 0.3 mg/kg, 5 mg/kg, and 0.5 mg/kg respectively. Meanwhile, the average Pb, Cd, Hg, and As content in capsules were –2.5618 mg/kg, –0.8585 mg/kg, 0.03 mg/kg, and 0.3013 mg/kg respectively. Therefore, the results showed that these heavy metals were not detected.

3.5. Microbial contamination

Determination of microbial contamination results as shown in Table 3. fulfilled the requirements of USP 42 as well as product standards from the Food and Drug Administration for capsule preparations as follows, ALT ($\leq 10^5$ CFU/g), AKK ($\leq 10^3$ CFU/g), *Escherichia coli* (≤ 10 CFU/g), Enterobacteriaceae ($\leq 10^3$ CFU/g), *Salmonella sp* (negative/gr), *Clostridium sp.* (negative/g), and *Shigella sp* (negative/gr).

3.6. Propolis toxicity test results

The observation of clinical symptoms from the day before treatment (D0 = 24 h) and day 14 (D14) revealed no symptoms of toxicity in any of the four groups with different doses. The rats were active, aggressive, had normal eyes and fine hair, and had no hair loss. This appearance is typical in normal rats. Furthermore, the observation of the presence or absence of death was also observed from D0 to D14. The results revealed that for 14 days, there were no deaths in any group, from the low-dose group that received 50 mg/kg body weight to the highest-dose group that received 5000 mg/kg body weight. During 14 days of observation, the development of body weight rats indicated an increase in each group of doses from the lowest dose to the highest dose. The results showed that there were no deaths or toxicity symptoms. Rats are active or aggressive animals with normal eyes and fine hair that do not stand or fall out. The average weight of the rats increased at the end of observation (Table 4). The results of the recapitalization of histopathological analysis descriptively for each group can be seen in Table 5. Pathological examination showed no abnormalities in rats' vital organs, such as the heart, lungs, liver, spleen, stomach, intestines, and kidneys. The value was LD₅₀ \geq 5000 mg/kg BW (the largest dose given). The toxicity level of propolis capsule containing South Sulawesi propolis from *Tetragonula sapiens* is classified as essentially non-toxic for Sprague Dawley rats. Therefore, it was concluded that the product was non-toxic and safe. The histopathological results can be seen in the Figs. 2–3. Based on the results of the histopathological descriptive analysis of the kidneys and liver, it showed that the administration of the propolis capsule did not have a toxic effect on the test animals.

Table 2
Total polyphenol and flavonoid content of Indonesian propolis.

Compound	Concentration (mg/g)	Concentration (%w/w)
Polyphenol	98.0821 ± 0.0465 mg GAE/g	9.79 ± 0.1100
Flavonoid	15.89 ± 0.9200 mg QE/g	1.59 ± 0.0058

Data were presented as means ± SD, n = 3.

Table 3
Propolis capsule microbial contamination test results.

Test	Result	Requirement
Total Plate Count	<10 CFU/gr	≤10 ⁵ colony/gr
Yeast and Mold Plate Count	<10 CFU/gr	≤10 ³ colony/gr
Identification of <i>Eschericia coli</i>	Negative	≤10 colony/gr
Identification of <i>Clostridium sp</i>	Negative	Negative/gr
Identification of <i>Salmonella sp</i>	Negative	Negative/gr
Identification of <i>Shigella sp</i>	Negative	Negative/gr

Table 4
The average weight of the SD rats for 14 days.

Dose of Propolis (mg/kg BW)	Week-0	Week-1	Week-2
5000	151.80 ± 11.74	158.60 ± 14.99	162.40 ± 16.81
2000	155.20 ± 14.22	155.00 ± 10.94	158.40 ± 10.91
300	158.00 ± 12.93	157.80 ± 11.09	165.00 ± 9.88
50	156.60 ± 10.01	167.20 ± 21.03	169.00 ± 22.46

Data were presented as means ± SD, n = 5.

Table 5
The results of the organ weight.

Dose of Propolis (mg/kg BW)	The relative weight of liver and kidney (gram)		
	Liver	Right kidney	Left kidney
5000	4.60 ± 0.49	0.42 ± 0.04	0.42 ± 0.04
2000	4.24 ± 0.32	0.43 ± 0.05	0.43 ± 0.05
300	4.14 ± 0.44	0.44 ± 0.05	0.42 ± 0.04
50	3.91 ± 0.32	0.44 ± 0.07	0.44 ± 0.05

3.7. Results of the volume profile on rat’s soles

Edema volume measurements were performed every 30 min. The value at each measurement time was obtained from the differ-

ence of the current value and before the measurement. Table 6 showed the volume of rat foot edema in all groups at each observation time.

3.8. Average percentage increase in edema volume

The average percentage increase was calculated to examine the rise in inflammation volume at each measurement time. Table 7 and Fig. 4 showed the average increase for all groups at each observation time. The percentage increase in the negative control group showed the largest edema, while the dose 2 group indicated the lowest. Meanwhile, Fig. 4, showed that there was no significant increase in the edema volume on the groups given doses 1, 2, and 3.

Table 7 showed an increase in the edema volume percentage at various times and a decrease in all groups excluding the normal. The negative control group had the largest percentage increase compared to others at approximately 39.77% to 91.19%. The positive control group showed a percentage increase ranging from 37.22% to 48.06%. The administration of dose 1 treatment increased the volume by approximately 30.18% to 51.77%, while dose 2 was 20.94% to 48.48%, and dose 3 with 30.11% to 53.25%. This indicated that the three had a range of percentage increases which was not different from the positive control.

3.9. Average edema inhibition percentage

This measurement aims to determine the anti-inflammatory activity with different treatments in each group. Fig. 5 showed a graph of the edema percentage inhibition in rat paws. Dose 2 had the greatest activity of 63.33% at the 180th-minute measurement. In addition, the group showed a significant difference at 180th minutes with others in each measurement time (p < 0.05). Generally, the treatment group at doses 1, 2, and 3 exhibited the activity of inhibiting the occurrence of edema.

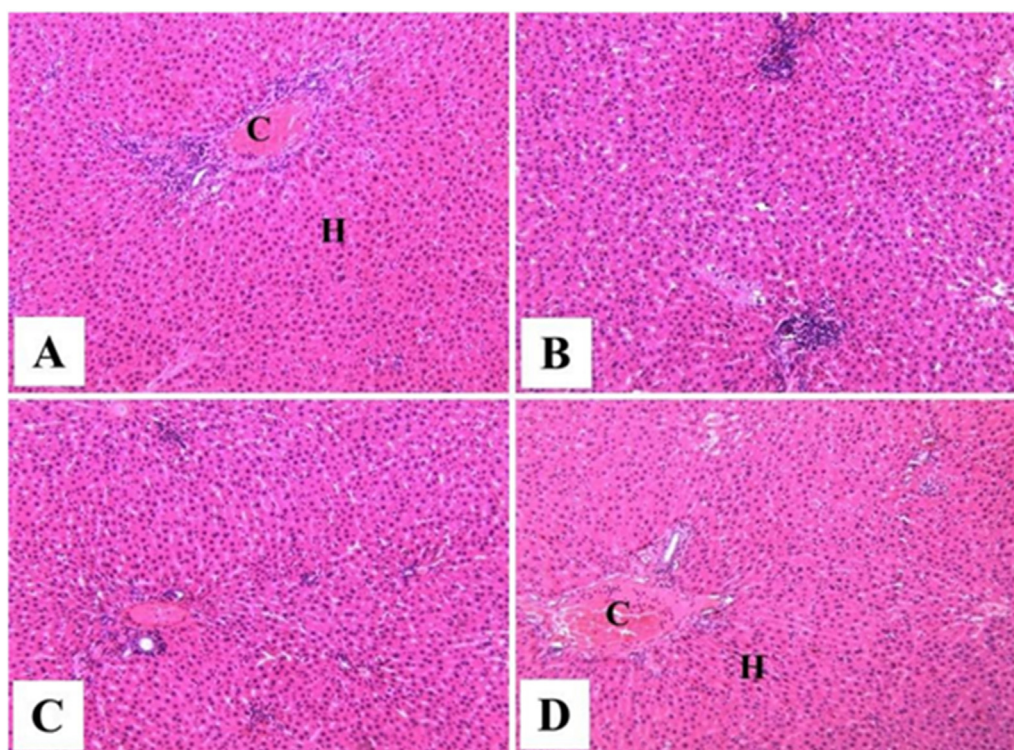


Fig. 2. Histopathology of liver: A. Dose 5000 mg/kg BW, B. Dose 2000 mg/kg BW, C. Dose 300 mg/kg BW, D. Dose 50 mg/kg BW. C: vena centralis, H: hepatosis.

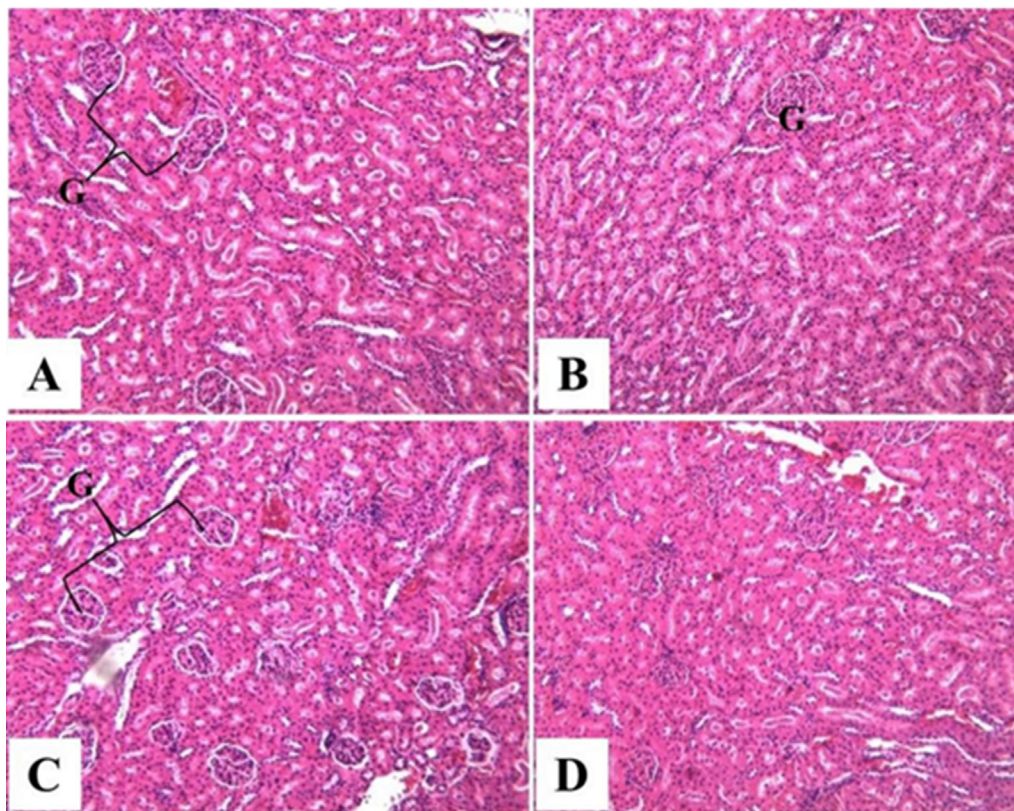


Fig. 3. Histopathology of kidney: A. Dose 5000 mg/kg BW, B. Dose 2000 mg/kg BW, C. Dose 300 mg/kg BW, D. Dose 50 mg/kg BW. G: Glomerulus.

Table 6
The volume of rat foot edema in all groups at each observation time.

Observation time (minutes)	Treatment Group (mean ± SD)					
	Normal	K-	K+	Dose 1	Dose 2	Dose 3
60	0.00 ± 0.00	0.22 ± 0.06	0.20 ± 0.04	0.18 ± 0.02	0.13 ± 0.05	0.18 ± 0.03
90	0.00 ± 0.00	0.27 ± 0.09	0.22 ± 0.05	0.18 ± 0.02	0.13 ± 0.05	0.19 ± 0.02
120	0.00 ± 0.00	0.28 ± 0.12	0.23 ± 0.05	0.21 ± 0.03	0.17 ± 0.05	0.21 ± 0.03
150	0.00 ± 0.00	0.29 ± 0.09	0.21 ± 0.05	0.24 ± 0.06	0.18 ± 0.06	0.18 ± 0.06
180	0.00 ± 0.00	0.50 ± 0.43	0.20 ± 0.06	0.24 ± 0.03	0.18 ± 0.06	0.21 ± 0.06
210	0.00 ± 0.00	0.29 ± 0.09	0.22 ± 0.05	0.24 ± 0.05	0.18 ± 0.08	0.22 ± 0.06
240	0.00 ± 0.00	0.33 ± 0.11	0.23 ± 0.07	0.26 ± 0.07	0.27 ± 0.09	0.29 ± 0.08
270	0.00 ± 0.00	0.33 ± 0.11	0.23 ± 0.06	0.27 ± 0.04	0.26 ± 0.05	0.25 ± 0.08
300	0.00 ± 0.00	0.28 ± 0.10	0.23 ± 0.09	0.28 ± 0.06	0.29 ± 0.07	0.28 ± 0.07
330	0.00 ± 0.00	0.32 ± 0.10	0.23 ± 0.08	0.30 ± 0.05	0.28 ± 0.04	0.31 ± 0.08
360	0.00 ± 0.00	0.34 ± 0.11	0.24 ± 0.08	0.29 ± 0.08	0.28 ± 0.06	0.28 ± 0.06
390	0.00 ± 0.00	0.33 ± 0.12	0.25 ± 0.08	0.29 ± 0.03	0.24 ± 0.05	0.26 ± 0.07
420	0.00 ± 0.00	0.32 ± 0.11	0.26 ± 0.07	0.28 ± 0.08	0.23 ± 0.08	0.24 ± 0.05

Description: K- (negative control); K+ (diclofenac sodium dose 13.5 mg/KgBW); Dose 1 (propolis 72 mg/KgBB); Dose 2 (propolis 144 mg/KgBB); Dosage 3 (propolis 288 mg/KgBW). Data were given in mean + SD, n = 6.

3.10. Calculation of the anti-inflammatory effectiveness percentage

The AUC value can be used to calculate the anti-inflammatory effectiveness percentage, by comparing the average AUC of the test group with the positive control (diclofenac sodium). The anti-inflammatory effectiveness value of propolis compared to positive control is shown in Table 8.

4. Discussions

4.1. Antioxidant activity

The IC₅₀ results for propolis indicate that propolis belongs in the category of very strong antioxidants. For comparison, the IC₅₀ value

obtained for propolis was not much different from the established IC₅₀ value for ascorbic acid. Antioxidant strength is grouped into the following classifications: strong (IC₅₀ < 50 ppm), strong enough (IC₅₀ 50–100 ppm), moderate (IC₅₀ 101–250 ppm), weak (IC₅₀ 250–500 ppm), and very weak (IC₅₀ > 500 ppm) (Jun et al., 2003). The DPPH assay is based on the reaction of the DPPH radical with the hydrogen-donor molecules from propolis. The phytochemical content in propolis inhibits the oxidation of other molecules, depending on its concentration and reactivity toward the reactive oxygen species. Lower IC₅₀ values were correlated with higher DPPH radical scavenging activity, which represents the concentration of the extract needed to decrease 50% of the DPPH solution's initial absorbance. Antioxidant potency is usually associated with the content of phenolic compounds because of their extensive conjugated π-

Table 7
The average percentage increase in the edema volume on rat foot at each observation time.

Observation time (minutes)	Treatment Group (mean ± SD)					
	Normal	K-	K+	Dose 1	Dose 2	Dose 3
60	0.00 ± 0.00	39.77 ± 11.11	37.22 ± 10.39	30.18 ± 5.27	20.94 ± 8.14	30.11 ± 4.57
90	0.00 ± 0.00	48.86 ± 16.79	40.28 ± 11.64	30.18 ± 5.27	22.22 ± 7.76	33.14 ± 4.70
120	0.00 ± 0.00	51.89 ± 22.97	41.94 ± 12.19	35.86 ± 6.54	28.03 ± 7.98	36.17 ± 7.75
150	0.00 ± 0.00	53.56 ± 16.95	38.89 ± 12.83	41.41 ± 9.98	30.44 ± 9.67	31.73 ± 9.97
180	0.00 ± 0.00	91.19 ± 77.49	37.50 ± 13.39	41.41 ± 5.49	30.83 ± 9.18	36.15 ± 10.94
210	0.00 ± 0.00	53.56 ± 16.95	40.28 ± 11.20	41.67 ± 9.97	29.03 ± 11.80	37.56 ± 11.64
240	0.00 ± 0.00	59.67 ± 22.25	42.22 ± 16.55	44.70 ± 12.97	44.31 ± 14.44	50.33 ± 14.67
270	0.00 ± 0.00	61.44 ± 20.98	41.94 ± 14.32	45.96 ± 7.75	43.01 ± 8.02	43.49 ± 15.23
300	0.00 ± 0.00	51.74 ± 18.01	43.89 ± 20.34	48.99 ± 12.57	48.48 ± 9.33	47.55 ± 12.59
330	0.00 ± 0.00	58.41 ± 19.75	43.61 ± 17.38	51.77 ± 10.47	45.93 ± 5.06	53.25 ± 14.14
360	0.00 ± 0.00	63.26 ± 23.49	44.72 ± 16.28	50.51 ± 15.54	46.25 ± 11.04	47.57 ± 11.93
390	0.00 ± 0.00	61.74 ± 24.28	46.67 ± 17.43	50.25 ± 7.58	40.57 ± 8.91	44.56 ± 12.98
420	0.00 ± 0.00	58.56 ± 23.06	48.06 ± 16.54	49.12 ± 15.89	37.77 ± 12.36	41.66 ± 9.68

Description: K- (negative control); K+ (diclofenac sodium dose 13.5 mg/KgBW); Dose 1 (propolis 72 mg/KgBB); Dose 2 (propolis 144 mg/KgBB); Dosage 3 (propolis 288 mg/KgBW). Data were given in mean + SD, n = 6.

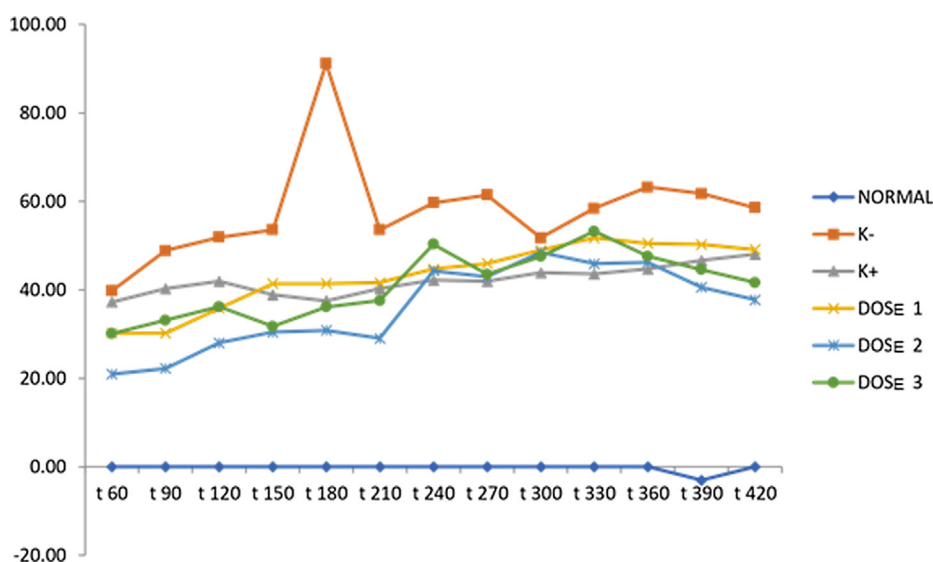


Fig. 4. The relationship graph between time (minutes) and the percentage increase in edema volume (%). Description: K- (negative control); K+ (diclofenac sodium dose 13.5 mg/KgBW); Dose 1 (propolis 72 mg/KgBB); Dose 2 (propolis 144 mg/KgBB); Dosage 3 (propolis 288 mg/KgBW).

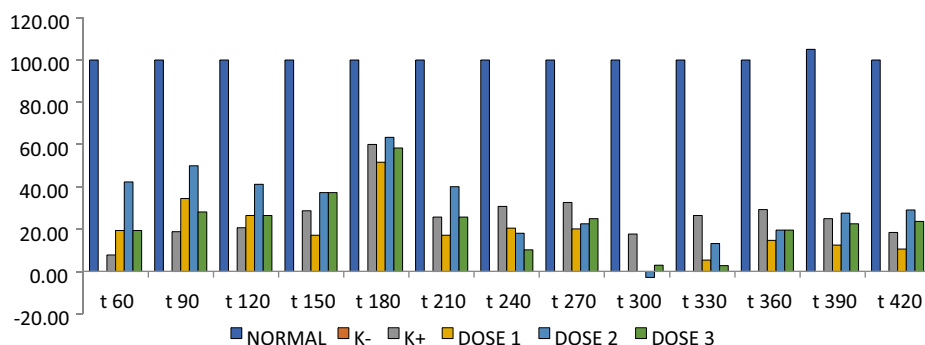


Fig. 5. Edema inhibition percentage. Description: K- (negative control); K+ (diclofenac sodium 13.5 mg/KgBW); Dose 1 (propolis 72 mg/KgBW); Dose 2 (propolis 144 mg/KgBW); Dose 3 (propolis 288 mg/KgBW).

electron systems, which facilitate the donation of electrons from the hydroxyl moieties to oxidizing radical species. The DPPH IC₅₀ radical scavenging activity of the propolis in this study was 9.694 ppm, greater than that of other types of propolis, including Bolivian propolis, which ranged from 4.54 to 48.27 ppm (Nina et al., 2016); propolis from the Tocantins, Brazil, which ranged

from 29.81 ± 2.49 to 50.23 ± 1.60 ppm (Saturnino da Silva Araujo et al., 2016); Chinese propolis, which ranged from 15.49 ± 70.59 to 28.69 ± 71.52 ppm (Huang et al., 2014); and Brazilian propolis, which ranged from 21.50 to 78.77 ppm (Bittencourt et al., 2015).

The scavenging activity of ABTS obtained from propolis was correlated with a lower IC₅₀, the concentration needed to reduce 50%

Table 8
Anti-inflammatory effectiveness percentage.

Treatment group	Anti-inflammatory effectiveness percentage (%)
Dose I Propolis 72 mg/kg BW	55.00
Dose II Propolis 144 mg/kg BW	65.83
Dose III Propolis 288 mg/kg BW	55.83

of the ABTS reagent initial absorbance. The ABTS IC₅₀ radical scavenging activity of propolis in this study was 2.213 ppm, greater than propolis from Algarve, South Portugal (ABTS IC₅₀ ranged from 6 to 36 ppm) (Miguel et al., 2014), Indian propolis (ABTS IC₅₀ ranged from 298.86 to 516.51 ppm) (Ramnath and Venkataramegowda, 2016), Brazilian green propolis (ABTS IC₅₀ was 72.10 ± 0.40 ppm) (Yuan et al., 2019), and Chinese propolis (ABTS IC₅₀ 20.0 ± 0.31) (Zhang et al., 2016).

The FRAP reducing power obtained for the propolis extract in this research was the same as that of propolis from Antioquia (Colombia), according to the FRAP method; the activity was between 40.9 ± 13.3 and 338.4 ± 22.4 μmol AAE/g of EEP (AEAC) (Palomino et al., 2009). The antioxidant capacity of the propolis extract was determined using the FRAP method, which is based on the reduction of potassium ferricyanide. The reducing agents in the PEE induced reduction of the ferric ions (Fe⁺³) to ferrous ion (Fe⁺²). Ionic Fe⁺³ chelated with nucleophilic aromatic rings as specific chelator groups present in the polyphenolic compound. An increase in absorbance indicated a high reducing power. The reducing power capacity of the samples was probably due to the phytochemical components present in propolis extracts (Pratami et al., 2018).

4.2. Results of physical quality evaluation

4.2.1. Organoleptic test

According to test results, the powder was light brown due to its raw material colour. It also had a distinctive smell like herbal medicine since it was derived from natural ingredients which have a distinctive aroma when processed. The particle form of the capsules (microencapsulated powder) is the most stable when used (Mangiring et al., 2018; Sahlan et al., 2019b). As a matter of fact, the capsules are slightly bitter due to alkaloids content (Pratami et al., 2018).

4.2.2. Disintegration time

For therapeutic effect, the shell should be crushed first in order to release the granules/powder, hence, it becomes smaller particles which can be easily absorbed in the gastrointestinal tract (Glube et al., 2013). According to the Regulation of the National Food and Drug Agency of Republic Indonesia, Number 32, 2019 concerning the safety and quality requirements of traditional medicine, the disintegration time for a good capsule is 30 min at 37 °C (BPOM RI, 2019). Therefore, the results indicated that the requirements were fulfilled. Capsules containing the powder coated with maltodextrin and gum arabic through spray drying had a slight difference in disintegration time of propolis hard gelatine and hard HPMC capsules. This was consistent with In vitro disintegration test by Zainal et al. (2021), which showed that the powder loaded into hard gelatine capsules disintegrated faster than those in the hard HPMC by 1.7 to 7.8 min and 3.7 to 8.4 min in the four media solutions (Zainal et al., 2021).

4.2.3. Water content

The water content result has passed the specification, lower than 10%, therefore, the probability of the capsules being contam-

inated by microorganisms' growth and some enzymes contained in active natural ingredients was less due to low water components. The product fulfilled the requirements by Regulation of the Food and Drug Supervisory Agency Number 32 of 2019 as well as the safety and quality of traditional medicines (BPOM RI, 2019).

4.2.4. Weight of content

The weight uniformity test was carried out to ensure that the formula contained the same amount and active substance with the assumption that the formula powder was homogeneously mixed. The weighted 20 capsules met the uniformity requirement according to USP 42 where % deviation was 0.82 to 7.02% (The United States Pharmacopoeia Commission Inc., 2019).

4.3. Determination of total flavonoid and polyphenol levels

Propolis is plant resin collected by bees that is mixed with enzymes in the bees' mouths. The results revealed the existence of polyphenols, indicating that the resin in plants collected by bees contains polyphenols. Therefore, the polyphenols and flavonoids are most likely obtained from plants (Bankova et al., 2000; Maleki et al., 2019). Based on the isolation conducted by Miyata et al (2020), propolis *Tetragonula spp* from South Sulawesi contained several flavonoid compounds as follows, 2',3'-dihydro-3'-hydroxypapuanic acid; (-)-papuanic acid, (-)-isocalolongic acid, isopapuanic acid, isocalopolyanic acid, glyasperin A, broussonflavonol F; (2S)-5,7-dihydroxy-4'-methoxy-8-prenylflavanone, and isorhamnetin (Miyata et al., 2020).

The anti-inflammatory activity was associated with propolis or compounds like polyphenols (flavonoids, phenolic acids, and their esters), terpenoids, steroids, as well as amino acids (Araujo et al., 2012). Phytochemical evaluation previously established that the polyphenols in propolis are present predominantly in the ethanol extract fraction (Pratami et al., 2018). Polyphenol profiles of propolis from many countries vary considerably with the geographical region of origin, reflecting the diversity of plant life in different areas (Bankova, 2005). Flavonoids and flavonoid esters, such as chrysin, galangin, and caffeic acid phenethyl ester (CAPE), are the main polyphenols in Chinese propolis. By contrast, Brazilian green propolis generally consists mainly of phenolic acids, including caffeic acid and prenylated p-coumaric acids (Artepillin C and Baccharin) (Wang et al., 2015). Previous research compared the in vivo activity of two different types of propolis. Both types of propolis showed in vivo anti-inflammatory activity against arachidonic acid (AA) and 12-O-tetradecanoylphorbol-13-acetate (TPA); the most active propolis was the type with higher activity levels than the other (Valenzuela-Barra et al., 2015). This difference is partly due to the variations in total phenols and flavonoids content and the phenolic profile.

4.4. Heavy metal contamination

The value of heavy metal determination was below the minimum limit. Pb (Lead), Cd (Cadmium), As (Arsenic), and Hg (Mercury) are examples of heavy metals that should be limited in preparation because they can interfere with health, and are toxic to the body when consumed in long term. The analysis results of their contamination fulfilled the requirements permitted by the Regulation of National Food and Drug Agency of Republic Indonesia No. 32, 2019 (BPOM RI, 2019).

4.5. Microbial contamination

Determination of microbial contamination fulfilled the requirements of USP 42 and the Regulation of the National Food and Drug Agency of Republic Indonesia No. 32, 2019 (BPOM RI, 2019). The

microbial contamination investigation are very important to ensure that the traditional medicine is safe for consumption and meets quality standards (Tjampakasari et al., 2021).

4.6. Propolis toxicity test results

To determine the dose of propolis for a hepatoprotective effect test, an acute oral toxicity test was performed. Based on the results of the acute oral toxicity and histopathological analysis, it showed that the administration of the propolis capsule safe and non-toxic.

Weighing of organs organ index is a parameter that can provide a general description of the effect of administering the test material. The size of the enlarged or shrunk organ can be known although it cannot be used as a standard in determining damage or repair of organ function. This parameter must be associated with the histopathological picture to determine the exact effect of the test material on the organs.

According to the test of 70% propolis ethanol extract conducted by AEZ Hasan et al. (2020), with a dose of as much as 720.3 mg/kg BW for 28 days, there were no abnormalities in the blood glucose, cholesterol, and triglyceride values of Sprague Dawley rats (Hasan et al., 2020). Furthermore, administration of the granules for 60 days as conducted by Khacha-ananda et al (2018) showed no acute or subchronic toxicity in Wistar rats (Khacha-Ananda et al., 2018).

An in vivo study conducted by Al Mukhlas Fikri et al (2019) concluded that Indonesian propolis at 380 mg/kg BW did not inhibit fetal development. However, it was likely to inhibit at 1400 mg/kg BW. An established level was not observed in adverse events (1400 mg/kg), therefore, this dose cannot be used as a safety level in pregnancy (Fikri et al., 2019). In 2021, another in vivo examination by AL Mukhlas Fikri et al (2021) showed that administration of the extract from South Sulawesi at the lowest with the highest dose of 380/1400 mg/kg BW during pregnancy did not appear to cause maternal toxicity in mother rat. Serum ALT, AST, urea, and creatinine did not differ between groups. In addition, histopathology examination showed no specific changes (Fikri et al., 2021).

4.7. Results of the volume profile on rat's soles

In vivo, an inflammatory activity test was initiated with the formation of artificial inflammation/edema on rats' soles. Some of the recommendations for propolis effective doses are in the range of 100–1500 mg/kg BW. Sahlan et al. (2019a) stated that it was effective as an anti-inflammatory at a dose of 100–200 mg/kg BW (Sahlan et al., 2019a).

Based on the results, the edema volume differs for all groups. Overall, the highest value was found in the negative control group, by 0.50 at 180 min. This indicated that carrageenan was able to induce the formation of edema in their soles.

According to the SPSS test results on the observation of the volume, the data were distributed normally at each observation time. Statistically, there was a significant difference for each observation time in all groups ($p < 0.05$).

4.8. Average percentage increase in edema volume

The average percentage increase was calculated to examine the rise in inflammation volume at each measurement time. The doses 1, 2, and 3 groups showed the ability to suppress volume which was quite large compared to the negative and positive control groups, therefore, the percentage increase was minimal. The negative control group had the largest percentage increase compared to others, then the positive control group showed a percentage increasing. This indicated that the three had a range of percentage increases which was not different from the positive control.

Carrageenan rat paw oedema model is widely used for research and development of new anti-inflammation NSAIDs with determination of effects after 3 to 5 h after oedema induction, neglecting longer-term effects (Cong et al., 2015). Local carrageenan injection induces a systemic response. The increasing of edema volume diverse systemic changes including increased levels of acute phase proteins, such as fibrinogen and C-reactive protein (Vazquez et al., 2015).

4.9. Average edema inhibition percentage

According to Sahlan et al. (2019), smooth propolis dry extract at 50 mg/kg BW extremely inhibited inflammation, followed by rough dry extract at a dose of 25 mg/kg. Both extracts indicated an inflammatory inhibition value of 62.24% and 58.12% at 25 mg/kg, which is comparable to the 70.26% of sodium diclofenac with the dose of 135 mg/kg (Sahlan et al., 2019a).

Sahlan et al. (2021), concluded that the anti-inflammatory activity test using an in vitro method showed the activity in reducing inflammation inhibition with the extract at a dose of 120 g/mL. The results of the anti-inflammatory test indicated that propolis SDE at 120 g/mL had an anti-inflammatory property as proven by TNF- α levels of 304.28 ± 30.25 pg/mL, iNOS of 5.42 ± 0.82 ng/mL, and NO 101.09 ± 1.49 mol/L (Sahlan et al., 2021b). The difference between in vitro and in vivo test results is because the former does not use experimental animals, hence, the results are not influenced by physiological factors. However, in vivo test results are strongly influenced by the physiological response and metabolism of experimental animals.

The results above showed that the curve profile from the average edema volume of the rat paws can assess the effectiveness of an anti-inflammatory drug, but the average value in each treatment group can only be concluded at a certain time (not overall). This analysis was conducted by calculating the AUC (Area Under The Curve) value and statistically testing with the SPSS (Statistical Package for the Social Sciences) program.

4.10. Calculation of the anti-inflammatory effectiveness percentage

Dose II showed a higher anti-inflammatory effectiveness value than the others. This is consistent with Da Silva et al (2015) and Sahlan et al. (2019), where doses > 200 mg/kg BW showed a decrease in anti-inflammatory effectiveness (da Silva et al., 2015; Sahlan et al., 2019a). This can be considered as an imbalance of estrogen hormone in female rats and high content of isoflavones, which causes inflammation. Estrogen is a sex hormone associated with high inflammatory activity (Lessey and Young, 2014). As matter of fact, it can depress inflammation by suppressing pro-inflammatory factors such as IL-6 or TNF- α and binding with a transcription factor such as NF-kB, therefore, preventing it from binding DNA (Weitzmann and Pacifici, 2006). As a result, when an imbalance occurs in this hormone, the inflammatory inhibition will decrease. The Propolis of *Tetragronula sapiens* used in this study had a high flavonoid content, such as isoflavones (a type of flavonoid). An increase in isoflavone content beyond a certain limit can trigger oxidative stress, therefore, causing inflammatory reactions (da Silva et al., 2015).

5. Conclusion

The antioxidant activity of propolis spray dry extract can be considered a strong antioxidant. It demonstrated the IC₅₀ values of the DPPH and ABTS scavenging activity were 9.694 ppm and 2.213 ppm, respectively. The FRAP reducing power was 189.05 mg AaE/g. The evaluation results for the physical, chemical,

and microbiological quality of propolis capsules fulfilled the requirements as traditional medicinal preparations in accordance with the Head of BPOM Regulation No. 32, 2019. The acute toxicity test indicated that there were no symptoms of toxicity, while the pathological examination showed no abnormalities in all rats at the end of observation. The LD₅₀ value was ≥ 5000 mg/kg BW, which means that acute toxicity potential in female rats was practically non-toxic (≥ 5 g). Administration of propolis capsule at doses of 5000, 2000, 300 and 50 mg/kgbw, which was observed for 14 days in female rats of the Sprague Dawley strain, proved safe, did not cause toxic effects, did not cause significant weight loss in animals, generally did not cause permanent damage/lesion to the liver and kidneys. The inhibition percentage value of the anti-inflammatory test in experimental animals for the positive control group > propolis 144 mg/kg BW > 288 mg/kg BW > 72 mg/kg BW was 11.86% > 7.81% > 6.63% > 6.53%, respectively. Furthermore, the effectiveness percentage value of the anti-inflammatory drug propolis 144 mg/kg BW > 288 mg/kg BW > 72 mg/kg BW compared to the positive control was 65.83% > 55.83% > 55.00 respectively. Based on the results, it can be concluded that propolis capsules fulfilled the standardization requirements, were likely to be non-toxic, and effective as an anti-inflammatory. Therefore, they can be developed into standardized traditional medicines for anti-inflammatory.

CRedit authorship contribution statement

Siti Farida: Conceptualization, Data curation, Writing – review & editing. **Diah Kartika Pratami:** Project administration, Writing – original draft, Supervision. **Muhamad Sahlan:** Formal analysis, Funding acquisition, Resources. **Dian Ratih Laksmiawati:** Methodology, Validation, Writing – review & editing. **Etin Rohmatin:** Investigation, Visualization, Writing – review & editing. **Herbert Situmorang:** Data curation, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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