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Original article

Potential hepatoprotective effects of flavonoids contained in propolis from South Sulawesi against chemotherapy agents

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ABSTRACT

The use of doxorubicin and epirubicin as chemotherapy agent causes side effects such as liver damage due to oxidative stress by reactive oxygen species (ROS) that cause increased of ALT and AST level as liver parameter. One source of natural antioxidants as ROS neutralizer comes from flavonoid that contain in propolis. Most researchers claim that flavonoid can be used to protect the liver. The aim of this study was to test the hepatoprotective effect of flavonoid in propolis from South Sulawesi against doxorubicin and epirubicin. The experiment included male Sprague dawley rats divided into nine groups. The rats received the microcapsule propolis or the quercetin orally for 15 days. The hepatotoxicity was promoted by injection epirubicin and doxorubicin (i.v.) with a cumulative dose of 9 mg/kg. In this study, total polyphenol and flavonoid tests of propolis have been carried out, there were 1.1% polyphenols and 2.7% flavonoids, the antioxidant activity tests showed IC50 value of 9849 ppm and LCMS/MS tests supported the presence of phenolic compounds in propolis from South Sulawesi. Liver parameter was measured and the results showed that the propolis 200 mg/kg group produced the lowest ALT and had potential protective effect against doxorubicin and epirubicin-induced hepatotoxicity.

1. Introduction

The incidence of cancer (excluding non-melanomatous skin cancers) is projected to rise from 17 million to 26 million between 2018 and 2040 (Wilson et al., 2019). Chemotherapy is one of the most common treatment efforts for therapeutic cancer. The increase in cancer cases is expected to increase the need for chemotherapy as a cancer treatment. Between 2018 and 2040 it is estimated that the need for chemotherapy drugs will increase from 9.8 million to 15 million, with a relative increase of 53% (Wilson et al., 2019). One of the most widely used chemotherapy agents comes from the group of anthracycline drugs. Anthracycline such as doxorubicin and epirubicin are first-line chemotherapy agents that are widely used for breast cancer. Epirubicin is a derivative of doxorubicin, but epirubicin has a lower level of cardiotoxicity (Tareen and Taneja, 2010). Epirubicin is metabolized by the liver to form Epirubicinol metabolites and glucuronide

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epirubicin (Weenen et al., 1984) Research shows that long-term use of doxorubicin and epirubicin can cause liver damage. This damage is thought to be due to oxygen free radicals and lipid peroxidation produced during metabolism (Singal and Iliskovic, 1998).

The Bee species are divided into 2 namely stinging bees and stingless bees. Examples of stinging bee is a bee that comes from the genus *Apis*, while one type of stingless bee comes from the genus *Tetragonula*. Both types of bees are known to produce a product called propolis, but the use of stingless bee is still rare compared to stinging bee. Propolis is a complex resin mixture collected by honey bees from various gums from plants, then enriched again by saliva and enzyme secretions and used for the construction and protection of beehives (Hasan et al., 2014; Al-Waili, 2012; Anjum, 2019). The composition of each propolis from various types of bees varies, depending on the type of plant resin available and substances that are synthesized and secreted by bees.

Lately, propolis is known as one of traditional medicine to treat many diseases. The biological activity of propolis includes its function as an antioxidant, antibacterial, antifun- gal, antiviral, anti-inflammatory, and photoprotector (Sahlan and Supardi, 2013; Sahlan et al., 2017; Sahlan et al., 2019; Khayrani, 2021; Pant, 2021; Sahlan, 2021a,b). There are various benefits of propolis, but therapeutic applications are still limited due to variability of the chemical composition and its weakness such as the low solubility in water, the taste is bitter and has a strong smell. Microcapsule propolis was enhanced the physical characteristic of propolis (Diah Kartika et al., 2020). There are more than 108 active compounds in propolis, some of which are antioxidants from phenolic compounds such as polyphenol and flavonoids (Segueni et al., 2016). Phenolic compounds are secondary metabolites that are found in plants. Phenolic compounds consist of several large groups such as Polyphenols, Flavonoids and Terpenoids. Phenolic compounds (flavonoids and polyphenol compounds) are one of the secondary metabolites that are more widely distributed in plants (Vuolo et al., 2019). Phenolic compounds from plants are derived from the pentose phosphate pathway, shikimate, and phenylpropanoid (Harborne, 1980). These compounds play an important role in plant growth and reproduction, giving protection against pathogens and predators.

Flavonoids have the ability to capture free radicals and inhibit lipid peroxidation (Sofna and Nina, 2015; Treml and Šmejkal, 2016). The biological activity of propolis is often associated with the presence of flavonoids. There are several types of flavonoids that are known as various effects on health. Therefore, flavonoid content can be used as a parameter or index in evaluating the quality of propolis used. Phytochemical analysis of extracts from 40 active compounds in various kinds of propolis based on their region showed the presence of general constituents such as phenol, tannins, and flavonoids (Bankova and Popova, 2007). These antioxidant compounds can be used to neutralize free radicals.

Thus, the authors decided to examine the study of the effects of propolis from Indonesian bees, Tetragonula sp. against liver damage due to doxorubicin and epirubicin.

2. Materials and methods

2.1. Material

Folin–Ciocaltue's reagent, gallic acid, sodium carbonate (Na_2CO_3), quercetin, aluminium chloride ($AlCl_3$), potassium acetate (CH_3COOK) purchased from Sigma-Aldrich. Epirubicin (EPI, 50 mg) and Doxorubicin (DOX, 50 mg) obtained from the PT Kalbe Pharma. Microcapsule propolis obtained from PT RINBIOTEK. The crude propolis obtained from South Sulawesi, Indonesia.

2.2. Animal protocol

The animal treatment protocol based on the protocol carried out by (Chaa et al., 2019) under the National Institute of Health (NIH) guidelines with slight modifications. All animal protocols were approved at January 13th 2020 by the Animal Ethics Commission of the Centre for Tropical Biopharmaca Studies, IPB University (Bogor Agricultural University), Bogor, West Java, Indonesia (No: 035-2020 KEH TROP BRC). Sprague- Dawley (200–300 g) male rats test animal model was used that adapted for 4 weeks at ambient temperature and photoperiodism for 12/12 h. Then the rats were divided into 9 groups consisting of the negative and positive control groups and sample groups. Each group consisted of 3 rats and given physiological saline by gastric gavage treatment for 15 days. For the positive control groups given physiological saline concomitant with quercetin, while for the sample groups given physiological saline was concomitant with a variation of propolis dose of 100 mg/kg and 200 mg/kg. On the 15th day, the positive control and sample groups were injected with doxorubicin and epirubicin for 3 times a week at 48 h intervals until the dose reached 9 mg/kg. The rats will be euthanized after 15th day to reduce severe pain that cannot be controlled. Grouping and treatment of animals shown in Table 1. This procedure carried out in the Laboratory of IPB.

2.3. Total polyphenol and flavonoid

The polyphenol test carried out using the Folin-Ciocalteu method which used 1000 ppm gallic acid as a standard. The first step was made the gallic acid standard by dissolving 50 mg gallic acid powder with 50 mL methanol. From the standard solution, variations in the standard concentration with volume variations formed. Furthermore, making sodium carbonate (Na_2CO_3) with a concentration of 1 M by weighing 5.3 g of sodium carbonate and then diluted with 50 mL of distilled water. Then folin solution was made in the ratio of 1:10 with distilled water. Each sample used (all standard concentrations and propolis samples) was 0.5 mL pipetted into each test tube and 5 mL folin was added. Then, the mixture is vortexed and allowed to stand for 5 min. After that, add 4 mL of Na_2CO_3 1 M and vortexed, then let it stand again for 15 min at room temperature. Next, the solution was measured by a spectrophotometer with a wavelength of 765 nm. TPCs in the sample extract were calculated by referring to the standard calibration curve and expressed as mg gallic acid equivalent (mg GAE/g) of extract sample.

Table 1
Animal protocol.

Group	Treatment	
	Day 1–15	Day 15–22
1	Physiological saline	–
2	Physiological saline + Propolis 100 mg/kg	Doxorubicin
3	Physiological saline + Propolis 200 mg/kg	Doxorubicin
4 (Positive control)	Physiological saline + quercetin 50 mg/kg	Doxorubicin
5 (Negative control)	Physiological saline	Doxorubicin
6	Physiological saline + Propolis 100 mg/kg	Epirubicin
7	Physiological saline + Propolis 200 mg/kg	Epirubicin
8 (Positive control)	Physiological saline + quercetin 50 mg/kg	Epirubicin
9 (Negative control)	Physiological saline	Epirubicin

The flavonoid test carried out following the colorimetric method of Aluminum chloride using 1000 ppm quercetin as standard. The first step is to make a standard solution by dissolving 50 mg of quercetin powder in 50 mL of methanol. From the standard solution, various concentration variations can be made. Second, making aluminium chloride with a concentration of 10% by means of 5 g of $AlCl_3$ being treated with 50 mL of distilled water. Then made a solution of 1 M CH_3COOK. After all the solutions made then take as much as 0.5 mL of standard solution and each sample into a test tube and then added with 1.5 mL of methanol, 0.1 mL of 10% $AlCl_3$, 0.1 mL of CH_3COOK, and 2.8 mL of distilled water. After that, it was vortexed and allowed to stand for 30 min, measured by a spectrophotometer with a wavelength of 415 nm. TFC was calculated based on quercetin calibration curve and expressed as mg quercetin equivalent (mg QE/g) of extract.

2.4. Antioxidant activity

Antioxidant activity test used DPPH free radicals. The method carried out following the method that has been applied by the Biopharmaca Study Center Laboratory, which consists of 3 steps, making 125 μM DPPH stock, 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

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

2.5. Phenolic compound identification

The purpose of LC-MS/MS usage was to determine the content of compounds in propolis samples. Microcapsule propolis was dissolved in 100 mg/mL ethanol then diluted to a concentration of 1 mg/mL. Then the solution was filtered by a filter. The filtrate obtained was analyzed using LC-MS/MS. This test used the Acquity UPLC H-Class System (Waters) instrument with the UPLC BEH C18 Acquity column. The inside diameter of the column is 2.1x50 mm with the mass spectrometer used is Xevo G2-ST Qtof (Waters). The column temperature is 50 °C with a flow rate of 20 mL/min. The two solvents used are 0.05% formic acid in water (A) and 0.05% formic acid in acetonitrile (B). The operating mode of the mass spectrometer is electrospray ionization positive mode, with a mass analysis range of 50–1500 *m*/*z*. The source temperature is 100 °C and desolvation temperature is 350 °C. Low collision energy used is 4 V, while for high collision energy is in the range of 25–70 V. Desolvation gas flow is 793 L/hr.

2.6. Toxicity acute test

Conduct a toxicity test to determine the level of propolis against rats by the fixed-dose method following the rules of the National BPOM RI (2014). Preparing 20 female Sprague-Dawley strain rats aged 8–10 weeks, then the rats were fasted for 16 h and divided into 4 groups randomly, namely: (1) the group was given propolis extract at a dose of 50 mg/kg. (2) the group of rats given propolis extract at a dose of 300 mg/kg. (3) the group was given propolis extract at a dose of 2000 mg/kg. (4) the group was given propolis extract at a dose of 5000 mg/kg. The dose was given once on the first day by gastric gavage then observed for 14 days. These rats were observed individually for at least the first 30 min after given propolis extract and periodically every 4 h for the first 24 h and once a day for 14 days. Observations include the time of occurrence and disappearance of toxic symptoms and the time of death. LD_{50} values were calculated based on the number of rats that died. All animals must be detained for pathology examination.

2.7. ALT and AST test

Stages of testing follow the IFFC protocol (International Federation of Clinical Chemistry and Laboratory Medicine) with the Biomaxima reagent, starting from taking samples of rats blood serum. Then 50 μm of serum sample mixed with 1000 μm of biomaxima reagent, stirring until well mixed. Next, measured the absorbance value at 340 nm for 1 min using a biochemistry analyzer under the ICubio brand, IChem-535Vet. The reading repeated after 1,2,3 min.

2.8. Statistic

Statistics SigmaStat software (Graphpad Prism 8) used for the analysis. The data were presented as mean ± SD and were evaluated by one-way ANOVA with Dunnett's post hoc test.

3. Result

3.1. Total polyphenol and flavonoid

Folin– The polyphenol test was carried out using the Folin-Ciocalteu method, used gallic acid as the standard while the flavonoid test was carried out following the colorimetric method of Aluminum chloride that used quercetin as the standard. The results of concentration obtained were determined (Table 2).

3.2. Antioxidant activity

In this study to determine the antioxidant activity of propolis using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). IC_{50} values obtained were determined (Table 3).

3.3. Phenolic compound identification result

LC-MS/MS test was carried out to the identified any compounds that contained in propolis from South Sulawesi. The LC-MS/MS results were interpreted by MassLynx 4.1. (Fig. 1; Table 4)

3.4. Toxicity acute test

The toxicity test was carried out using the fixed dose method in which the observations included symptoms of toxicity and death, body weight before treatment and every week during treatment and pathology observation (Table 5). Animal pathological tests see the presence or absence of damage to vital organs such as the liver, heart, lungs, stomach, intestines, kidneys. From the observations in (Fig. 3) showed that no abnormalities in these organs.

3.5. ALT and AST test

ALT and AST levels obtained and provided in Table 6.

Table 2
Total polyphenol and flavonoid content of propolis south sulawesi.

Compound	Concentration (mg/g)	Concentration (%w/w)
Polyphenol	11.024 ± 20.62	1.10
Flavonoid	27.589 ± 105.92	2.76

Table 3
IC_{50} of propolis and ascorbic acid.

Title 1	IC_{50}
Propolis	9.849 ppm
Ascorbic acid	4.368 ppm

4. Discussion

The Based on the results shown on the Table 1 the total content of polyphenol and flavonoid compounds contained in propolis is 11.024 mgGAE/g or 1,1% and 27.589 mgQuer/g or 2.7%. As previously known the definition of propolis itself is the resin collected by bees from plants and mixed with enzymes in bees' mouths. The existence of polyphenols which is proven from the test results shows that the resin in plants collected by bees contains polyphenols. Based on this, polyphenols and flavonoid are most likely to be obtained from plants. Then the antioxidant activity of the phenolic compounds was tested by DPPH method. The result showed the IC_{50} of propolis indicate that propolis is included in the category of very strong antioxidants, its value is not much different from the IC_{50} value of ascorbic acid as a comparison. This is based on the grouping of antioxidant strength levels classification that the antioxidant strong (IC_{50} < 50 ppm) strong enough (IC_{50} 50–100 ppm), moderate (IC_{50} 101–250 ppm), weak (IC_{50} 250–500 ppm), and very weak (IC_{50} > 500 ppm) (Jun et al., 2003).

The identification result by LC-MS/MS showed that the phenolic compounds were the flavonoid and triterpenoid groups. The identified molecule was confirmed if spectra from the reading results are matched with literature spectra, with at least parent ion and 2 daughter ion were identical (KIVRAK et al., 2016). Each molecule has a different pattern of fragment (White V, 1982); (Sahlan et al., 2019). In this study, the possible compounds that have a hepatoprotective effect are suspected compounds belonging to phenolic compounds such as Mollicellin H, Macarangin, Heteroflavanone C, Glyurallin B, Muscanone, Ganoderic acid R, Ganoderic acid Me, D8′-Merulinic acid A and camaryolic acid. The discovery of this phenolic compound is in line with the above test of polyphenol and flavonoid content. Only Ganoderic acid R has a hepatoprotective effect (Hirotani et al., 1986). Mollicellin H is a depsidone com-

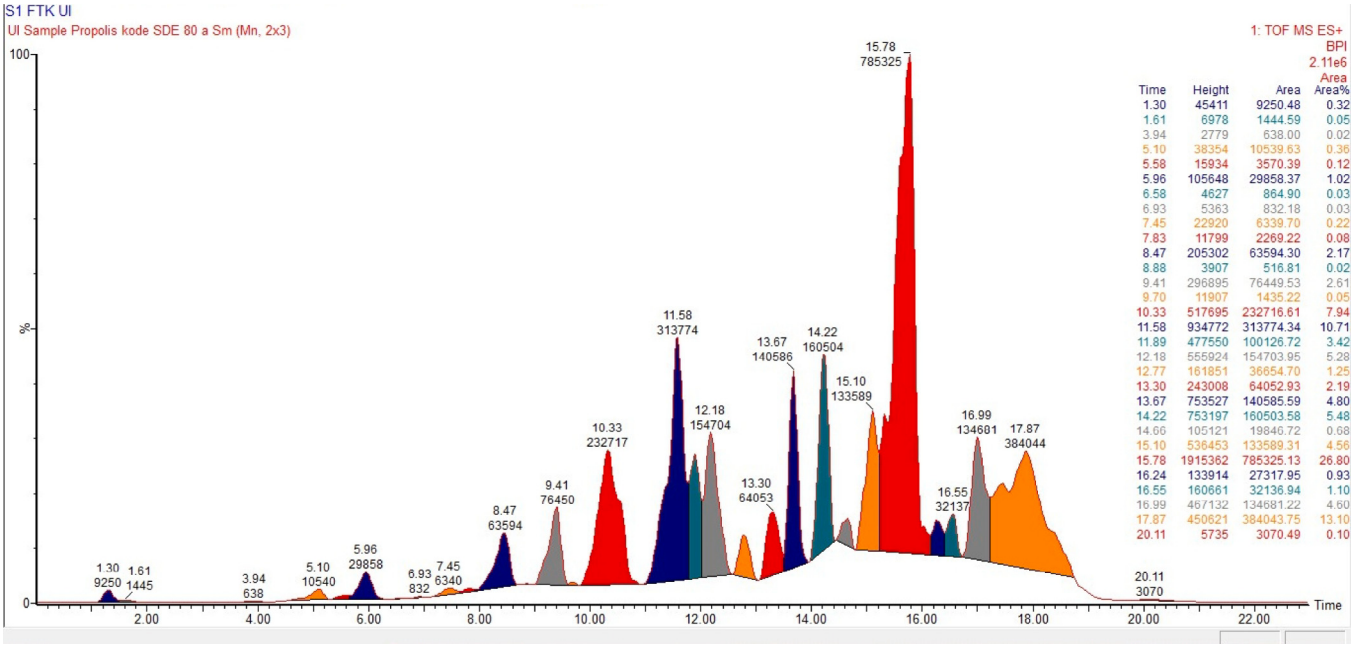


Fig. 1. Chromatogram %Area.

Table 4
Suspected compounds that are estimated to have hepatoprotective effects.

No	Retention Time	Molecular Formula	Chemical's Name	Compound	Parent and Daughter Ion	Identification Status and Category
1	10.33	$C_{21}H_{20}O_6$	Mollicellin H	Depsides and depsidones epsidine	369.1337; 119.0497; 311.0556	Identified, confirmed
2	11.58	$C_{25}H_{26}O_6$	Macarangin	Flavonoid	423.1819	Identified, tentative
3	11.89	$C_{23}H_{26}O_7$	Heteroflavanone C	Flavonoid	415.1752; 211.0965	Identified, tentative
4	12.18	$C_{25}H_{26}O_6$	Glyurallin B	Flavonoid	423.1789; 367.1182; 247.0970; 247.2059	Identified, confirmed
5	15.10	$C_{34}H_{50}O_6$	Muscanone Ganoderic acid Me Ganoderic acid R	Flavonoid Triterpenoid Triterpenoid	555.3701	Identified, tentative
6	16.24	$C_{24}H_{38}O_4$	D8′-Merulinic acid A	Triterpenoid	391.2841; 345.2788; 149.0233	Identified, confirmed
7	16.55	$C_{36}H_{54}O_6$	Camaryolic acid	Triterpenoid	583.4015	Identified, tentative

Table 5
Body weight per week.

Dosage (mg/kgBB)	Body Weight (gram)		
	Day0	Day7	Day14
50	198.0 ± 19,89	208.2 ± 22,86	212.8 ± 23,64
300	198.4 ± 10,60	209.4 ± 14,76	214.0 ± 16,43
2000	213.4 ± 9,555	218.4 ± 12,50	218.4 ± 12,30
5000	202.8 ± 7,887	207.6 ± 11,08	212.4 ± 10,85

Table 6
ALT and AST result.

Treatment	ALT (U/l)	AST (U/l)
Normal	94.82 ± 5.910	186.04 ± 28.69
Propolis 100 mg/kg + 9 mg/kg DOX	58.25 ± 11.21	196.99 ± 12.00
Propolis 200 mg/kg + 9 mg/kg DOX	48.86 ± 3.900	208.63 ± 9.820
quercetin 50 mg/kg + 9 mg/kg DOX	58,49 ± 4.730	200.46 ± 12.18
9 mg/kg DOX	67.35 ± 8.950	194.75 ± 43.32
Propolis 100 mg/kg + 9 mg/kg EPI	65.51 ± 8.140	218.54 ± 34.08
Propolis 200 mg/kg + 9 mg/kg EPI	63.59 ± 19.63	175.04 ± 12.65
quercetin 50 mg/kg + 9 mg/kg EPI	68.78 ± 9.380	176.69 ± 42.68
9 mg/kg EPI	71.21 ± 5.430	188.33 ± 7.200

pound. Depsidone is known to have antibacterial, antiproliferative, cytotoxic, antioxidant, antimalarial, antihypertensive, aromatase and and antifungal bioactivity (Ouyang et al., 2018). The next compound is a Macarangin that can be isolated from Macarangga plants (Ilimu and Syah, 2019). In Indonesia, Macaranga plants can be found in several regions, namely Papua, Maluku, Sulawesi, Kalimantan, Sumatra, Bangka, and Java. Heteroflavanone C is a compound that belongs to the class of organic compounds known as 8-prenylated flavanones. This compound can be found in Artocarpus champeden plants which are commonly used as malaria medicines (Widyawaruyanti et al., 2007). Glyurallin B belongs to the group of organic compounds known as isoflavones. Gyurallin B compound has been shown to act as an anticancer that inhibits KB cell proliferation, and has antioxidant and anti-inflammatory activity (Fu et al., 2013; Inami et al., 2017; Ito et al., 2020). Muscanone is an antifungal compound of flavones. This compound can be found in the Commiphora wightii plant. Muscanone shows activity against candida albicans (Fatope et al., 2003). Ganoderic acid R and Genoderic acid Me are compounds isolated from the fungus Ganoderma lucidum which belong to the class Polyporaceae. Ganoderic acid Me has known hypercholesterolemic activity (Hajjaj et al., 2005; Xu et al., 2010). D8′-Merulinic acid A is a phenolic lipid isolated from basidiomycetes such as Hapalopilus mutans, Phlebia radiata, and Merulius tremellosus (Khadem and Marles, 2010; Sontag et al., 1999). It has antibacterial activity and acts as an amphiphilic molecule (Stasiuk et al., 2004; Zjawiony, 2004). Camaryolic acid is a compound that can be isolated from the Lantana camara plant. This plant is used for the treatment of various diseases in humans such as itching, sores, boils, swelling, swollen fever, eczema, tetanus, malaria, tumor, and rheumatism. Camaryolic acid has antioxidant activity (Begum et al., 2003; Shikhar Katiyar et al., 2013).

In determining the dose of propolis that will be used for a hepatoprotective effect test, an acute oral toxicity test was done first. Observation of clinical symptoms from the day before treatment (D0 = 24 h) and day 14 (D14) revealed no symptoms of toxicity were observed in all four groups with different doses. The rats were active, aggressive, had normal eyes, fine hair and had no hair loss. This appearance is commonly found 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 was no death either from the low dose group of rats that is 50 mg/kg body weight up to the highest dose of 5000 mg/kg body weight (Fig. 2). During 14 days of observation, the results of the development of body weight rats indicate an increase in each group of doses from the lowest dose to the highest dose. Animal pathological tests also proclaimed that no abnormalities to the vital organs such as the liver, heart, lungs, stomach, intestines, kidneys. Based on these observations, the LD50 value of propolis

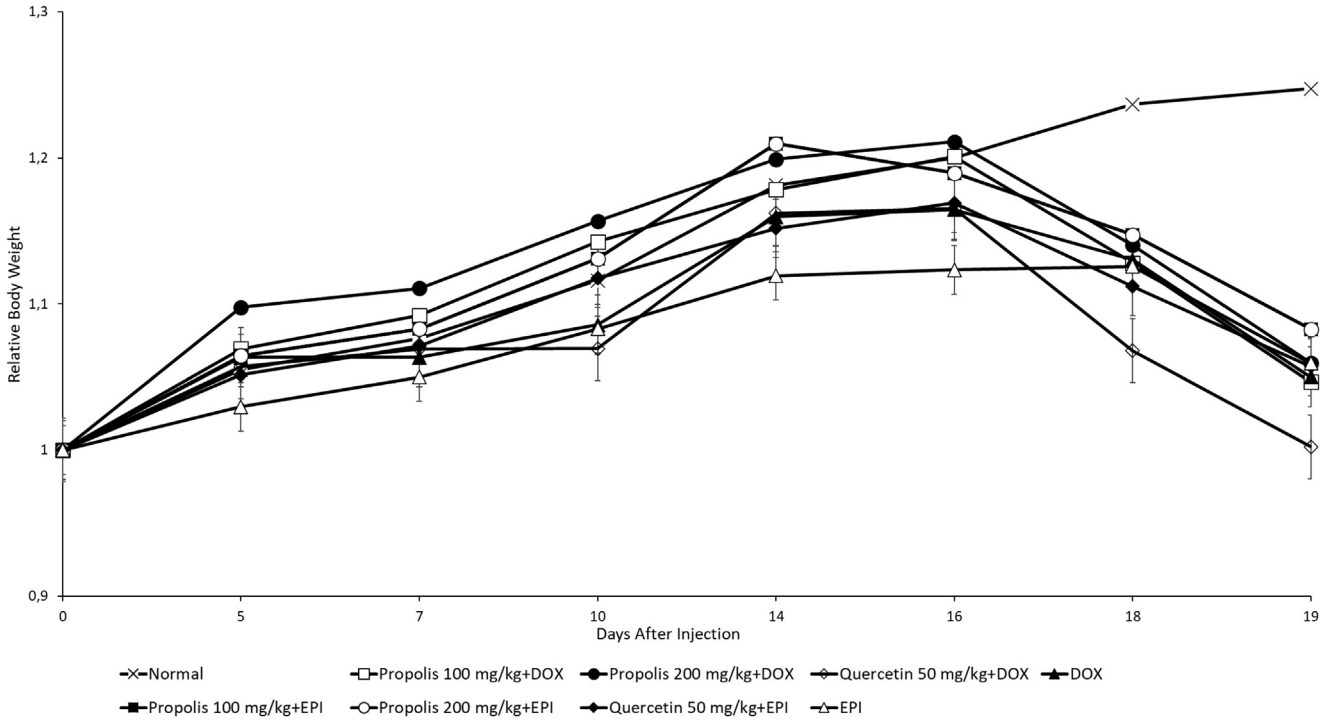


Fig. 2. The body weight relative of rats for 19 days.

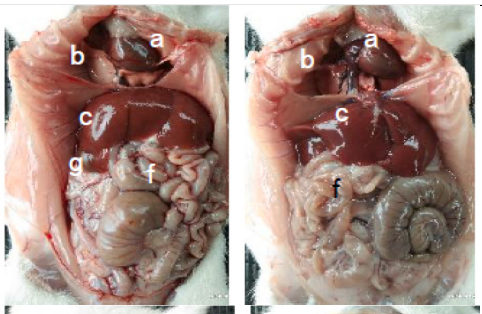
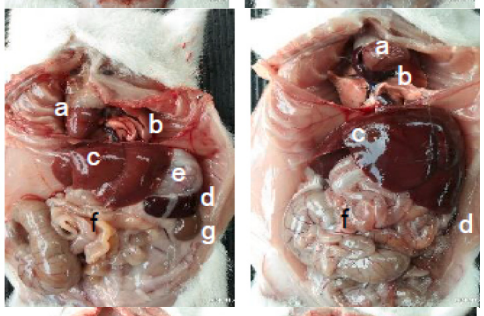
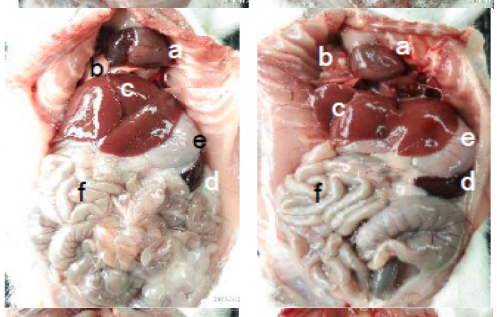
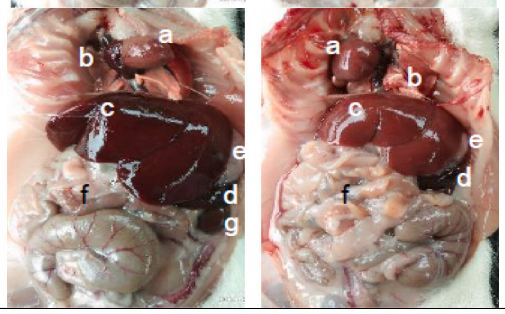
No.	Group	Picture	Result
1	50 mg/kg		No abnormalities
2	300 mg/kg		No abnormalities
3	2000 mg/kgBB		No abnormalities
4	5000 mg/kgBB		No abnormalities

Fig. 3. The result of propolis toxicity acute.

is ≥ 5000 mg/kg and the toxicity level of South Sulawesi propolis is classified as a practically non-toxic compound for Sprague Dawley rats.

The measurement results intimated that the ALT and AST values in the group of rats given propolis at a dose of 200 mg/kg had the smallest ALT value and the difference was significant in the normal group as a control. Furthermore, for the dose of propolis 100 mg/kg, the ALT value was not much different from the positive control, but both revealed significant differences in the normal group as a control. Based on that, propolis from South Sulawesi with a dose of 100 mg/kg and 200 mg/kg has the potential to provide a hepatoprotective effect, where the flavonoid compounds contained in it act as antioxidants that caused low ALT values. Antioxidants in propolis are believed to prevent hepatocyte damage due to lipid peroxidation produced by doxorubicin and epirubicin. The previous study proclaimed the extract propolis from South Sulawesi had antioxidant effects. The propolis was examined for xanthine oxidase (XO) inhibitory activity and its exhibited XO inhibitory activity (Miyata et al., 2019). Furthermore, the AST values obtained did not show significant differences according to the ANOVA test. The absence of this significant difference is due to AST levels obtained that do not represent the condition of the liver only

because AST in the blood can originate from other organs such as the heart, skeletal muscles, kidneys, and brain (Woreta and Alqahtani, 2014).

The negative control conveyed that the ALT value was smaller than the ALT value of the normal group rats. This probably due to the doses of doxorubicin and epirubicin too small to cause toxicity to the liver. The phenomena indicated by the bodyweight of rats during propolis and doxorubicin administration. Bodyweight is a parameter that can be used to determine the effect of drugs on test animals. Statistical test results by ANOVA test continued with the Dunnett test in which the relative weight of the normal group as a control revealed there was no significant difference in each treatment to the control.

The usage of 9 mg/kg dose in this study based on previous research that claimed sufficient enough to create a toxic effect, but the types of rat that used were other strain rats and have different body weights (Dobbs et al., 2003; Chaa et al., 2019). There are various studies on the effects of toxicity on doxorubicin and epirubicin on the liver, where each study used different doses with different methods. Most likely the dosage of doxorubicin and epirubicin used is not large enough to cause a toxicity effect on the liver because the use of rats strains and the body weight can affect the dose that must be used.

The selection of types of doxorubicin and epirubicin used also affect the toxicity caused. There are two types of doxorubicin and epirubicin drugs, which are pure and encapsulated. Doxorubicin and epirubicin hydrochloride are the types of doxorubicin and epirubicin that have been encapsulated in the liposome where the surface is coated by methoxy-polyethylene glycol which moves to tumor cells to a certain extent escaped from immune control, promoting the release of drug drugs in which continues for a longer time and reduces drug content in the liver tissue (Prasanna et al., 2020). However, it is necessary to have other parameters besides ALT and AST values to more ensure the toxicity effects produced by doxorubicin and epirubicin.

5. Conclusion

100 mg/kg and 200 mg/kg dose of propolis can suppress ALT levels in the blood below the normal ALT levels, based on that propolis has the potential hepatoprotective effect. The hepatoprotective effects caused by some phenolic acid which had found in propolis as antioxidant. Based on DPPH test Sulawesi propolis also include as one of strong antioxidant. One suggestion for advanced research in the future is to use more than one liver damage parameter such as Gamma-glutamyl transferase (GGT) levels and Malondialdehyde (MDA) levels.

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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