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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

IMMUNOMODULATORY EFFECT OF INDONESIAN PROPOLIS IN PREGNANT MICE: A PRELIMINARY RESULT

Endonezya Propolis'in Hamile Farelerde İmmünomodülatör Etkisi: Bir Ön Sonuç

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ABSTRACT

Propolis has been widely accepted to exhibit immunomodulatory activity. However, the activity during pregnancy has not been investigated yet. This study was a preliminary study that aimed to analyze the effect of several types of Indonesian propolis on the histological changes of maternal mice spleen. A total of 25 pregnant mice were divided into 5 groups, control (1% Tween 80) group, low (380 mg/kg) and high dose (1400 mg/kg) ethanol extract of South Sulawesi propolis groups, and low (380 mg/kg) and high dose (1400 mg/kg) water extract of Banten propolis groups. Propolis samples were administered daily during pregnancy. At day 18 of gestation, the mice were sacrificed to obtain spleen which was used for histological evaluation using hematoxylin and eosin staining. The number and diameter of white pulp were observed under the 10x magnifying of microscope. The results showed that all propolis extracts at low dose significantly increased the number of white pulp ($p < 0.05$). However, an increase in the diameter was found not significant in all propolis administered groups. This study suggests that Indonesian propolis may modulate maternal immune system.

Keywords: Immunomodulatory, Histology, Pregnancy, Propolis

ÖZ

Propolisin immünomodülatör aktiviteye sahip olduğu yaygın olarak kabul edilmiştir. Bununla birlikte, hamilelik sırasındaki aktivite henüz araştırılmamıştır. Bu çalışma, hamilelik sırasında propolis uygulamasının anne fare dalağının histolojik değişiklikleri üzerindeki etkisini analiz etmeyi amaçladı. Toplam 25 hamile fare, Güney Sulawesi propolisinin kontrol (%1 Tween 80), düşük (380 mg/kg) ve yüksek doz (1400 mg/kg) etanol özütü ve düşük (380 mg/kg) ve yüksek dozlu (1400 mg/kg) Banten propolisinin su özütü olmak üzere 5 gruba ayrıldı. Propolis uygulaması gebelik boyunca günlük olarak yapıldı. Gebeliğin 18. gününde fareler, hematoksilen ve eozin boyaması kullanılarak histolojik değerlendirme için kullanılan dalak elde etmek için sakrifiye edildi. Beyaz pulp sayısı ve çapı 10x büyütmeli mikroskop altında gözlendi. Sonuçlar, düşük dozdaki tüm propolis ekstraktlarının beyaz

pulp sayısını önemli ölçüde arttırdığını gösterdi (p < 0.05). Bununla birlikte, propolis uygulanan tüm gruplarda çaptaki bir artış anlamlı bulunmadı. Bu çalışma, Endonezya propolisinin annenin bağışıklık sistemini modüle edebileceğini düşündürmektedir.

Anahtar kelimeler: Immunomodulatori, Histoloji, Hamilelik, Propolis

GENİŞLETİLMİŞ ÖZET

Amaç: Propolis, yoğun fitokimyasal bileşenleri nedeniyle çeşitli sağlık yararları olan doğal bir üründür. İmmünomodülatör aktivite, araştırmacılar tarafından geniş çapta rapor edilen propolisin ana aktivitelerinden biridir. Ancak gebelikte yapılan inceleme henüz değerlendirilmemiştir. Bu çalışma, günlük Endonezya propolis uygulamasının anne fare dalağının histolojik değişiklikleri üzerindeki etkisini değerlendirmeyi amaçladı.

Gereç ve Yöntem: Toplam 25 gebe fare kullanıldı ve kontrol grubu (%1 Tween 80), Güney Sulawesi'nin düşük (380 mg/kg) ve yüksek doz (1400 mg/kg) propolis etanol özü ve Banten'in düşük (380 mg/kg) ve yüksek doz (1400 mg/kg) propolisin su özütü grubu olmak üzere 5 gruba ayrıldı. Propolis, gebeliğin 0. gününden 18. gününe kadar günlük olarak uygulandı. Uygulamanın sonunda, fareler dalak elde etmek için sakrifiye edildi. Dalak, hematoksilen ve eozin boyama ile muamele edildi ve beyaz pulpanın sayısını ve çapını hesaplamak için mikroskop altında 10x büyütme ile gözlendi.

Bulgular: Bu çalışma, yalnızca düşük doz dışında tüm propolis ekstraktlarının beyaz pulp sayısını önemli ölçüde artırdığını gösterdi (p < 0.05). Ancak, propolis uygulanan tüm gruplarda beyaz pulp çapındaki artış anlamlı değildi (p > 0.05). Ayrıca, tüm gruplarda spesifik histopatolojik değişiklikler gözlenmedi.

Sonuç: Bu çalışma, düşük doz Endonezya propolisinin (380 mg/kg) annenin bağışıklık sistemini aktive edebileceği sonucuna varmıştır. Bununla birlikte, yüksek dozda (1400 mg/kg) alanlar daha az önemli değişiklikler göstermiştir. Bu çalışmada sadece histolojik değerlendirme kullanıldığı düşünülerek daha fazla çalışma yapılması gerektiği kanısına varılmıştır.

INTRODUCTION

Propolis is a bee product derived from resins of various plants and provides wide range of health benefits (Sforcin et al. 2017). Propolis is actually used by bees for self-defense and nest construction (Mohammadzadeh et al. 2007). Nonetheless, propolis has been used for centuries in the fields of medicine due to its pharmacological properties, such as anti-microbial, anti-ulcer, anti-inflammatory, antioxidant, anti-tumor, and cytotoxic activity (Fikri et al. 2019b, Król et al. 2013, Rao Muvva et al. 2021, Sevim et al. 2021). Moreover, several studies have reported propolis is safe for consumption (Burdock 1998).

The evaluation of propolis on pregnant conditions has attracted considerable attention since it has a great potential on supporting physiological well-being in pregnancy. Our previous study showed propolis has a potential to suppress emesis during early stage of pregnancy (Fikri et al. 2018). Also, propolis (380 mg/kg) has been reported to support fetal development and did not show maternal toxicity in mice (Fikri et al. 2019a, Fikri et al. 2021). In addition, propolis may improve the pregnancy outcomes and placental oxidative stress of diabetic rats (Usman et al. 2018).

However, the effect of propolis on the maternal immune system has limited evidence. On the other hand, the immunomodulatory property is one of the main activities of propolis (Sforcin, 2007). An adequate immune system is important to prevent maternal and fetal infection (Morelli et al. 2015). However, over-activation of the maternal immune system may lead to fetal rejection and other negative pregnancy outcomes (Burwick et al. 2021). Thus, this study aimed to evaluate histological changes in the spleen after the administration of Indonesian propolis during pregnancy.

MATERIALS AND METHODS

Propolis Preparation

Propolis samples originated from Banten Province, and South Sulawesi Province produced by *Tetragonula laeviceps* and *Tetragonula biroi*, respectively. Ultrasound-assisted extraction was applied to obtain two types of propolis extracts,

water extract of Banten propolis and ethanol extract of South Sulawesi propolis which were previously described to be potential in the treatment of emesis during pregnancy (Fikri et al. 2018). The procedure of extraction followed the method of Fikri et al. (2018). The propolis sample was cut into small 0.5 cm pieces and subsequently dissolved in a solvent at a ratio of 1:10 Ultrasound was applied for 3 hours and the samples were evaporated until dry. Water extract of Banten popolis contained phenolics (15.64 mg/g), flavonoids (1.80 mg/g), and IC_{50} of 503.93 mg/ L, whereas ethanol extract of South Sulawesi propolis found to be higher in total phenolics (22.30 mg/g), and total flavonoids (3.39 mg/g) content, but lower in antioxidant activity with IC_{50} of 543.93 mg/L (Fikri et al. 2019b).

Animals

The present study used mice (*Mus musculus*) weighed 25-20 g and aged 8-10 weeks. Female mice at proestrus and estrus were mated with male mice for a night and the vaginal plug was checked in the next morning. If the vaginal plug was found, day 0 of pregnancy was determined. A total of 25 pregnant mice were equally divided into 5 groups to receive 1% Tween 80, ethanol extract of South Sulawesi propolis at low dose (380 mg/kg) and high dose (1400 mg/kg), and water extract of Banten propolis at low dose (380) and high dose (1400 mg/kg), respectively. Dose of 380 mg/kg is an active dose that commonly used in biological activity studies, whereas dose of 1400 mg/kg is a non-observed adverse effect level (NOAEL) of propolis (Burdock, 1998; Eda et al. 2005). Propolis sample was dissolved in 1% Tween 80 and administered in 5 ml/kg daily during pregnancy. The mice were sacrificed at day 18 of gestation using 10% ketamine and 2% xylazine at a ratio of 20:1. Spleen was harvested after laparotomy and fixed with 10% neutral buffer formalin.

Histological Evaluation

Histological evaluation was performed using hematoxylin and eosin (HE) staining following the method of Pillai et al. (2011). The spleen was further dehydrated with alcohol and cleaned with xylene. It was then infiltrated with paraffin and cut using a microtome at a thickness of 4-6 µm. The number and diameter of white pulp were calculated under a microscope with a 10x magnify and Java Image J program.

Data Analysis

Data were reported as mean ± standard deviation. The differences between the groups were determined using ANOVA with Duncan's multiple range tests. A significant difference was determined at a p-value < 0.05.

RESULTS

Our result indicates that propolis administration during pregnancy could affect the immune system of mice. Low dose of both types of extract significantly increased the total number of white pulps ($p<0.05$). However, high dose extracts did not change the number. In addition, a non-significant increase of white pulp diameter was seen in all groups administered with propolis ($p>0.05$) (Table 1). According to univariate analysis of variance, the type and dosage of propolis had significantly effect on the number of white pulps ($p<0,05$). Regardless of the dosage, the number of white pulps were found to be higher in group administered with water extract of Banten propolis compared to ethanol extract of South Sulawesi propolis. Meanwhile, regardless of the type of propolis, higher number of white pulps were notably observed in low dose group. Both the type and dosage of propolis, and its interaction did not have significant effect on the diameter of white pulp (Table 2). Histopathologically, no specific changes in the spleen were observed in all groups. The histological section of the mice's spleens can be seen in Figure 1.

Table 1. Number and diameter of maternal white pulp after propolis administration

Groups	Number	Diameter (µm)
Control	9.40 ± 1.82[c]	326.18 ± 51.12[a]
Low dose EE	13.60 ± 2.61[ab]	391.95 ± 31.36[a]
High dose EE	9.33 ± 3.78[c]	376.42 ± 68.85[a]
Low dose WE	19.00 ± 2.83[a]	348.19 ± 91.42[a]
High dose WE	10.25 ± 2.75[c]	395.13 ± 84.03[a]

*Superscript with the different letter in the same column shows significant different at p-value < 0.05 using ANOVA with Duncan's multiple range test

Control : 1% Tween 80

Low dose EE : Ethanol extract of South Sulawesi propolis (380 mg/kg)

High dose EE : Ethanol extract of South Sulawesi propolis (1400 mg/kg BB)

Low dose WE : Water extract of Banten propolis (380 mg/kg BB)

High dose WE : Water extract of Banten propolis (1400 mg/kg BB)

Table 2. Univariate analysis of variance among factors

Factors	p-value	
	Number of white pulps	Diameter of white pulps
Type of propolis	0.046	0.757
Dosage	0.001	0.327
Interaction	0.143	0.551

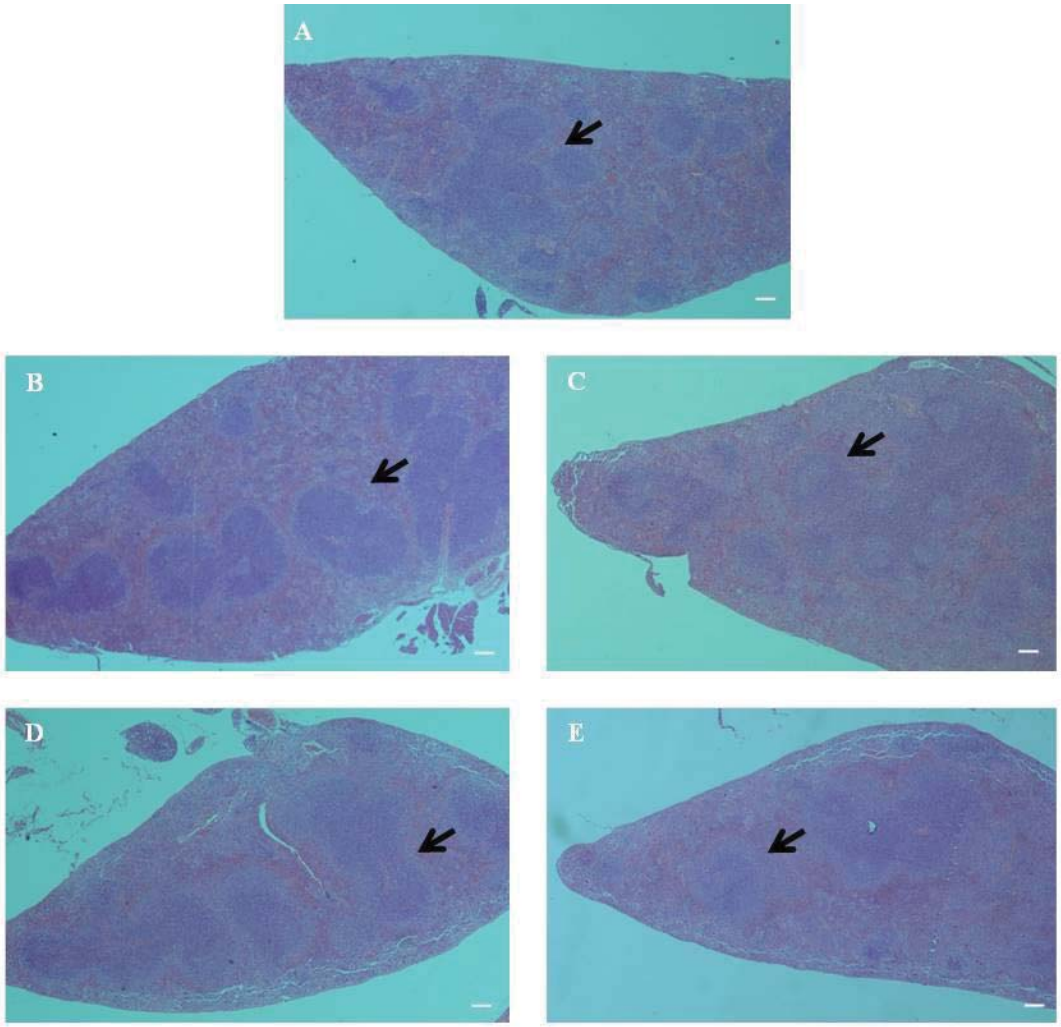

Figure 1. Histological section of maternal mice spleen at day 18 of gestation after propolis administration during pregnancy (hematoxylin and eosin staining; black arrow shows white pulp; 10x magnifying). A: control group (1% Tween 80); B: low-dose of ethanol extract of South Sulawesi propolis (380 mg/kg); C: high-dose of ethanol extract of South Sulawesi propolis (1400 mg/kg); D: low-dose of water extract of Banten propolis (380 mg/kg); E: high-dose of water extract of Banten propolis(1400 mg/kg).

DISCUSSION

Spleen is the largest immune organ in the body which responsible for immune system activation. The spleen is composed of two main compartments, the red pulp and the white pulp (Cesta 2006). The red pulp contains a large number of macrophages which efficiently remove foreign materials, cellular debris, and aging erythrocyte. Likewise, the white pulp contains a periarteriolar lymphatic sheath (PALS), lymphoid follicles, and a marginal zone, where the adaptive immune response can be initiated (Mebius & Kraal, 2005).

Our study suggests that propolis may activate the immune system during pregnancy. An increase in the number and diameter of the white pulp indicates the activation of the immune system (Horton & Manning 1974, Makiyah et al. 2014). This is actually not a surprising result because a lot of studies report the immunomodulatory action of propolis (Al-Hariri 2019, Conti et al. 2016, Dimov et

al. 1991, Oršolić & Bašić 2003). Using the same samples, Kalsum et al. (2017) described *Trigona* propolis at a low dose (0.16%) could regulate the nitric oxide, IgG antibody production, and phagocytic index of rats infected with *Staphylococcus aureus,* whereas high-dose (1.44%) did not make any changes. Moreover, propolis at high dose may act as an immunosuppressant where the results of the previous study analyzing the effect of propolis on the concentrations of CD4, CD8 T cells, and T cell memory (Rohmawati & Rifa'i 2014).

As the immune system highly affects the maternal and fetal health, the activation of maternal immune system by propolis should be further discussed. An inadequate maternal immune system may increase the risk of infection, whereas over-activation may cause fetal rejection (Burwick et al. 2021, Morelli et al. 2015). However, instead of causing negative outcomes, the activation of the immune system as the present study found might support fetal development. Our previous studies which used the same type and dose showed that propolis did not inhibit fetal development and did not show any indication of maternal toxicity (Fikri et al. 2019a; Fikri et al. 2021). Specifically, the present results are in line with our previously published study reporting that water extract of Banten propolis had better pregnancy outcomes than ethanol extract of South Sulawesi propolis (Fikri et al. 2019a). Using pure water in propolis extraction may produce an extract with less wax and resin content leading to less concentrated phytochemical compounds. In addition, propolis extracts prepared using water (100% and 80%) may not contain gallic acid and *p*-OH benzoic acid compared to ethanol extract (Kara et al. 2022). However, the extract may have more vitamins and minerals that extracted from bee pollen (Najafi et al. 2007). Nevertheless, water extract of Banten propolis was reported to possess higher antioxidant activity than the ethanol extract (Fikri et al. 2019b). Kara et al. (2022) found that caffeic acid phenethyl ester (CAPE) content was higher in the water extract than ethanol extract. Thus, water extract of Banten propolis might be more tolerated in pregnant condition which having favorable outcomes compared to the ethanol extract of South Sulawesi propolis. Moreover, propolis at high dose provides no beneficial effect. The previous study showed that propolis extracts at 1400 mg/kg disrupted placental development and caused intrauterine growth retardation (Fikri et al. 2019a). Therefore, consuming propolis at high dose are not recommended during pregnancy.

Conclusion

This preliminary study concludes that the maternal immune system may be modulated by all samples of Indonesian propolis. Moreover, remarkable activation was found in the group administered with propolis at low dose (380 mg/kg), whereas propolis at high dose (1400 mg/kg) did not provide beneficial effect. However, the results were only based on histological evaluation of the spleen, thus further investigation needs to be done.

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Conflict of interest: The authors declare no potential conflict of interest

Ethics issue: All protocols have been approved by Animal Care and Use Committee, IPB University

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