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Purified Gambier (*Uncaria gambir* Roxb.) and Propolis Performance in Male White Mice (*Mus musculus* L.) Antibody Titer with Measles Vaccine

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ABSTRACT

Gambier (Uncaria gambir Roxb.) and propolis showed a beneficial effect on infectious diseases. This study aims to examine the effect of gambier and propolis on the antibodies of white male mice given measles vaccine using the antibody titer method, number, and percentage of different types of leukocytes. Thirty white male mice were used and grouped into six groups (100 mg/kg BW of purified gambier, 200 mg/kg BW of purified gambier, 200 mg/kg BW of purified gambier + 195 mg/kg BW of propolis, 195 mg/kg BW of propolis, 0.5% Na CMC and no treatment). After 14 days of preparation, each group was vaccinated with measles vaccine. On the 28th day, the antibody titer, total leukocyte cells, and the percentage of leukocyte cell types were determined. The results showed that gambier and propolis could increase antibody titer of male white mice. The purified gambier group at a dose of 200 mg/ kg BW + propolis 195 mg/ kg BW was the most optimal compared to the other doses. There was a significant difference ($p < 0.05$) between antibody titer, the total leukocyte cells, and the percentage of lymphocytes of mice that received the combination of purified gambier and propolis. It can be concluded that purified gambier can increase antibody titer, the number of leukocytes, and the percentage of lymphocytes of male mice.

Keywords: *antibody titer, purified gambier, propolis, measles vaccine*

1. INTRODUCTION

Several indicators determine the public health status in a country. Among significant indicators of health status are maternal mortality rate, infant mortality rate, and nutritional status. In Indonesia, the infant mortality rate may be caused by infection, asphyxia, and vaccine-preventable diseases [1].

Among the diseases that belong to vaccine-preventable diseases are Measles and Rubella. Measles is a highly infectious disease caused by Morbillivirus. Since 2000, more than 1 billion children in high-risk countries have been vaccinated through immunization programs, causing a 78% decrease in the death rate. Indonesia is one of the countries with the most cases of Measles in the world. Rubella is caused by the Togaviridae family, genus Rubivirus. The virus can pass through the placenta blood barrier, infect the fetus, and cause abortus or congenital rubella syndrome [1].

From 2010-2015, it is estimated that there were 23.164 cases of measles and 30.463 cases of rubella. These numbers may not reflect the actual number due to a high number of unreported cases and low surveillance reports. The Indonesian government has a national immunization program to control the cases of measles and rubella [1].

Measles-Rubella (MR) vaccine is a vaccine that is used to prevent measles and rubella in the population. MR vaccine is a live attenuated vaccine in dry powder form with a solvent [2,3]. Each vial of the MR vaccine contains ten doses. Each dose of MR vaccine contains 1000 CCID50 measles virus and 1000 CCID50 rubella virus. The vaccine is used subcutaneously at a dose of 0.5 ml. On the top of the vaccine vial, there is an indicator called Vaccine Vial Monitor. The only vaccine with VVM A or B category that can be used [4].

Although vaccines have successfully eradicated several viral infections, immune responses to vaccination are highly variable. Often, vaccine immunogenicity is suboptimal in specific populations more prone to diseases, including infants and people who live in low-income/middle-income countries [5,6].

Several strategies such as vitamins, minerals, and probiotics enhanced immunity towards viral infections [6]. Besides, other bioactive natural products, such as gambier and propolis, also have an immunomodulatory effect. Gambier which is an extract obtained from *Uncaria gambir (Hunter) (Roxb))* contains catechin [7,8], which showed an immunomodulatory effect [9,10]. Propolis, a resin collected by honey bees, contains polyphenols, phenolic aldehyde, sesquiterpene, quinines, coumarins, amino acids, steroids, and inorganic compounds. The immunomodulatory activity of propolis is exhibited by flavonoids and phenolic acids, mainly caffeic acid phenethyl esters and artepillin C (3,5-diprenyl-4-hydroxycinnamic acid) [11].

This study aims to examine the effect of gambier and propolis on the antibodies of white male mice given measles vaccine using the antibody titer method, number, and percentage of different types of leukocytes.

2. MATERIALS AND METHODS

2.1. Animal preparation

Thirty male white mice (2-3 months old, 20-30 g) which have not been treated with any drug were prepared. The mice were acclimated for seven days for adaptation purposes. Then, the mice were grouped into six treatment groups.

2.2. Preparation of purified gambier suspension

Purified gambier was obtained from PT. Andalas Sitawa Fitolab, Padang, Indonesia. The purified gambier was given in the dose of 100 mg/kg BW and 200 mg/kg BW [10]. The purified gambier (PT. Andalas Sitawa Fitolab, Indonesia) was suspended in 0.5% Na CMC.

2.3. Preparation of propolis

The propolis that is used is liquid propolis (PT. Melia Sehat Sejahtera, Indonesia).

2.4. Animal treatment

Each group of mice was given treatment described as below:

Group I: treated with 0.5%NaCMC for 14 days, then were evaluated

Group II: treated with 0.5% NaCMC for 14 days, vaccinated with MR vaccine (Health Office, Padang, Indonesia) on day 15, observed for 14 days, then the mice were evaluated.

Group III: treated with purified gambier (100 mg/kg BW) for 14 days, vaccinated with MR vaccine on day 15, observed for 14 days, then the mice were evaluated.

Group IV: treated with purified gambier (200 mg/kg BW) for 14 days, vaccinated with MR vaccine on day 15, observed for 14 days, then the mice were evaluated.

Group V: treated with propolis (195 mg/kg BW) for 14 days, vaccinated with MR vaccine on day 15, observed for 14 days, then the mice were evaluated.

Group VI: treated with purified gambier (200 mg/kg BW) + propolis (195 mg/kg BW) for 14 days, vaccinated with MR vaccine on day 15, observed for 14 days, then the mice were evaluated.

2.5. Measurement of antibody titer

Preparation of goat erythrocytes

Goat blood was washed with physiological saline solution (Andeska Laboratory, Indonesia) (1:1), 5 ml each, and stirred until homogenous, then centrifuged (2000 rpm, 15 minutes). The supernatant was removed. These steps were repeated three times by adding 5 ml physiological saline solution for each repeat.

Goat erythrocytes were then incubated with the vaccine for 30 minutes. Then, 5% suspension of erythrocytes was prepared by adding 0,2 ml goat erythrocytes with physiological saline solution until 4 ml.

Preparation of antibody titer

After 24 hours from the last day of treatment (day 15), mice blood was collected by cutting the jugular vein, let for 30 minutes, and centrifuged (2000 rpm, 15 minutes). Ten test tubes were filled with 0.2 ml of the physiologic saline solution each. The first tube was added 0.2 ml of serum, mixed until homogenous. Then 0.2 ml of solution from the first tube was moved to the second tube. After that, 0.2 ml of solution from the second tube was moved to the third tube, mixed until homogenous. These steps were repeated until the tenth test tube. On the tenth test tube, 0.2 ml of solution was removed. The final dilution from the ten tubes was 1/8, 1/16, 1/32, 1/64, 1/128, 1/256, 1/512, 1/1024.

Then, 0.1 ml of 5% goat erythrocyte suspension was added to each test tube. The mixture was then centrifuged (2000 rpm, 5 minutes). The agglutination was observed. The titer number was determined by the highest dilution in which the serum was agglutinated with the goat erythrocytes.

The titer number was determined using this equation:

$$[\,2\log(titer)]$$

2.6. Determination of leukocyte percentage

Blood smears were prepared to determine the leukocyte percentage after seven days of treatment (with purified gambier, propolis, or combination). The mice blood was collected from the tail. Then the blood was dropped on the object-glass. Another object-glass was used to make the smear. The dried blood smear was then added with methanol and let them for 5 minutes. A 10% Giemsa solution was then added to the blood smear, then rested for 20 minutes. The blood smear was then washed by aqua dest (Andeska Laboratory, Indonesia), added immersion oil, and observed under the microscope.

2.7. Determination of leukocyte count

Fresh blood was pipetted by leukocyte pipette until 0.5 number, then added with Turk solution until reached the 11 number, then mixed for 3 minutes. One to two drops were removed. On the hemocytometer, one drop of blood was dropped. The leukocyte count was determined with this equation:

$$\text{Total leukocyte count} = \text{cell count x } \frac{20}{40}$$

2.8. Data analysis

The data were analyzed using one-way ANOVA. A significant difference was considered when $p<0.05$. This data analysis was continued with Duncan's post hoc test.

2.9. Ethical consideration

This study obtained ethical approval from the Research Ethic Committee, Faculty of Medicine Universitas Andalas (registration number 257/UN.16.2/KEP-FK/2021).

3. RESULTS AND DISCUSSION

This study examines the performance of purified gambier and propolis through three parameters: total leukocyte count, differential leukocyte percentage, and antibody titer. The results of those three parameters are described in Table 1.

Table 1. Determination of antibody titer

Treatment	Mean antibody titer	p-value
0.5% NaCMC [a]	1.00	0.000
MR Vaccine[b]	2.80	
Purified gambier 100 mg/kg BW[b]	3.40	
Purified gambier 200 mg/kg BW[c]	5.60	
Propolis 195 mg/kg BW[c]	5.60	
Purified gambier 200 mg/kg BW + Propolis 195 mg/kg BW[d]	7.60	

Table 1 showed the mean of antibody titer was higher compared to group 1 (NaCMC 0.5%). There was a significant difference in mean antibody titer between each group ($p<0.05$). According to Duncan's post hoc test, the mean antibody titer in the group that received purified gambier 200 mg/kg BW + Propolis 195 mg/kg BW was significantly different from other treatment groups (Figure 1).

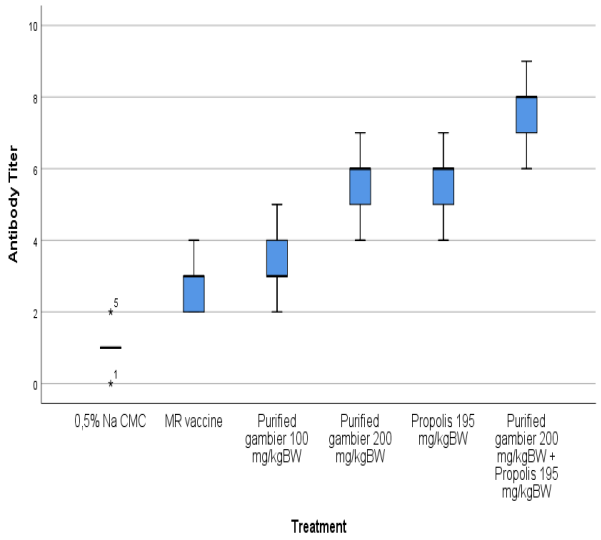

Figure 1. Mean of antibody titer in mice across different treatment groups

Measles vaccine can induce humoral and cellular immune responses, like natural measles virus infection. The antibodies first appear between 12 and 15 days after vaccination and typically peak at 21-28 days. This explains why the parameters were evaluated 14 days after being vaccinated.

The antibodies that are produced after immunizations are IgM, IgA, and IgG. IgM appears transiently in blood, IgA appears in mucosal secretions, and IgG stays in the blood for years. Apart from antibodies, vaccination also induced measles virus-specific CD4+ and CD8+ T-lymphocytes [12,13].

Meanwhile, catechin, which is one of the compounds abundant in gambier, increases immunoglobulin. A study in India suggested that catechin in the dose of 25, 50, and 100 mg/kg (p.o.) significantly increased the antibody titer of the rat model in the hemagglutination test. [14]. Propolis increases antibody production, which may contribute to its use as an adjuvant in vaccines [11,15].

Table 2. Determination of total leukocyte count

Treatment	Mean of total leukocyte count (/µL blood)	p-value
0.5% NaCMC [a]	7,190	0.000
MR Vaccine[b]	9,230	
Purified gambier 100 mg/kg BW[b]	9,470	
Purified gambier 200 mg/kg BW[c]	10,700	
Propolis 195 mg/kg BW[c]	10,740	
Purified gambier 200 mg/kg BW + Propolis 195 mg/kg BW[d]	11,990	

As shown in Table 2, the mean of total leukocyte count was higher compared to group 1 (NaCMC 0.5%). There was a significant difference in mean antibody titer between each group ($p<0.05$). According to Duncan's posthoc test, the mean antibody titer in the group that received purified gambier 200 mg/kg BW + Propolis 195 mg/kg BW was significantly different from other treatment groups (Figure 2).

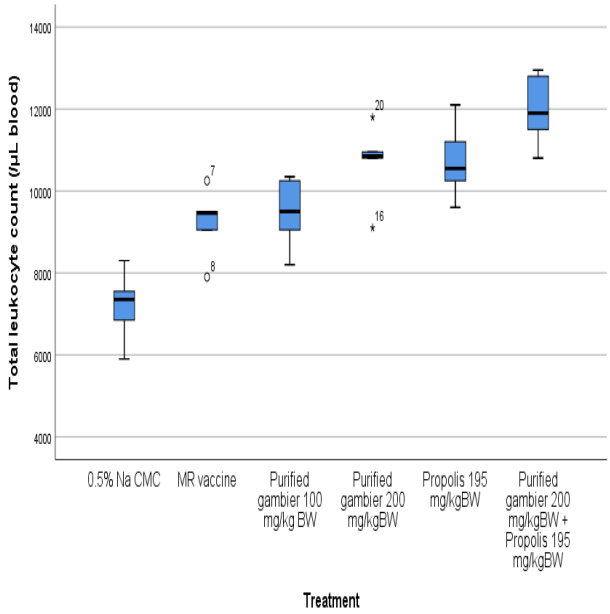

Figure 2. Mean of total leukocyte count in mice across different treatment groups

As described in Table 3, the segmented neutrophil, band neutrophil, lymphocyte, and monocyte were significantly different across treatment groups ($p <0.05$). After Duncan posthoc test was performed, the percentage of segmented neutrophils of purified gambier, propolis, and purified gambier+propolis group were different with 0.5% Na CMC and MR vaccine. Meanwhile, the percentage of band neutrophil in the group of purified gambier 200 mg/kg BW, 0.5% Na CMC, and MR vaccine were located at the same subset, although they were higher than purified gambier 200 mg/kg BW+propolis 195 mg/kg BW.

The lymphocyte percentage in mice that received purified gambier 200 mg/kg BW + Propolis 195 mg/kg BW was significantly different compared to groups that received purified gambier and propolis. The monocyte percentage in mice that received 0.5% NaCMC was located in a different subset than other groups, while the percentage was also higher.

A previous study suggested that propolis increases leukocyte count in white Wistar rats [16]. In a study conducted in Korea, CAPE in propolis decreased the total leukocyte, eosinophil, lymphocyte, and macrophage, suggesting its effect in attenuating asthmatic reaction [17]. This may explains the finding in this study, in which the eosinophil in propolis group is lower than other group, although not significant.

Table 3. Differential leukocyte percentage across treatment groups

Treatment	Differential leukocyte percentage (%)				
	Segmented neutrophil	**Band neutrophil**	**Lymphocyte**	**Monocyte**	**Eosinophil**
NaCMC 0.5%	27.40	27.00	29.80	10.40	5.00
MR Vaccine	30.20	28..00	38.40	2.00	1.40
Purified gambier 100 mg/kg BW	33.20	24.40	39.20	2.20	1.40
Purified gambier 200 mg/kg BW	34.40	18.00	43.40	2.80	1.40
Propolis 195 mg/kg BW	35.80	17.20	43.80	1.40	1.80
Purified gambier 200 mg/kg BW + Propolis 195 mg/kg BW	37.60	11.20	48.20	3.43	1.20
p-value	**0.000**	**0.000**	**0.000**	**0.000**	**0.079**

4. CONCLUSIONS

The administration of purified gambier and propolis can significantly increase the antibody titer, total leukocyte count, and lymphocyte.

AUTHORS' CONTRIBUTIONS

Lailaturrahmi: providing feedback to study methodology and discussion, writing the manuscript

Rabani Ahmad: prepared materials, conducting the experiment, data analysis and interpretation

Dwisari Dillasamola: study conception, supervision, providing feedback to the study methodology and data analysis

Almahdy A: study conception, supervision, providing feedback to the study methodology and data analysis

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REFERENCES

[1] Indonesian Ministry of Health. Situasi Campak dan Rubella di Indonesia 2018 (Measles and Rubella Situation in Indonesia 2018). Kemenkes RI 2018:1–10.

[2] Moss W, Scott S. WHO immunological basis for immunization series: module 20: Measles. World Health Organization; 2008.

[3] Lanzieri T, Haber P, Icenogle J, Patel M. Rubella. Epidemiol Prev Vaccine-Preventable Dis 2021:301–14. https://doi.org/10.1002/9781119467748.ch11.

[4] Kemenkes RI (Ministry of Health Indonesia). Petunjuk Teknis Kampanye Imunisasi Measles Rubella (MR)-Technical Guidance of Measles Rubella (MR) Immunization Campaign. 2017.

[5] Lynn DJ, Benson SC, Lynn MA, Pulendran B. Modulation of immune responses to vaccination by the microbiota: implications and potential mechanisms. Nat Rev Immunol 2021;0123456789. DOI: https://doi.org/10.1038/s41577-021-00554-7.

[6] Jayawardena R, Sooriyaarachchi P, Chourdakis M, Jeewandara C, Ranasinghe P. Enhancing immunity in viral infections, with special emphasis on COVID-19: A review. Diabetes Metab Syndr Clin Res Rev 2020;14:367–82.

[7] Ani Isnawati, Mariana Raini, Ondri Dwi Sampurno, D. Mutiatikum, Lucie Widowati RG. Characterication of 3 Types Gambir Extract ((Uncaria gambir Roxb). Bul Penelit Kesehat 2012;40:201–8.

[8] Kurniatri AA, Sulistyaningrum N, Rustanti L. Purifikasi katekin dari ekstrak gambir. Media Litbangkes 2019;29:153–60.

[9] Pambayun R, Gardjito M, Sudarmadji S, Kuswanto KR. Kandungan fenol dan sifat antibakteri dari berbagai jenis ekstrak produk gambir (Uncaria gambir Roxb) Phenolic content and antibacterial properties of various extracts of gambir (Uncaria gambir Roxb). Maj Farm Indones 2007;18:141–6.

[10] Musdja MY. Immunomodulatory Effect, Antibacterial Activity of Materials and the Mixture Materials of Betel Chewing and Comparative of Composition Between Betel Leaf Essential Oil with the Mixture Materials of Betel Chewing. University of Indonesia, 2012.

[11] Wolska K, Górska A, Antosik K, Ługowska K. Immunomodulatory effects of propolis and its components on basic immune cell functions. Indian J Pharm Sci 2019;81:575–88. https://doi.org/10.36468/pharmaceutical-sciences.548.

[12] Ovsyannikova IG, Dhiman N, Jacobson RM, Vierkant RA, Poland GA. Frequency of measles virus-specific CD4+ and CD8+ T cells in subjects seronegative or highly seropositive for measles vaccine. Clin Diagn Lab Immunol 2003;10:411–6. DOI: https://doi.org/10.1128/cdli.10.3.411-416.2003.

[13] Wong-Chew RM, Islas-Romero R, García-García M de L, Beeler JA, Audet S, Santos-Preciado JI, et al. Induction of cellular and humoral immunity after aerosol or subcutaneous administration of Edmonston-Zagreb measles vaccine as a primary dose to 12-month-old children. J Infect Dis 2004;189:254–7. https://doi.org/10.1086/380565.

[14] Ganeshpurkar A, Saluja AK. Protective effect of catechin on humoral and cell mediated immunity in rat model. Int Immunopharmacol 2018;54:261–6. https://doi.org/https://doi.org/10.1016/j.intimp.2017.11.022.

[15] Scheller S, Gazda G, Pietsz G, Gabrys J, Szumlas J, Eckert L, et al. The ability of ethanol extract of propolis to stimulate plaque formation in immunized mouse spleen cells. Pharmacol Res Commun 1988;20:323–8. https://doi.org/10.1016/s0031-6989(88)80068-7.

[16] Radiati LE, Umam K, Awwaly A, Kalsum U, Jaya F. Pengaruh Pemberian Ekstrak Propolis Terhadap Sistem Kekebalan Seluler Pada Tikus Putih. J Teknol Pertan 2008;9:2.

[17] Jung W-K, Lee D-Y, Choi YH, Yea SS, Choi I, Park S-G, et al. Caffeic acid phenethyl ester attenuates allergic airway inflammation and hyperresponsiveness in murine model of ovalbumin-induced asthma. Life Sci 2008;82:797–805. DOI: https://doi.org/10.1016/j.lfs.2008.01.014.