

Original Research Article

Effect of aqueous infusion of *Plectranthus amboinicus* (LOUR) Spreng leaves on humoral immune system in rats

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Sent for review: 9 May 2022

Revised accepted: 29 April 2023

Abstract

Purpose: To examine the effect of an aqueous infusion of *Plectranthus amboinicus* (AIP) on the immunological development of rats in response to Bacille Calmette-Guerin (BCG) antigen induction.

Methods: Twenty-four male rats, aged 3 months, were separated into six groups. Group 1 was normal control group without treatment, whereas groups 2 and 3 were the treatment groups induced with 100 μ L BCG on days 7 and 14, followed by oral administration of AIP at doses of 19 and 31.5 g/kg, respectively. Groups 4 and 5 were treated only with AIP at doses of 19 and 31.5 g/kg respectively. Group 6 was a negative control group induced with 100 μ L BCG on days 7 and 14. On day 31, IgG activity, antibody titer, lysozyme activity, relative organ weight, and lung histology were determined.

Results: The results showed that IgG levels, antibody titers, and lysozyme activity were significantly elevated in groups 4, 5, and 6 compared to normal control group ($p < 0.05$). Aqueous infusion of *Plectranthus amboinicus* and BCG had no effect on the relative weight of spleen. However, the therapy administered to rats in group 3 significantly increased their liver and lung mass ($p < 0.05$).

Conclusion: Aqueous infusion of *Plectranthus amboinicus* leaves stimulates the immune system in BCG-induced rats.

Keywords: *Plectranthus amboinicus*, Bacille Calmette-Guerin, IgG, IgM, lysozyme

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INTRODUCTION

Numerous medicinal plants have been utilized for treating a variety of illnesses, especially in Indonesia. *Mucuna pruriens*, for example, has been used as a treatment for Parkinson's disease [1]. *P. amboinicus* Lour is a member of the Lamiaceae family and has been used by the Batak Tribe for centuries to boost breast milk production. After various research, it was reported that *P. amboinicus* had been utilized

specifically as a hepatoprotective, anti-diabetic, cholesterol-reducing, and antioxidant [2,3] medicine. In addition, *P. amboinicus* exhibited immunostimulant activity in sheep red blood cells as an antigen-treated model [4], and previous research indicated that aqueous extract of *P. amboinicus* leaf was able to increase leukocyte and erythrocyte production and maintain blood profile in BCG-induced rats [5]. Furthermore, supplementing chicken feeds with *P. amboinicus* leaf flour exhibited greater

activity to reduce cholesterol levels in the blood and chicken flesh [6].

Plectranthus amboinicus works as a possible immunostimulant due to the presence of flavonoids and other phytochemicals [7]. Flavonoids are phytochemicals that have been shown to influence the gut immune system's function [8]. *Plectranthus amboinicus* contains the flavonoid class, which includes quercetin, luteolin, and apigenin. In this instance, apigenin demonstrates antiviral and immunostimulant properties [9]. While luteolin has demonstrated anti-inflammatory capabilities [10], and quercetin effectively stimulates the immune system against cancer [11]. In this case, the antigen also plays a role in increasing the activity of the body's immune system such as Bacille Calmette-Guerin (BCG). The BCG is a TB vaccine consisting of an attenuated tuberculosis bacillus (*Mycobacterium bovis*) that boosts IgG and IgM levels [12]. On the bases of this information, this study assesses the effect of the combination of AIP and BCG, or BCG alone, on the humoral immune system of rat models.

EXPERIMENTAL

Plant material collection

Plectranthus amboinicus leaves were obtained from the medicinal plants garden at Universitas Negeri Medan's, Faculty of Mathematics and Natural Sciences. The plants were plucked for their leaves, washed, and air-dried for one night. The leaves were dried in a drying cabinet before being mashed using a blender.

Preparation of aqueous infusion

Dried powdered *P. amboinicus* leaves (1000 g) were blended with 5000 mL of distilled water (1:5) in a boiling pan and then cooked over a water bath for 30 min at 90 °C with intermittent stirring. The solution was filtered 30 min later. Four cycles of filtration were performed to produce the desired solution [13].

Animals

Twenty-four male rats weighing approximately 180 - 200 g were divided into six groups and subsequently acclimatized for 1 week. Rats were housed in a typical room temperature setting with a constant relative humidity cycled every 12 h. The rats were fed a conventional laboratory pellet diet supplemented with distilled water. Prior to the investigation, 1 week of acclimation

of the test animals was performed. Animal care and handling conformed to accepted guidelines [14]. This study was approved as per Ethical Agreement no. 195/KEPH-FMIPA/2016.

Chemicals and reagents

Various chemicals and reagents were used in this study such as Elisa Rat IgG Kit (Cat No. E111-100-Sigma); 96-well plate (Cat No. CLS3370-100EA-Sigma); ELISA Coating Buffer (Cat No. E107-Sigma); ELISA wash solution buffer (Cat No. E106-sigma); and ELISA blocking buffer (Cat No. E107) (Cat No. E104), Lysozyme Kit (Sigma).

Treatment regime and procedure

The rats were divided into a normal control without treatment (group 1), treatment groups induced with 100 µL BCG at days 7 and 14 followed by oral AIP administration at doses of 19, and 31.5 g/kg, respectively, for 30 consecutive days (Groups 2 and 3). Groups 4 and 5 were treated only with AIP at doses of 19, and 31.5 g/kg respectively for 30 consecutive days while Group 6 was a negative control group induced with 100 µL BCG at days 7 and 14 without AIP treatment.

Serum collection

On day 31, blood serum collection was started by anesthetizing rats with diethyl ether. The blood was collected by inserting a needle into the rat tail vein. Once the blood has been collected, it was allowed to clot at room temperature for 20 to 30 min. After the blood has coagulated, the serum was removed from the clot by centrifuging the blood for 10 to 15 min at high speed. The recovered serum was then transferred to a clean tube for IgG and IgM tests.

Evaluated indices

Spleen, lung, and liver parameters

The relative weights of the spleen, lung, and liver organs were determined.

Antibody titers, and lysozyme activity

The IgG and IgM testing technique was conducted according to the kit's instructions. Hemagglutination method was used to determine the antibody titer. A 25 µL of serum was dripped into the first hole of the microtitration plate well 96, where 25 µL of PBS had been added to each hole, and then 25 µL of the first hole was transferred to the second hole. A 25 µL of PBS

was extracted from the second hole and transferred to the third hole, and so on for the remaining holes containing 25 μ L of PBS (1:2; 1:4; 1:8; 1:16; 1:32; 1: 64; 1: 128; 1:256; 1:512; 1:1024; 1:2048). Each hole was then filled with 25 μ L of 1 % cow red blood cells. The sample was then allowed to stand for one hour to visually see hemagglutination. The antibody titer was determined by utilizing the final dilution at which the antibody was still detectable by visually visible hemagglutination. After that, the antibody titer was multiplied by (2log(titer)+1) [15].

Statistical analysis

The acquired data were subjected to statistical analysis using one-way analysis of variance (ANOVA). All information is provided as a means of standard deviation. *P*-values less than 0.05 indicate statistical significance.

RESULTS

Relative weight of organs

Measurement of organ weight relative to body weight is essential to minimize bias caused by body weight. Therefore, the relative weight of organs was determined in this study.

Antibody titer

Antibody titer showed the amount of humoral antibody that is formed in response to antigens that enter the body. As shown in Figure 1, antibody titers increased significantly in all treatments.

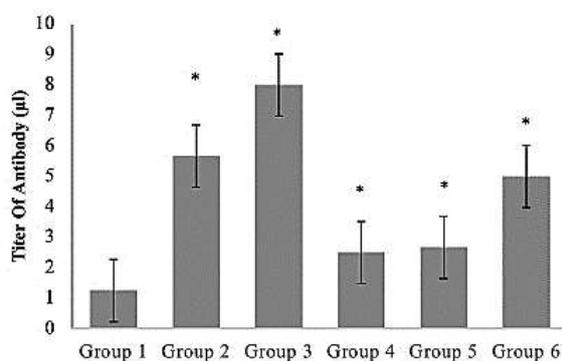


Figure 1: Effect of aqueous extract of *Plectranthus amboinicus* leaves and BCG on antibody titer. **P* < 0.05 versus normal group

The administration of AIP increased antibody titers significantly in groups 2, 3, 4, and 5 when compared to normal control. The treatment of AIP and BCG in groups 2 and 3 showed higher activity in antibody titer when compared to AIP, only treatments in groups 4, and 5. It is indicated

that the addition of an aqueous infusion of *Plectranthus amboinicus* leaves increases the antibody titer response.

Immunoglobulin (IgG and IgM) response

Antibodies are protein globulins that react specifically with the antigens that stimulate their production. The main immunoglobulin in human serum is IgG for 70 to 75 percent of immunoglobulins [16]. The results of IgG response is shown in Figure 2.

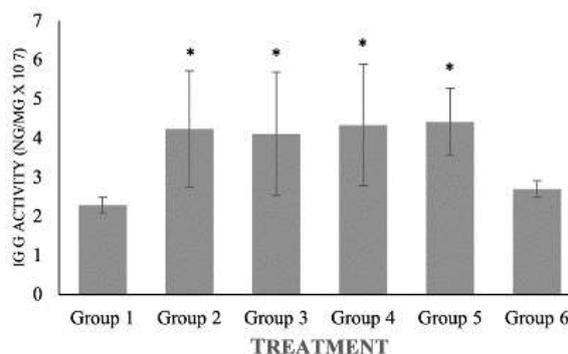


Figure 2: Effect of *Plectranthus amboinicus* aqueous infusion on IgG activity. **P* < 0.05 versus normal group

Figure 2 shows that IgG activity increased significantly in all AIP treatments, while Groups 2, 3, 4, and 5 indicated that the administration of AIP increased the IgG response. However, in groups 2, and 3 induced by BCG, it did not show any significant difference from groups 4, and 5 which were on AIP treated only (*p* > 0.05). Additionally, IgM was lowest in groups 2, and 3 (Figure 3).

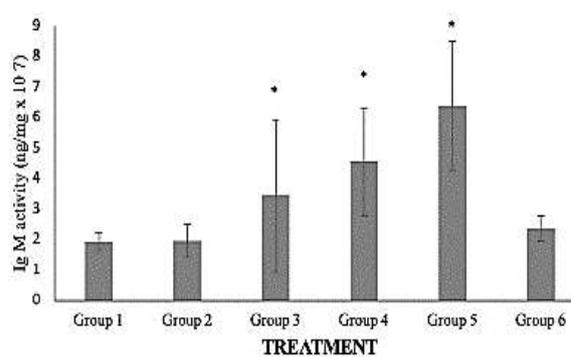


Figure 3: Effect of *Plectranthus amboinicus* aqueous infusion on IgM effect, **P* < 0.05 versus normal control

IgM is the first antibody secreted by the adaptive immune system in response to an antigen. In this study, the IgM response increased after administration of AIP by as much as 31.5 g/kg, but group 2 indicated no increasing IgM response compared to normal control group.

Lysozyme

Lysozyme increased due to administration of AIP (Figure 4). The increase in lysozyme in this study showed the same pattern as antibody titer, Lysozyme activity was significantly increased in all treatments compared to normal control group.

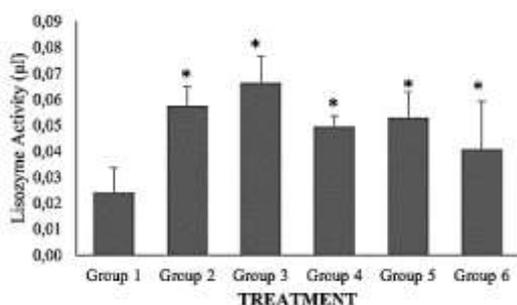


Figure 4: Effect of *Plectranthus amboinicus* aqueous infusion on lysozyme activity. * $P < 0.05$ versus normal control

DISCUSSION

Plectranthus amboinicus contains numerous phytochemical compounds including quercetin. Numerous *in vitro* studies have shown that quercetin acts as an anti-inflammatory and immune booster. The activities of leukocytes and a variety of signaling kinases, intracellular phosphatases, enzymes, and membrane proteins are also enhanced [17]. In this study, the administration of AIP increased antibody titers significantly in groups 2, 3, 4, and 5 when compared to normal controls. In another study, rodents administered an ethanol extract of *Plectranthus amboinicus* leaves exhibited a significant increase in leukocytes. Moreover, this study further revealed that IgG activity increased significantly in all AIP treatment groups. IgM production is the initial stage, followed by IgG production and IgM activity was lowest in group 2. The primary humoral immune response is characterized by a decrease in IgM followed by an increase in IgG during the second phase, this case was related to present study.

The study used BCG (Bacille Calmette-Guérin) as an immune response inducer. When rodents are vaccinated with BCG, they produce antibodies known as IgM as part of their immune response. Prior to the immune system's ability to mount a more specific response, IgM is especially essential for neutralizing pathogens in the early stages of infection [18]. The increase in IgG and IgM in AIP-treated groups might be due to some flavonoids contained in *Plectranthus amboinicus* such as apigenin and luteolin. Another study revealed that apigenin has an

immunostimulatory effect [19] and luteolin also boosts the body's immune system by increasing leukocytes number in the body.

The results of lysozyme activity are related to another study that explained the administration of *Plectranthus amboinicus* ethanol extract on sheep red blood cells. The induced animal model significantly increased lysozyme compared to control [20]. Lysozyme is an enzyme present in numerous physiological fluids, including tears, saliva, and mucus. It serves a crucial role in the innate immune response by destroying bacterial cell walls, thereby preventing infection. During an infection, for instance, lysozyme production frequently increases in response to the presence of microorganisms [21].

The results for relative organ weights between treated and untreated groups of animals are crucial for determining the potential adverse effects of substances on different organs in the body. The changes in relative organ weights can indicate organ toxicity or injury caused by the tested substance [22]. Table 1 shows that the relative weight of the spleen was significantly higher in group 3 treatment while the other groups did not show a significant difference. The

Table 1: Effect of *Plectranthus amboinicus* aqueous infusion on the relative weight of organs

Treatment group	Relative weight of organ {organ weight (g)/100 g body weight}		
	Spleen	Liver	Lungs
1	0.27±0.03	3.11±0.31	0.88±0.26
2	0.27±0.04	3.39±0.83	0.70±0.14
3	0.39±0.05*	4.21±0.35*	1.05±0.27*
4	0.29±0.02	3.55±0.64	0.81±0.16
5	0.29±0.03	3.66±0.61	0.75±0.09
6	0.28±0.07	3.78±0.55	0.91±0.15

Data represent as Mean ± SD; * $p < 0.05$ versus normal group

relative weight of the liver and lungs in the group 3 treatment also showed significant increases when compared to control. In toxicity tests, weighing the lungs, spleen, and liver provides valuable information about the potentially deleterious effects of a substance on these organs. These organs are essential for maintaining normal physiological functions in the body, and alterations in their weight may indicate substance-induced injury or toxicity. An increase in lung weight, for instance, may indicate pulmonary edema or inflammation, whereas an increase in splenic weight may indicate immunomodulatory effects. An increase in liver mass may be indicative of liver toxicity, such as inflammation or hepatocellular hypertrophy. In general, the greater the dose of a toxic

substance, the more pronounced the alterations in organ mass.

CONCLUSION

Plectranthus amboinicus aqueous infusion is able to stimulate the response of IgG, IgM, and lysozyme. Furthermore, results in the relative weight of organs indicate that AIP had no potential toxicity. As with any toxicological study, the results must be carefully evaluated to ascertain the potential health risks to humans.

DECLARATIONS

Acknowledgements

The authors gratefully acknowledge the financial support given for this work by the Directorate of Research and Community Service (DRPM), Directorate General of Research and Development Strengthening, Indonesian Ministry of Research, Technology, and Higher Education, through a 2016 Fundamental Grant.

Funding

None provided.

Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Melva Silitonga analyzed and interpreted the data. Pasar Maulim Silitonga and Erlintan Sinaga prepared the manuscript for publication.

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