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# Activity of arabica green coffee bean (*Coffea arabica*) extract as an immunomodulator in mice (*Mus musculus*) infected with *Staphylococcus aureus*

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## Abstract

Arabica green coffee bean is the most widely produced coffee bean in Indonesia. Studies have shown that arabica green coffee beans are rich in polyphenols and antioxidants, stimulating immune cell proliferation. This study investigated the immunomodulatory activity of arabica green coffee beans (*Coffea arabica*). Thirty mice (*Mus musculus*) with an average body weight of 30 g were divided into five groups: negative control, positive control, and groups administered arabica green coffee bean powder extract at doses of 0.03, 0.06, and 0.18 mg/30 g BW. It was orally administered once daily for 14 days. On the 15th day, the mice were intraperitoneally injected with non-pathogenic *Staphylococcus aureus*. After an hour, mice were necropsied for peritoneal fluid collection. Peritoneal fluid was stained with a peripheral blood smear, and the number of macrophages and lymphocytes was observed under a microscope. Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's test. The group administered with 0.18 mg/30 g body weight (BW) dose showed the highest average of macrophage and lymphocytes compared to the negative control group and any other groups. Arabica green coffee bean powder extract was shown to have immunomodulatory activity, with the highest activity observed at a dosage of 0.18 mg/30 g BW.

**Keywords:** arabica green coffee bean | immunomodulator | macrophages | lymphocytes | mouse

## Introduction

The prevalence of various diseases worldwide has increased in recent decades. These diseases consist of communicable and non-communicable diseases. The diseases that experience this increase vary, both caused by bacteria, such as salmonellosis, cholera, and dysentery, and those caused by viruses, such as influenza and SARS (Ali *et al.*, 2022). This increase in disease prevalence can be prevented in several ways, including by enhancing the immune system. The immune system comprises a collection of cells, chemical compounds, and processes that protect the skin, respiratory tract, intestinal tract, and other areas from foreign bodies or antigens, such as bacteria, fungi, parasites, viruses, cancer cells, and toxins (Marshall *et al.*, 2018). Immune effector cells such as lymphocytes, macrophages, dendritic cells, natural killer cells (NK cells), and cytotoxic T lymphocytes work synergistically to protect the body by detecting antigens expressed on foreign surfaces. Immunomodulators can be used to enhance the immune system by stimulating the function of these effector cells.

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Immunomodulators are substances or components that stimulate or suppress the immune system components in the body (Catanzaro *et al.*, 2018). Immunomodulators are divided into synthetic and biological immunomodulators based on their source and manufacturing methods. Synthetic immunomodulators are in the form of drugs such as levamisole, isoprinosin, and muranil peptidase, whereas biological immunomodulators consist of cytokines, monoclonal antibodies, animals, and medicinal plants (Moghadam *et al.*, 2020). Plants have been widely used in daily human life for hundreds of years as food and medicinal ingredients based on their active metabolite contents. They are used to treat and prevent diseases by increasing the immune system (Gomaa *et al.*, 2019). Some of the main compounds used in commercially patented immunomodulators can also be found in herbal plants. Indonesia is a country with high biodiversity, and many herbal plants have potential as immunomodulators (Setiawan *et al.*, 2021). One of the herbal plants that have potential as herbal immunomodulators is arabica green coffee beans.

The Arabica green coffee bean (*Coffea arabica*) is one of Indonesia's most widely produced green coffee beans. Indonesia produces 800 kg/hectare/year of arabica coffee beans (Muchtardi *et al.*, 2021). Arabica coffee beans are rich in polyphenols, which have various bioactive properties such as antioxidant, antiviral, and anti-inflammatory activities (Amrullah & Sandi, 2022). Green coffee beans are those that have not yet been processed. The polyphenol content of unprocessed coffee beans was higher than that of processed coffee beans. Therefore, this study aimed to investigate the immunomodulatory activity of arabica green coffee bean powder extract. This research is expected to provide scientific information about arabica green coffee bean powder extract and its use as an immunomodulator and can be a natural alternative for immunomodulators.

## Methods

### Experimental animals

This study was approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences (SVMBS), IPB University (approval number 043/KEH/SKE/V/2023). This study was conducted at the Laboratory Animal Management Unit of SVMBS, IPB University, and the eLRosa Laboratory Research Facility of the IRatCo Group.

This study used 30 male mice (*Mus musculus*) of Deutschland Denken Yoken (DDY) strain with a body weight of 25–30 g, aged two months old. Male mice were used to avoid physiological variations related to the estrus cycle in female mice. The mice were maintained at 18–26°C, relative humidity of 40–70%, and lighting settings of 12 h light and 12 h dark (Nugroho, 2018; Pierson *et al.*, 2018). Five mice cages were used, all made of plastic, with dimensions of 35 cm×25 cm×10 cm, equipped with 80 mL drinking bottles, wood shavings as the base, and cage covers in the form of mesh wires. Mice were fed 10% of their body weight. The cage bedding was replaced every seven days.

Mice were acclimatized for seven days prior to the study to adapt to the environment and reduce stress levels. Mice were administered the anthelmintic ivermectin (0.04 mg/kg, diluted with distilled water) once a day for seven days. The mice were provided with *ad libitum* feed and water.

### Experimental design

DDY strain male mice with a total of 30 mice were divided into five groups, six mice each group (**Table 1).** The treatment was carried out by administering arabica green coffee bean powder extract, which was purchased commercially and prepared by dissolving it in warm water. Arabica green coffee bean powder extract was administered once a day for 14 days using the micropipette-guided oral

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**Table 1** Treatment groups of research

| Groups | Treatments |
|---|---|
| Group I (Negative control) | Distilled water as placebo |
| Group II (Positive control) | Commercial immunomodulator with volume of 250 µL |
| Group III | Arabica green coffee bean powder extract with a dosage of 0.03 mg/30 g BB |
| Group IV | Arabica green coffee bean powder extract with a dosage of 0.06 mg/30 g BB |
| Group V | Arabica green coffee bean powder extract with a dosage of 0.18 mg/30 g BB |

drug administration (MDA) method. The dosage was determined based on the conversion of Human Effective Dose (HED) to mouse effective dose (MED), referring to the effective dose of arabica green coffee beans consumed daily by humans according to Reagan-Shaw *et al.* (2008), namely 1460 mg/60 kg BW per day. On the 15$^{th}$ day, the mice were intraperitoneally injected with $10^8$ CFU/mL non-pathogenic *Staphylococcus aureus* via the intraperitoneal route. Non-pathogenic *S. aureus* was obtained from the Medical Microbiology Division of SVMBS IPB University.

The mice were euthanized using the cervical dislocation method one hour after *S. aureus* induction. The mice were dissected for peritoneal fluid collection. The abdominal cavity of the mice was opened and rinsed with physiological NaCl to facilitate the collection of peritoneal fluid. The mice were moved slightly to mix peritoneal fluid with physiological NaCl. Peritoneal fluid was collected using a 1 mL syringe and a 26G needle.

The object glasses were cleaned with 70% alcohol and dried. Samples of peritoneal fluid were dripped onto the object glass, with one drip of one sample per glass. The second object glass with a flat edge was placed on one side of the first object glass end at an angle of 30 degrees. The second slide was withdrawn until it touched the peritoneal fluid drop and spread along the side at moderate speed to form a thin layer of peritoneal fluid. Peritoneal fluid preparations were then stained with a set of peripheral blood morphological dyes consisting of three reagents: methanol, eosin, and methylene blue. The slides were immersed in a methanol solution for two seconds, dried in room temperature, immersed in eosin solution for 20–30 s, and then immersed again in methylene blue solution for 15–30 s. The glass slides were then dried and marked for each group.

The peritoneal fluid slide was observed using a light stereomicroscope at 40×10 magnification. Macrophages and lymphocytes were counted in five views for each glass object. The number of macrophages and lymphocytes from each mouse in each group was recorded.

## Data analysis

Data were analyzed using one-way Analysis of Variance (ANOVA), followed by Tukey's test. Data analysis was performed using Microsoft Excel 2019 and Minitab 19 software.

## Results

The numbers of macrophages and lymphocytes in each smear preparation were recorded, as shown in **Figure 1**. The number of macrophages and lymphocytes was calculated and averaged for each treatment group, as presented in **Table 2** and **Figure 2**. The highest number of macrophages and lymphocytes was in group V, with a dose of 0.18 mg/30 g BW with an average number of macrophages of 4.30 ± 1.45 and an average number of lymphocytes of 4.30 ± 1.45. The lowest number of macrophages and lymphocytes was found in the group I, which was only administered distilled water as a placebo, with an average number of macrophages of 1.10 ± 1.15 and an average number of lymphocytes of 16.5 ± 10.51. The average number of macrophages and lymphocytes in groups II, III, and IV was not different from that in group I; however, in group V,

at a dose of 0.18 mg/30 g BW, there was a significant difference compared with the other groups.

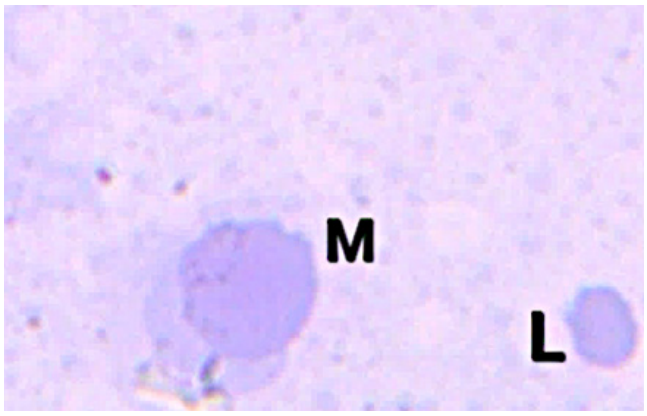


**Figure 1** Macrophages and lymphocytes found in the smear preparation of Group III. Magnification 40×10. M: Macrophage. L: Lymphocyte.

## Discussion

*S. aureus* was used in this study to trigger the immune system. *S. aureus* is both a commensal bacterium and a human pathogen, which can be a leading cause of bacteremia and infective endocarditis (IE), as well as osteoarticular, skin and soft tissue and pleuropulmonary infections (Tong *et al.* 2015). Before bacteremia and other diseases occur, the immune system reacts with *S. aureus*. As the first line of defence, the innate immune response is rapidly activated by pattern recognition pathways that detect nonspecific markers of microbial infections. Consequently, phagocytic cells such as macrophages and neutrophils are activated (Karauzum & Datta, 2017).

**Table 2** The average number of macrophages and lymphocytes found on the smear preparations of peritoneal fluid of mice after administration of arabica green coffee bean powder extract for 14 days

| Treatment groups | The avarage number of | |
|---|---|---|
| | Macrophages | Lymphocytes |
| Group I | 1.10 ± 1.15 [a] | 1.26 ± 0.58 [a] |
| Group II | 3.06 ± 1.98 [ab] | 1.76 ± 0.81 [a] |
| Group III | 3.06 ± 1.46 [ab] | 3.60 ± 2.95 [a] |
| Group IV | 3.06 ± 1.48 [ab] | 3.06 ± 2.47 [a] |
| Group V | 4.30 ± 1.45 [b] | 16.5 ± 10.51 [b] |

Different superscript letters in the same row indicate significant differences (P<0.05)

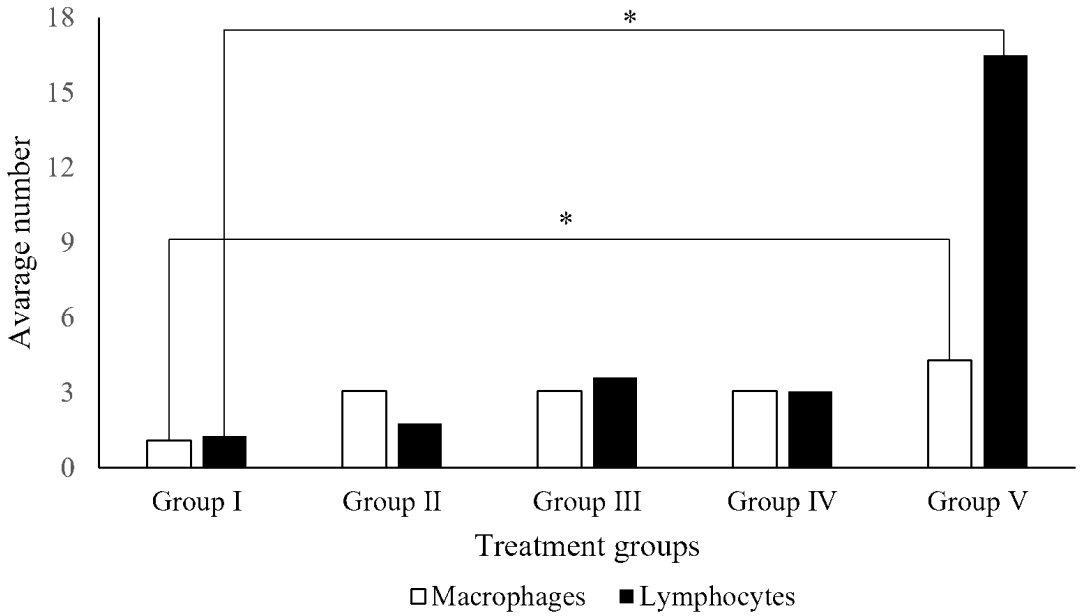


**Figure 2** The average number of macrophages and lymphocytes in mouse peritoneal fluid one hour after *Staphylococcus aureus* induction. *Significant difference (P<0.05).

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Peritoneal macrophages are the most numerous cells among other peritoneal cells, function in innate immunity, and are present in the peritoneal cavity (Liu *et al.*, 2018). Peritoneal macrophages are often used to observe innate immune responses. Peritoneal fluid collection is an accurate and frequently used method for collecting large numbers of fully differentiated macrophages from rodents (Pavlou *et al.*, 2017).

The immunomodulatory activity of arabica green coffee bean powder extract increased the innate immune response of individuals. This condition can be observed by an increase in the average number of macrophages and lymphocytes in the group treated with green coffee bean powder extract. *S. aureus* induction stimulates the presence of macrophages, accompanied by lymphocyte proliferation. Macrophages act as antigen-presenting cells by phagocytosing and processing antigens into the lymphocytes. This phenomenon causes stimulated lymphocytes to produce interleukin-2 (IL-2), which mediates the activation and proliferation of more lymphocytes (Chang *et al.*, 2008).

Arabica green coffee beans have a high polyphenol content. Polyphenols are known to have several health benefits, including antioxidant and anti-inflammatory effects that can prevent cardiovascular disease, neurodegenerative disease, cancer, and obesity (Cory *et al.*, 2018). They can improve lipid profiles, blood pressure, insulin resistance, and systemic inflammation (Rana *et al.*, 2022). Previous research has shown that polyphenols can affect dendritic cells, have immunomodulatory effects on macrophages, and increase the proliferation of B and T cells (Shakoor *et al.*, 2021).

The highest polyphenol content in arabica green coffee beans is chlorogenic acid, which accounts for 35.11% of the total polyphenol content (Kamel & Ali, 2011). Previous studies have shown that chlorogenic acid has health benefits, including neuroprotective, cardiovasculoprotective, gastrointestinoprotective, renoprotective, hepatoprotective, and anticarcinogenic effects, and can regulate glucose and lipid metabolism (Lu *et al.*, 2020). Chlorogenic acid has also been shown to have anti-inflammatory, anti-genotoxic, and antioxidant effects (Bagdas *et al.*, 2020). Apart from chlorogenic acid, arabica green coffee beans contain ferulic acid, a polyphenol. Ferulic acid has been reported to have an immunomodulatory effect by acting as a chemoattractant that can stimulate neutrophils to enter the area of inflammation and function in the host's innate immune system. In addition to promoting the innate immune system, ferulic acid can increase delayed-type hypersensitivity (DTH) response (Singh *et al.*, 2016).

## Conclusion

This research proved that arabica green coffee bean powder extract has immunomodulatory activity. The most effective dose was 0.18 mg/ 30 g BW, significantly different from that of the other groups in terms of the number of macrophages and lymphocytes.

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**Competing interests:** The authors have no conflicts of interest to declare.

**Author contributions:** AA and MS designed and supervised SA during the research; SA analyzed the data and wrote the paper.

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