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Original Research Article

The Potential of Lampung Robusta Green Coffee (*Coffea Canephora*) Extract Toward T Cell Activation in ISA-brown Laying Chickens

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ABSTRACT

Coffee (*Coffea canephora*) is one of the world's most popular beverages containing active chemicals such as caffeine, phenol, trigonelline, diterpenes, and water-soluble substances. Chlorogenic acids are a type of phenol found in coffee that can stimulate the immune system within the body. As a result, this natural substance promotes poultry growth by strengthening the immune system and preventing disease. There is a dearth of information on coffee as an immunomodulator in chickens, necessitating this investigation. The present study was therefore conducted to investigate the effect of Lampung Robusta green coffee extract on T lymphocyte cell activation in ISA-brown laying chickens. Ethanolic extract of the Lampung Robusta green coffee was prepared and subjected to liquid chromatography-mass spectrometric analysis. One-day-old ISA-brown laying chickens were divided into four groups: control (C), T1, T2, and T3 groups, which received extract coffee doses of 500, 1000, and 1500 mg/kg BW, respectively. The relative numbers of $TCD4^+$, $CD8^+$, and $CD45^+$ cells were determined using a flow cytometer. The results of the LC-MS analysis revealed that the Lampung Robusta green coffee extract also contained chlorogenic acids. With a dose of 1500 mg/kg BW, Lampung Robusta green coffee extract had an immunomodulatory effect on the T3 group, increasing the relative number of $TCD4^+$ and $CD45^+$ while decreasing $CD8^+$. The relative number of $TCD8^+$ was higher than that of $TCD4^+$, suggesting immunosuppressive potential. The findings of this study revealed that Lampung Robusta green coffee extract is effective as an immunomodulator in chickens.

Keywords: Antioxidant, T lymphocytes, Chicken, Coffee, Immunomodulator.

Introduction

Indonesia is rich in natural resources and feed additives containing natural compounds, which are beneficial to health. One of such substances is the Lampung Robusta green coffee, which contains antioxidant and anti-inflammatory agents. They can be utilized as an alternative treatment option. As a result, this natural substance aids poultry growth by boosting the immune system and preventing potential diseases.[1] Coffee *(Coffea canephora)* is one of the most consumed beverages in the world because it contains antioxidants that can lower reactive oxidative species (ROS) or oxidative stress, which occurs when oxidant molecules produced by cells are higher than those of antioxidants. Food that contains high antioxidants, such as coffee, has an essential role in neutralizing free radicals. Caffeine, phenol, trigonelline, diterpenes, and water-soluble chemicals are among the bioactive substances found in coffee. Caffeine, a psychoactive chemical such as methylxanthine, gives coffee its bitter taste. Chlorogenic acid (CGA) accounts for 98% of the total phenols in coffee, with alkylmethoxyphenols, alkylphenols, methoxyphenols, and other phenolics making up the remaining 2%.[1]

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Prior research on coffee as an immunomodulator in chickens has been limited, necessitating the need to fill the gap. Therefore, this study was aimed at determining the effect of Lampung Robusta green coffee as an immunomodulator on T lymphocyte cell activation in ISA-brown laying chickens.

Materials and Methods

Source of animal
This research used one-day-old ISA-brown laying chickens as the animal model. The chickens were obtained from PT Charoen Phokphand, Indonesia.

Ethical clearance
Ethical approval for this study was obtained from the Research Ethics Committee of the Universitas Brawijaya, Indonesia, with the ethical clearance number 1142-KEP-UB.

Preparation of Lampung Robusta green coffee extract
The extraction was made by soaking 414 grams of Lampung Robusta green coffee in 15,000 ml of 90% ethanol in a covered jar. The mixture was homogenized by centrifugation at 50 rpm. Afterward, the liquid extract was filtered and heated in a rotatory evaporator once it was ready, yielding the brown liquid used in this study.

Liquid chromatography-mass spectrometric analysis
The liquid coffee extract was subjected to phytochemical analysis and liquid chromatography-mass spectrometry (LC-MS) at UPT Materia Medika in Batu, Indonesia. The LC-MS test was used to determine the chlorogenic acid content. Hypersil Gold specifications were used for

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the columns (50 mm x 2.1 mm x 1.9 µm). Thermo Scientific's UHPLC brand ACCELLA type 1250, which consists of a vacuum degasser, quaternary pump, and thermostatic autosampler controlled by a personal computer via the program x-calibur 2.1 was employed for the analysis. The coffee extract was vortexed for 30 seconds and then centrifuged at 13,000 rpm for 30 minutes before being transferred to the autosampler vial. The clear liquid was then injected into the LC-MS system, which contained solvents A (0.1% formic acid in aquabidest) and B (0.1% formic acid in acetonitrile). The mobile phase was adjusted linearly with Gradient at a speed of 300 l/min as follows: a) 0-0.6 minutes, 95% A; 0.6-3.0 minutes, 75% B; 3.0-3.5 minutes, 75% B; 3.5-4.0 minutes, 75% B; and 4.0-5.5 minutes, 95% A. On LC, the injection volume was 2 µL The column was set to 30°C, while the autosampler compartment was set to 16°C.

Preparation of animal model

The chickens were acclimated, fed with food and *ad libitum* water, and given the supplement on days 1, 2, 3, 4, 5, and 11 to reduce stress. The chickens were vaccinated with ND-IB on day 4, and ND G7B and AI H5N1 on day 10. The treatment was divided into 4 groups with 12 treatment replications, consisting of negative control group C (chickens without extract coffee), T1 group (with extract coffee of 500 mg/kg BW), T2 group (with extract coffee of 1000 mg/kg BW) and T3 group (with extract coffee of 1500 mg/kg BW). The coffee extract was given for 14 days (from day 3 to day 16).

Analysis of the relative numbers of CD4, CD8, and CD45

The chicken spleen was extracted on day 20 and suspended by mashing it with a mortar and mixing it with PBS. The liquid suspension was pipetted into an Eppendorf tube and analyzed by flow cytometry. A pipette was used to mix 50 µL of liquid suspension with 10 µL of antibody CD4, CD8 FITC, and CD45 PerCP reagent in a conical tube. The solution was homogenized using a vortex mixer before being incubated in the dark for 15 minutes at 20-25 °C. The solution was then homogenized after being diluted with 50 pL of I Ox FACS lysis liquid and 450 µL aqua dest. After incubation, 450 µL FACS (lx) diluting reagent was further used to homogenize the solution and was incubated for 15 minutes in a dark room at 20-25°C. FACS equipment was used for the analysis.[2]

Statistical analysis

The relative numbers of CD4, CD8, and CD45 were statistically analyzed using the ANOVA test ($p < 0.5$). The statistical analysis was performed using the Statistical Package for Social Sciences (IBM SPSS Statistics; version 21).

Results and Discussion

The active chemical in Lampung Robusta coffee extract

According to the LC-MS analysis, the Lampung Robusta green coffee extract contained chlorogenic acids (CGA). CGA is an antioxidant found in roasted coffee that has been shown to reduce inflammation caused by oxidative stress.[3] Furthermore, previous research indicated that the Lampung Robusta green coffee extract contains active compounds such as tannin and alkaloids, based on phytochemical screening.[4]

The relative level of TCD4$^+$ cells

The effect of the coffee extract on the relative number of TCD8$^+$ cells in the chicken spleen showed a significant difference between the groups. The relative number of TCD4$^+$ cells in the normal group (C) did not differ significantly from that of the T2 group but significantly differed from that of the T1 and T3 groups. The average relative cell number of TCD4$^+$ in the chicken spleen of T1, T2, and T3 groups did not differ significantly, but those in the T3 treatment group had the highest average of all treatment groups. This indicates that Lampung Robusta green coffee extract with a 1500 mg/kg BW had the highest average of all treatment groups (Figure 1). The T3 group, with the relative number of TCD^{4+} cells was the highest of all other groups due to increased CGA absorption, resulting in high stimulation of the relative number of CD^{4+}. Despite the high content of CGA in coffee, the amount absorbed by the chicken body was minimal. CGA would be absorbed by molecules in the intestine, but microbes in the colon would metabolize some of it. Also, CGA is subsequently absorbed and conjugated in the liver before being transported to the tissues.[2]

The relative number of TCD8$^+$ cells

The relative number of TCD8$^+$ cells was affected by Lampung Robusta green coffee extract. The relative number of TCD8$^+$ in the chicken spleen was observed to be significantly different between the normal group (C) and the T1 and T2 groups. Nevertheless, the T1, T2, and T3 groups were significantly different. As shown in Figure 2, T1 group has the highest relative number of TCD8$^+$ cells. T1 groups have the highest relative number of TCD8$^+$ cells as presented in Figure 2. On regeneration cells, the TCD8$^+$ cells kill infected cells, tumor cells, and normal cells.[5] Furthermore, previous research has shown that long-term and high-dose herbal medication can influence the average relative TCD8$^+$ cells.[6]

Coffee contains more than 1000 bioactive compounds (antioxidants, anti-inflammation, anti-fibrotic, and anticancer) and active compounds (caffeine, CGA, diterpene, vitamin B3 and magnesium).[7] CGA is one of the antioxidants that activates macrophages by producing cytokines like IL-1, TNFα, and IFN-γ.

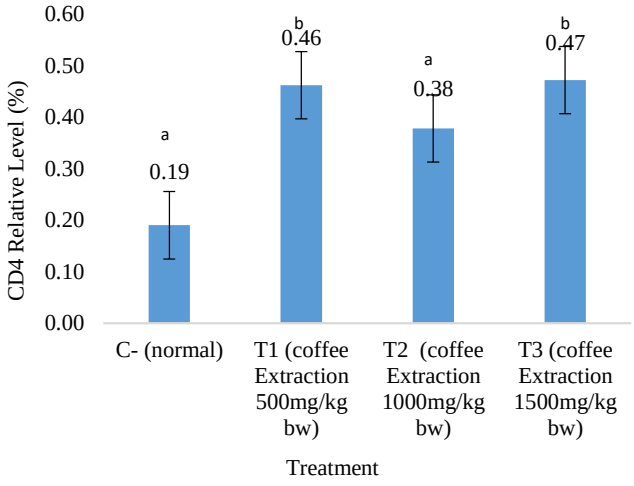

Figure 1: The effect of Lampung Robusta green coffee extract on the relative number of TCD4$^+$ cells. There was significant difference between treatment groups, with a, ab, and b notations ($P < 0.05$).

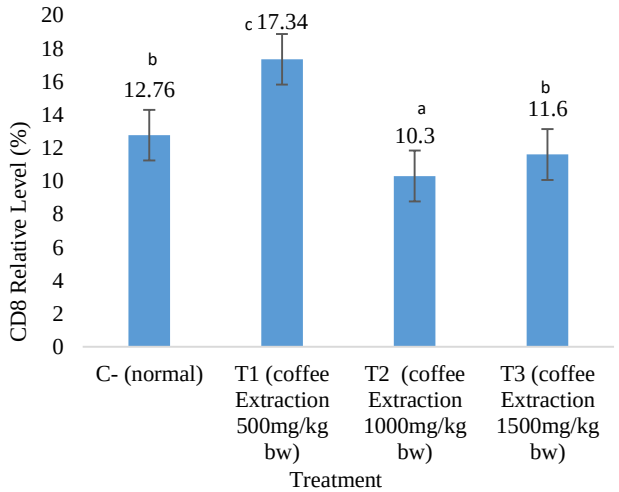

Figure 2: The effect of Lampung Robusta green coffee extract on the relative number of TCD8$^+$ cells. There was no significant difference between the groups, as indicated by the a, b, and c notations ($P < 0.05$).

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The phagocytic macrophage produces nitric oxide (NO) that kills bacteria and foreign bodies.[8] Cytokine of IL-1, TNFα, and IFN-γ also stimulates TCD4 and TCD8 cells.[9] IFN-γ stimulates macrophages as Antigen Presenting Cell (APC) to present antigen to TCD4. Then TCD4 also activates the Tc CD8 for phagocyte antigen.[10] Activated macrophages secrete IL-12, stimulating proliferation and activation of CD4, CD8, NK, and TNF to kill pathogens.[11] This study indicated that the relative number of $TCD8^+$ was higher than that of $TCD4^+$, which were C (12.76±0.19), T1 (17.34±0.46), T2 (10.3±0.38), and T3 (11.6±0.67). It was shown that Lampung Robusta green coffee extract had the potential to be an immunosuppressant to inhibit TNF and cyclooxygenase-2 (COX-2) production.[12]

The relative number of $TCD45^+$ cells

The relative number of $TCD45^+$ in the normal group (C) significantly differed from the T1, T2, and T3 groups. The T1, T2, and T3 treatment groups were higher than those of C. The coffee extract boosted TCD45 (Figure 3). The $TCD45^+$ is a glycoprotein found on the surface of leucocytes and other hematopoietic cells. According to several studies, CD45 plays an important role in lymphocyte T proliferation, stimulated by antigen and occurring within the thymus. More so, CD45 is linked to complex activation molecules like TCR-CD3, CD4, and CD8.[13] CD45 is required for signal transduction, antigen receptor formation, and lymphocyte development. Signal modulation is another function of CD45, which is linked to the cytokine receptor. Autoimmunity, immunodeficiency, and other damage (such as cancer) have all been linked to CD45 excess signal modulation in previous research.[13] Polyphenols scavenge free radicals and inhibit the generation of pro-inflammatory cytokines and leukocytes.[14]

This study revealed that administering a certain amount of Lampung Robusta green coffee extract can have an immunomodulatory effect, which is corroborated by other studies showing comparable results after consuming coffee for four weeks. However, that treatment could pose a harmful effect such as membrane damage, which correlates with oxidative stress presented by NO levels,[1] in which over-produced NO levels cause damage to the cells. The T3 group, with a 1500 mg/kg BW dose of Lampung Robusta green coffee extract, showed an increased relative number of $TCD4^+$ and $TCD45^+$ but decreased $TCD8^+$. The relative number of $TCD8^+$ cells was higher than that of $TCD4^+$ cells in the comparison, indicating that $TCD8^+$ cells are immunosuppressive.

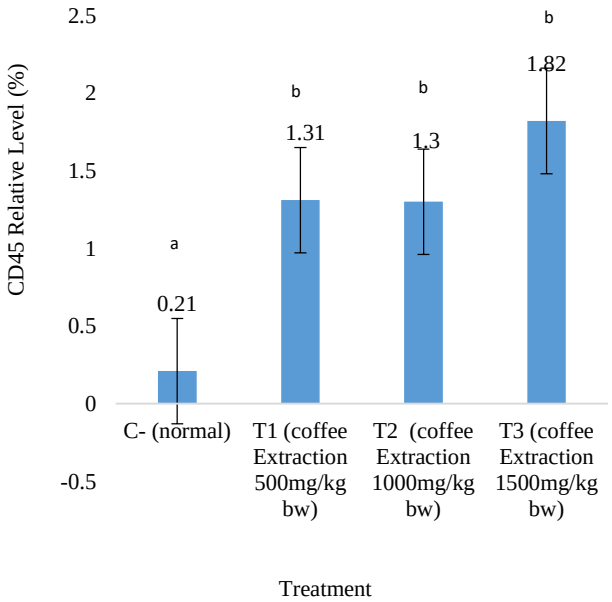

Figure 3: The effect of Lampung Robusta green coffee extract on the relative number of $TCD45^+$ cells. There was significant difference between groups with a and b notations ($P < 0.05$).

Conclusion

The findings of this study reveal that the T3 group, with a 1500 mg/kg BW dose of Lampung Robusta green coffee extract showed an immunomodulatory effect. The average relative number of $TCD8^+$ cells was higher than that of $TCD4^+$ cells in the comparison, suggesting that coffee extract could be immunosuppressive.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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