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Immunomodulation Effect of Meniran (*Phyllanthus niruri linn*) on Blood Profile of Broiler Chickens Infected with Enterotoxin of Antibiotic-Resistant *Escherichia coli*

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Abstract. **The use of antibiotics against *Escherichia coli* on broiler chicken should be re-evaluated because antibiotic-resistant *Escherichia coli* has been found. Meniran (*Phyllanthus niruri Linn*) is a plant that can be used as an alternative to the prevention and treatment of the diseases caused by *Escherichia coli* enterotoxin. In this study, 28-day old broiler chickens were divided into six groups. Group 1 was a positive control group infected by *Escherichia coli* while group 2 was a negative control group without infection. The next group (group 3) were infected with *Escherichia coli* and given an extract of meniran of several different doses including 20%, 25%, and 30%, respectively. The last group used only an antibiotic as a treatment. Blood collection was conducted five days after treatment. The data obtained was analysed using ANOVA and was continued by a Duncan Multiple Range Test. The results showed that there was a significant difference in the immunomodulation activity in the group with 30% doses of meniran extract. The 30% doses of meniran extract were able to decrease the number of lymphocytes in the blood. In conclusion, a 30% concentration of meniran extract (*Phyllanthus niruri L.*) can decrease the number of lymphocytes in the blood of broiler chicken infected with the enterotoxin of antibiotics-resistant *Escherichia coli*.**

Keywords: Immunomodulation, *Phyllanthus niruri* Linn.extract, Broiler, *Escherichia coli*

I. INTRODUCTION

Poultry farming is one of the most important businesses in the supply of animal protein, but there remain some constraints that can cause significant economic losses such as the presence of infectious pathogens. One of the most common infectious bacterial diseases in a farm is colibacillosis caused by pathogenic *Escherichia coli*. Colibacillosis or *Colisepticaemia* in multi-age broilers and breeders, and other types of poultry such as turkeys and ducks [1].

The cause of the *E.coli* infection may be derived from its enterotoxin. There is a specific strain of *E.coli* that can produce *Shiga toxin* known as *Shiga toxin-producing E. coli (STEC)* or *verocytotoxigenic E.coli (VTEC)* or *enterohemorrhagic E. coli (EHEC)*. Enterotoxin-induced diarrhoea may be produced by several strains of *E. coli* such as O157: H7 and O111 or non-enteric *E.coli*. Highly virulent *E.coli* can eventually lead to disease, the colonisation of bacteria in human and animal bodies, and increased morbidity and mortality.

Most of the Indonesian farmers usually use broad-spectrum antibiotics to treat infectious disease caused by *Escherichia coli*. The use of antibiotics might result in the deposition of residues in meat, milk, and eggs. Concern over antibiotic residues in animal origin proteins causing disease and the possible development of resistant strains which causes a failure of antibiotic therapy in clinical situations [2,3].

One of alternative medicines that may be used to prevent the infection of the bacterial pathogen is herbal medicine. As a tropical country, Indonesia has many potential medicinal plants. Many types of medicinal plants contain antimicrobial compounds that are able to serve as bactericidals, bacteriostatics and immunomodulators. Meniran (Phyllanthus niruri Linn) is one of the Indonesian medicinal plants that can be used as an alternative in the treatment and prevention of Escherichia coli infection. Meniran contains two important chemical compounds; flavonoid and tannin. Flavonoid is known to modulate the immune system, while tannin is known as an antiseptic and hemostatic [4]. Thus, the peroral administration of meniran extract in poultry may act as an immunostimulant affecting the function and activity of the immune system.

Therefore, based on the above issues, this research study was conducted to know the effectiveness of meniran (*Phyllanthus niruri Linn*) as an immunomodulator of broiler chickens infected with the enterotoxin of antibiotic-resistant *Escherichia coli.*

II. METHODS

A. Research Design

This experimental research study was conducted using thirty broiler chickens that were divided into six groups. Each

group consisted of 5 replicates. The group was prepared as follows:

P0^{+} : infected with *Escherichia coli* + no extract of meniran (as a positive control)
P0^{-} : no treatment (as a negative control)
P1 : infected with *Escherichia coli* + 20% concentration of extract of meniran
P2 : infected with *Escherichia coli* + 25% concentration of extract of meniran
P3 : infected with *Escherichia coli* + 30% concentration of extract of meniran
P-Ab : infected with *Escherichia coli* + Flemequin antibiotics.

The chicks were inoculated orally with a single dose of E.coli per chick containing an average of 10^8 cells/ml. The E.coli suspension was carefully inoculated by the use of a blunt cannula attached to a tubercullin syringe. Extract of meniran was given orally for five days into the 28-day old broiler chickens that had been previously infected with *E.coli*. Blood samples were collected aseptically from the brachialis vein using 3 ml sterile disposable syringes and needles. Blood was collected in EDTA tubes for blood smear preparation. The blood smears were stained [5,6] with Wright's stain in a concentration of 0.3% (Sigma Chemical Co., St. Louis, MO). Blood sample collection and the staining of the blood smear was performed to calculate the percentage of lymphocytes in a white blood cell (WBC) differential.

B. Calculation of Lymphocytes Obtained from the Examination of Differential Counting

Differential counting is an assessment method of leukocytes in the blood based on the proportion (%) of each type of leukocyte from the total number of leukocytes. The absolute number of each type of leukocyte is obtained from the calculation of its relative value multiplied by the total number of countable leukocytes. The results of this examination can specifically describe the occurrence and process of infection in the body. The differential counting was performed using the staining of the blood smear. The first step was making the blood smear, then staining it using Wright's stain. The observation was performed dry and with immersion oil using a microscope with a 100x objective lens. The calculation of leukocytes was conducted continuously to get 100 leukocytes using a blood cell counter. The number of each type of leukocyte was calculated as a percentage of the total number of leukocytes.

C. Data Analysis

The data obtained from the differential counting method was analysed statistically using one way ANOVA continued with a Duncan Multiple Range Test.

III. RESULTS AND DISCUSSION

The results of this research showed that there was a significant difference in the immunomodulation activity in the group with a 30% concentration of meniran extract. The 30% concentration of meniran extract was able to reduce the number of lymphocytes in the blood. A lymphocyte is one of the sub-types of white blood cell that has the ability to produce antibodies in an immune response after the maturation process.

TABLE I. AVERAGE VALUE OF LYMPHOCYTE NUMBER AMONG TREATMENTS

Treatment	Average
P0+	13344.320^{a}
P0-	13344.320^{ab}
P1	13524.720^{a}
P2	13084.840^{ab}
P3	11159.720^{b}
P-Ab	9574.840^{b}

Special cells presenting in the blood include erythrocytes, leukocytes, and platelets. These blood cells are grown from induced pluripotent stem cells. Leukocytes are responsible for the defence against infectious microorganisms. Leukocytes consist of five cells that have different roles in the inflammatory and immune response. One of the leukocytes types that has a fundamental important role in the immune system is a lymphocyte. Most of the newly formed lymphocytes will migrate to the thymus gland then become a mature lymphocyte which serves in the cellular immune response known as T cells. T cells that do not through the maturation process in the thymus gland will be processed in the bone marrow and distributed in the lymph nodes. These cells may have the ability to produce antibodies in the immune system after the maturation process and are known as B cells. Newly-formed T and B cells will circulate to blood vessels and lymphatic vessels [7].

In the immune response, T cells undergo a transformation into an immunoblast when the antigen of an infectious microorganism enters the body. On the other hand, activated B cells proliferate and differentiate into antibody-secreting plasma cells. Plasma cells, the end product of B cells, have no immunoglobulin in the cell surface.

T cells are an expression of the T-cell receptor (TCR) providing unique and specific antigens on the cell. Immature lymphocyte cells will leave the bone marrow to grow and mature in the thymus. Mature CD4+ or CD8+ T cells are discharged from the thymus and spread to the peripheral lymphoid tissue in the periarteriolar lymphoid sheath of spleen or the perifollicular area. T cells need to be activated. The antigen has generally no ability to stimulate the T cells. The activation of T cells requires an intracytoplasmic signal transmission after introducing the peptide antigen and the major histocompatibility complex (MHC) residues from TCR. There is an interaction between the antigen-presenting cell (APC) and T cells on the surface of other molecules, as they

release the costimulatory molecule to bind with the cytokine receptor on T cells. T cells migrate from the bone marrow to the thymus. The T cells will circulate in the blood vessels to bind the antigens. After the stimulation of the antigen, the T cells produce a chemical that inhibits or kills the growth of the microorganism and signalling about the infection to other white blood cells [8].

B cells mature in the bone marrow and circulate in the blood vessels until they recognise and bind to the antigens. At this stage, the B cells mature and become a memory cell or plasma cell producing antibodies [8]. Each antibody is specific to a particular antigenic because the antibody structure is composed of amino acids with a changeable region in the heavy and light chains. The arrangement of amino acids has a different shape for each specific-antigen [9].

IV. CONCLUSION

A 30% concentration of extract of meniran (*Phyllanthus niruri L.*) can decrease the number of lymphocytes in the blood of broiler chickens infected with the enterotoxin of antibiotics-resistant *Escherichia coli*.

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