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Is Cinnamaldehyde have an effect as immunostimulator ?

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Abstract : The aim of this research is to know the role of cynnamaldehyde contained in the cinnamon ethanol extract as immunostimulator against Salmonella enteridis. Cynnamaldehyde could improve immune system but until now the research about that has not been widely reported. The research used Completely randomized design which consists of 6 groups in curative treatment divided into 4 groups of Balb-c mice infected with amount of bacteria $0{,}25x10^8$ cfu/ml administered orally with cynnamaldehyde in different doses were 50, 100,150, 200 mg/ml consecutively, negative control given no bacteria and positive control only given bacteria. The cynnamaldehyde didn't impact to the expression of CD4, CD8, CD62L T lymphocyte and B220IgD, it means that cynnamaldehide has no effect as immunostimulator .

Key word : Herbal, Bacteria, T cell, Immune.

1. Introduction

Diseases caused by bacteria have caused high morbidity and mortality in infected chicken and also humans because of salmonella can be act as food borne disease (1). Indonesia is a tropical country with temperatures and humidity unpredictable could support the growth of a wide variety of microorganisms, in addition to unfavorable environmental conditions. Bacteria *Salmonella enteritidis* (S.enteritidis) is one of the bacteria pathogenic to humans and animals, especially poultry. These bacteria can be transmitted through the tool, eggs, feed and feces-contaminated drinking infective (2). Eggs and meat have been not cooked perfectly as a potential source of transmission in humans (3) cause acute gastroenteritis and other infections (2). Chicken is under immunosupressive condition is more susceptible than healthy condition because of imbalance microflora in gastrointestinal (1).

The use of chemotherapy and antibiotics for the treatment of diseases have a negative impact, such as resistance and a decrease in the body's immune system (immunosuppression) (4). Studies conducted in 1990 showed that 64% of the human population in the world use herbal remedies to overcome health problems. It is estimated that nearly 50% of the synthetic drugs derived from plants (5). Immunity is the body's ability to defend or protect themselves against infectious and non-infectious agents. Damage or failure immune system to fight foreign substances or pathogens causing the body will be weak. So, we need immunomodulator, which is a substance that regulate or optimize the body's immune system.

Cinnamon (*Cinnamomum burmannii*) or Cinnamon sticks are tree spice commonly used in Indonesian community in the form of bark and it has been used as seasoning, flavor enhancer and baking. The role of Cinnamon efficacious as analgesic, antibacterial, antidiabetic, antifungal, antioxidant, antirheumatik, antithrombosit, gastroprotective, antitumor and immunomodulatory (6). Active ingredients such as cinnamyl alcohol, coumarin, cinnamic acid, cinnamaldehyde, anthocynin, and essential oils as well as other nutritional content such as sugar, protein, crude fat, pectin, allegedly helped the workings of the immune response (7). According to research conducted by (8), cinnamaldehyde can be immunomodulator by inducing apoptosis through mitocondria permeability transition in human promyelocytic leukemia HL-60 cells and induce apoptosis through activation of proapoptotic Bcl-2 family proteins in human hepatoma cells. There is still no data proven scientifically about the use of cinnamaldehyde as immunostimulator to against bacteria infection.

2. Materials and Methods

The research is conducted in September - November 2013 in Microbiology and Immunology Laboratory, in Veterinary Medicine Program and Animal Physiology Laboratory, Faculty of Mathematics and Natural Sciences, Brawijaya University, Indonesia.

2.1 Procedure of cinnamon extract ethanol

The ethanol extract of Cinnamomum burmanii bark is obtained from Jatisari-Bekasi, Indonesia in dry extract powder and previously identified to know the existence of cinnamaldehyde by thin layer chromatography. One part of dry powder mess (28/24) cinnamon bark put in maserator, and then added ten parts 70% ethanol (9). The Concentrated extract is weighed, added 5% aerosil and 65% amylum, then it stirred until homogeneous and ready to be dried for 10 hours by the method of freeze drying.

2.2 Preparation of *Salmonella enteritidis*

Bacteria *Salmonella enteritidis* is obtained from a collection of Microbiology and Immunology Laboratory, Veterinary Medicine Program with the code 0405/03/2013 that has been tested biochemically and serologically using polyvalent and monovalent O and H antisera. The Concentration of bacteria suspension is used in the research accordance with turbidity of Mc Farland standard 0,5.

2.3 Treatment

The Research used mice Balb-C female, 6-7 weeks old, weighing 25-30 grams, not in a state of estrus and in a pregnant condition. The research is passed of Ethical clereance no. 551/EC/KEPK-PKH/1/2013. Mice were adapted for 7 days and then infected with bacteria suspension orally by gavage for one day with dose 0,25 x 10^8 cfu/ml. Clinical Sympthoms have been appeared after mice were inoculated with bacteria was diarrhea. Diarrhea is cultured in Salmonella Shigella Agar showed that salmonella in black colonies. At the 9^{th} day, mice were given dry extract of cinnamon that is mixed with sterile distilled water according to the multilevel dose groups: group/P1 (50 mg / kg body weight) (bw), P2 (100 mg / kg bw), P3(150 mg / kg bw), and P4 (200mg / kg) then there were conducted for 14 days. Mice in Positive control are given only bacteria (sick mice), and negative control only feed (healthy mice). BALB/c mice were given a drink and food standards form of biscuits are ad libitum. At the 22 nd days, Balb/c mice is sacrificed.

2.4 Procedure of flowcytometry

Flow cytometry technique is to determine the number of cells that express CD4, CD8, CD62L T lympocyte and B220IgD. Euthanasia in mice were conducted by dislocation servicalis to take spleen. Then Spleen was removed, rinsed with PBS twice. Pellet was added 1 ml PBS. Results of centrifugation, pellet was added to extracellular antibody (CD4, CD8, CD62L, and B220IgD) (10). Analyzed with a Becton-Dickson Fluorescence-Activated Cell Sorting (FACS) Calibur Flowcytometer .

2.5 Statistical Analysis

Data collected in the number of CD4 T cell, CD8 T cell, CD62L T cell, and B220IgD. Flowcytometer data were analyzed using BD cellquest PRO ™ software and further in Analysis using one-way ANOVA using SPSS (Statistical Product of Service Solution) 16.0 for Windows use. If the test results one way ANOVA shows significant results then tested post hoc test to determine the significance between the group treated with α = 0,05.

3. Results

The results showed that there is no difference between the expression of CD4 T cell treatment (Fig. 1) but in average show that in negative control and treatment have score higher than negative positive control. In control positive group, P3 and P 4 there was one mice dead before it was sacrificed.

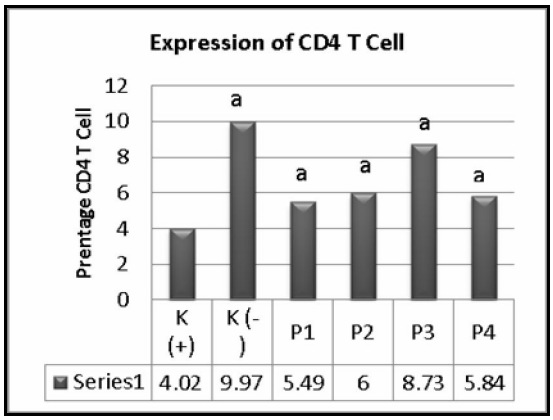

Figure 1. Expression of CD4 T cell relative

In this study indicate that the expression of CD8 + T cells showed the same expression in all treatment groups (Fig. 2) but in average between group showed that negative control showed higher score than positive control. It was like in expression of CD8 T cell that before it were sacrificed, one mice was dead in positive control, P3 and P4

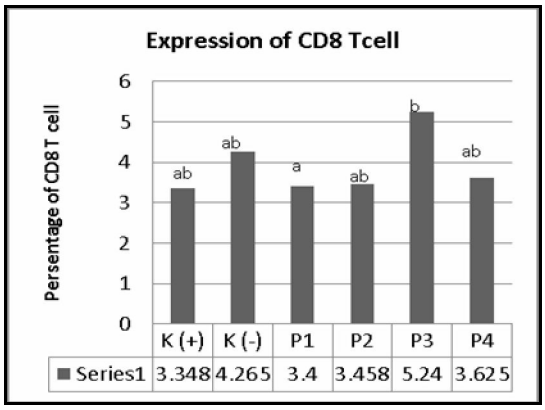

Figure 2. Expression of CD8 T cell relative

Based on the results of the study showed no difference in expression of CD62L T cell between control and treatment (Fig. 3). Based on Figure 3 showed that negative control least than positive control. CD62L T cell to know naif cell, cell not exposed by antigen after maturation cell.

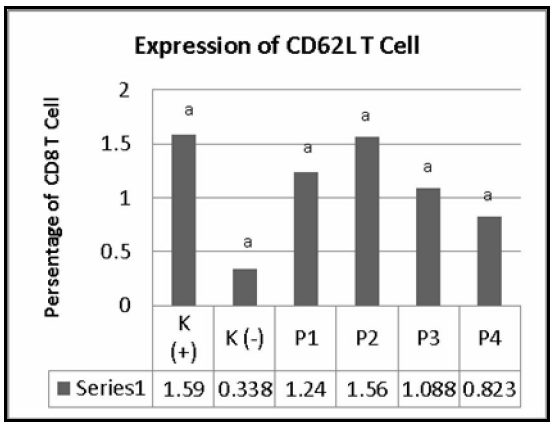

Figure 3. Expression of CD62L T Cell relative

The same result also shown by expression of B220IgD that all treatment didn't have difference (Fig. 4). Expression of B220IgD as marker of B cell is activated as immune cell. In average showed that negative control was higher than positive control.

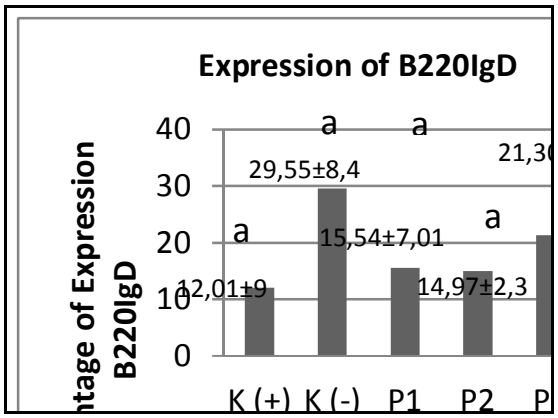

Figure 4. Expression of B220IgD cell relative

4. Discussion

The result in (fig. 1) is different with research conducted by (11) stated that a combination between eugenol and cinnamaldehyde can increase the number of lymphocytes in spite of causing weight loss. A decrease in the number of CD4 T cell expression associated with nutrients enter the body used for the formation of energy metabolism more is not to fight bacterial infections through immune system enhancement. Feed with low efficiency energy use can lead to more nutrients are not absorbed in the lumen therefore to encourage the growth of many microorganisms that can lead to indigestion (12). Conditions of impaired gastrointestinal tract can inhibit cell proliferation of T lymphocytes cell.

Both Positive Control and negative control are same caused by bacteria can not activate naïve CD4 T cells therefore the cells are still in a state not activated beside that T-cell maturation has not occurred that. It can not recognize potentially toward the antigen exposure. CD4 T cells are activated if the organ limphoid matured and there is exposure to antigen. Bacterial dose and duration of exposure given in this study can not activate CD4 T cells in the treatment group and the positive control whereas according to (13) that the Salmonella infection may induce antigen-specific responses of CD4 T-cells, CD8 T-cells, and B-cell which play a role inimmune system. Salmonella-specific CD4 + T cells were activated to express surface CD69 within 3 hours of oral infection and can secrete sitokine IL-2, 9-12 hour later. The dead mice in positive control group, P3 (150 mg / kg bw) and P4 (150 mg / kg bw) during the study likely caused its condition not well after exposure to bacteria and cinnamaldehyde. CD4 + T cells produce cytokines that act to stimulate the function of effector cells such as T helper to produce antibody in the process of inflammatory mechanisms. CD4 + T cells recognize antigens on MHC class II molecules expressed by antigen presenting cells (APC) (14).

CD8 T cells are the main components of the adaptive cellular provides protection against intracellular pathogens and infected tissue as well as tumors. It also serves secretion of proinflammatory cytokines (IFN-g and TNF) can cause inflammatio. CD8 T cell activation requires two signals, namely the interaction between T-specific antigen (TCR) with peptide antigens that bind to MHC1 presented by APC (antigen presenting cells). The second signal requires costimulator provided by the same APC to prevent anergy. Furthermore naive CD8 T cells undergo clonal expansion and differentiate into cytotoxice effector and memory T cells (15).

The curative treatment using cinnamaldehyde causing obstacles on cell viability and proliferation, and induces apoptosis of immune cells depending on the dose given (16). Apoptosis occurs during the process of development and aging as compensation cells to maintain homeostasis in the process of cell populations. Apoptosis occurs as the body's immune reaction or when the cell is damaged during an illness or noxious agents. Some cells expressing fas or TNF receptor that causes apoptosis through ligand binding and protein cross-linking (17). Cynnamaldehyd not function as an immunomodulator with the dose administered. This is contrast to (18,19) Gallois and Oswald, 2008 and Awaad et al., 2014 state in vitro, component cinnamaldehyde, and cinnamon essence has the effect of immunomodulator. Precursors of long-lived memory will be up-

Regulate CD8 T cell antiapoptosis molecule and the surface expression of IL-15R and IL-7R to maintain homeostasis against subsequent antigen (20).

Activation of naïve CD8 + T cells into effector cells and memory cells depends on the process of maturation, activation of dendritic cells, duration of antigen exposure minimum at least 1 week and the presence of Th lymphocyte cells to induce cell proliferation incompetent memory. Both CD4 + and CD8 + are surface molecules present in the activated T cells. Activated T lymphocytes cells will proliferate quickly then migrate into tissues where the presence of antigen and form effector cells that play a role in the immune system such as cell-mediated cytotoxicity and production of various cytokines. Cytotoxic CD8 + T cells play a role in virus-infected cells lyses. CD8+ CTL could also be characterized by expression of role perforin and granzymes, proteins required for cytolytic functions form hole (14).

B cells require two main types of signals to be active. First signal delivered by cross immunoglobulin receptors. The bone marrow cells pass through several different stages of development, in which they acquire antigen specificity. Reaching the adult stage, the B cells exit marrow and complete development to adult stage or naive. It is characterized by the emergence of IgD in addition to IgM on the cell surface. The whole sequence of this development occurs in the absence of contact with exogenous antigen. Therefore the development of B-cells called antigen-independent. This cross leads to the activation of intracellular signaling pathways that make cells capable of interacting with T cells and thus receives a signal 2. B cells expressing active as APC and peptide together with MHC class II on the surface. This peptide may arise from the processed antigen that is internalized after binding to the B-cell surface immunoglobulin receptor. When a B cell contacts CD4 + T cells specific for the peptides to self-MHC class II and having previously been activated by APCs, T cells are able to provide cognate (direct cell contact) assistance and activate B cells for further differentiation into memory cells or cells plasma (21).

Molecular surface markers can role the homing capacity of effector T cell receptor to migrate to rich follicular B lymphocyte cells of the lymph nodes and support the production of antibodies. Conversely, the absence of CCR7 and CD62L on lympocyte cell allows them to migrate to the lymphoid tissues such as organs are inflamed lungs and intestines to eliminate pathogenic agent in the tissue. Naïve T cells circulating in the blood, which is expressed by the surface marker molecule, L-selectin (CD62L), CC chemokine receptor 7 (CCR7) and leukocyte function antigen-1 (LFA-1 integrin αLβ2) if exposed to the antigen will undergo adhesion, and extravasation of cells through high endothelial venules in the peripheral lymph nodes and mucosal lymphoid organs [14].

5. Conclusions

Extract cinnamon, Cynnamaldehyde no effect on the expression of CD4, CD8 , CD62L lymphocyte cells T, and B220IgD cell.

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