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IMMUNOMODULATORY ACTIVITY INFUSE WHITE TURMERIC (*Curcuma zedoaria*) IN MICE INFECTED *Escherichia coli* Dwi Krihariyani, Wieke Sri Wulan, Edy Haryanto Health Analyst Department Health Polytechnic Surabaya Jl. Karangmenjangan No. 18A Surabaya Email: dkrihariyani@yahoo.co.id

ABSTRACT Background The cause of diarrhea are due to *Escherichia coli* infection and intoxication. During this are made to control diarrheal diseases is with antibiotics. But too-frequent use of antibiotics also cause problems that are difficult to handle. Curcumin is one of the compounds contained in white turmeric (*Curcuma zedoaria*) and has many health benefits. The purpose of this study is to investigate the immunomodulatory activity of turmeric white infuse in some concentration in mice infected bacterium *Escherichia coli*. Method To test the immunomodulatory activity of turmeric white infusion in mice infected bacterium *Escherichia coli*, was observed on the incidence of diarrhea by counting the number of mice with diarrhea and identify the bacteria that cause diarrhea. Result The results showed that there was the influence of immunomodulatory activity of turmeric white infusion in mice infected bacterium *Escherichia coli*. Conclusion Mice were given a drink infuse turmeric white 100% concentration did not experience diarrhea, found the average number of colonies of bacteria 102 CFU / ml, the culture results in media EMB there is growth of bacteria *Escherichia coli* characterized by the colony metallic sheen, this is because the bacteria *Escherichia coli* is a normal flora of the gastrointestinal tract bottom in warm-blooded animals and humans, and these bacteria become pathogenic if located outside the intestinal tissue Key word: White turmeric, immunomodulator, *Escherichia coli*

INTRODUCTION In Indonesia, diarrhea is still one of the major public health problem. Survey conducted by the Sub-Directorate morbidity of diarrhea, the Ministry of Health from 2000 to 2010, the tendency incidence rises. Extraordinary Events diarrhea is often the case,

with the percentage of mortality is still high (DG, Ministry of Health, 2011). Many risk factors are thought to cause diarrhea. The cause of diarrhea are due to *Escherichia coli* infection and intoxication. The WHO said there are about 4 billion cases of diarrheal infections each year with a mortality rate of 3-4 million per year (Zein et al., 2004). *Escherichia coli* is a normal flora in the intestinal digestive tract in humans or animals, but it will be a pathogen when out of their habitat, can cause diarrhea, urinary tract infections, respiratory disease, and pneumonia (Roesli et al, 2014). During this are made to control infectious diseases, especially diarrhea is the administration of antibiotics. Giving antibiotics indeed has many benefits in the control of bacterial infections. But too- frequent use of antibiotics also cause problems that are difficult to handle, among which the incidence of dual resistance, in addition to the cost of treatment currently quite expensive (Brooks et al., 2007). Other efforts that can be done to control infectious diseases is to increase endurance. Increased endurance to do with regulation of the immune response (immunopotentiating), using natural or synthetic material which is an attractive alternative for the prevention and treatment of infectious diseases (Roitt et al., 2001). One of the natural substances that can act as an immunomodulator is white turmeric. White turmeric as an immunomodulator is also expected to be used to repair or rebuild (imunorestorasi) immune system is deficient or dysfunctional, either as a primary or secondary cause. Curcumin is one of the compounds contained in turmeric white and have various health benefits (Rahmawati et al, 2013; Bahar E, 2009). In a study it was found that the mechanism of curcumin itself as immunostimulation is by inhibiting the proliferation of PHA-induced T cells, the production of [interleukin-2, NO generation, and lipopolysaccharide - induced nuclear factor- kB \(NF-kB\) and increase NK cell cytotoxicity](#). The results showed [that curcumin likely to inhibit cell proliferation and cytokine production by inhibiting NF-kB target genes involved in the induction of immune parameters](#) (Yadav, 2005) In another study curcumin can also serve as immunosuppression that can run the action via multiple molecular mechanisms, one of them in inhibiting the activity of NFkB, by blocking the signals that activate IKB kinase (IKK), which in turn will inhibit the synthesis of inflammatory mediators. In addition, other studies report that curcumin also inhibits activation of T lymphocytes by blocking Ca mobilization and inhibit the activation of NFAT (nuclear factor of activated T cells) (Sastroasmoro, 2004). In other words, here curcumin is able to give effect to an increase in phagocytic activity of macrophages. Therefore, the compound will further facilitate phagocytic cells to perform their function in antigen memfagosisopsonin for their function. Opsonin is a substance that serves to enhance the phagocytic activity (Jawetz, Et al., 2005). To test the immunomodulatoryactivity infuse white turmeric (*Curcuma zedoaria*) in mice infected bacterium *Escherichia coli*, was observed on the incidence of diarrhea by counting the number of mice with diarrhea and identify the bacteria that cause diarrhe. RESEARCH METHOD This research is the design of True Experimental Experimental Randomized Post Test Only Control Group Design. Materials research: infuse white turmeric; *Escherichia coli* ATCC 25 922 types; male mice strain Balb/C which has a weight of 20-30 grams, 8 weeks old and in good health, as many as 25 animals, were separated into five groups. The number of replication was determined by using the formula Federer. Media used: Selenith broth, Nutrient for Slant, and EMB media, Aquadest and sterile 0.9% NaCl. Location Research: Animal Research and Development Unit Try the Faculty of Veterinary Medicine Airlangga University and the Laboratory of Microbiology Department of Health Analyst Polytechnic Health Ministry of Health of Surabaya. Research variables are independent variables = infuse white turmeric and the dependent variable = Number of mice diarrhea and growth of the bacterium *Escherichia coli*. Data collection: performed with

experimental observation techniques. The tools required = Smit mounted sonde (1ml), laminar flow, analytical balance, autoclave, the sample container screw-cap closures, ose, thermometer, glass beaker, measuring cups, spatulas, test tube rack, Bunsen, tripod, gauze asbestos, erlenmeyer, incubator, sieve, knives, cutting boards, fridge, basin, petridisk, stove, sample transport sterofom box, aluminum foil, waterbath. Procedure Research: Sterilization of tools and materials; Making infuse Turmeric 100%, 75% and 50%; Determination infusion doses white turmeric; Making suspension of Escherichia coli; Determination of the administered dose of Escherichia coli bacterial infections in mice. Treatment of experimental animal mice: Adaptation of experimental animal; the treatment of the five groups of experimental animals. In this process using 25 mice were divided into 5 groups, each group contains 5 mice. The first group were given a drink each day infusa white turmeric concentration of 50%, 75% the second group, third group of 100% for 21 days. The fourth group is the negative control group, were given food and drink as normal, untreated. The fifth group is the positive control group, the group treated with the infecting bacterium Escherichia coli. On day 22, mice from the treatment groups 1, 2, 3, and 5 are separated by providing insulation and mat aluminum foil on the cages of mice and each bulkhead filled only the mice then 5 mice infected with the bacterium Escherichia coli as much as 0.325 ml oral later incubated 24 hours. After 24 hours the mice were observed whether the faeces released by the mouse has a liquid consistency (diarrhea), if the diarrhea mice faeces immediately fledgling done isolation and identification, which is used to control mice remained taken faecesnya and isolated and identified. Escherichia coli bacteria culture process: initiated when the mice defecation, and faeces were inoculated in the culture medium selenite broth and incubated at 37C for 1x24 hours. Bacterial culture that has rejuvenated EMB inoculated into selective media, incubated at 37oC for 1x24 hours, observed the growth of the colony. Interpretation of the results of bacterial culture on EMB media; A positive result, if there are colonies with traits have rainbow colors sparkling metallic or metallic sheen. Negative results if there are no colonies with traits have rainbow colors sparkling metallic or metallic sheen. Data Analysis: Using descriptive qualitative data analysis. RESULT From the observation of the mice that had been given infusa turmeric white with a concentration of 50%, 75% and 100%, for 21 days and were then infected with the bacterium Escherichia coli is carried out in Unit Development and Research Centre Try Faculty of Veterinary Medicine Airlangga University and the Laboratory of Microbiology Analyst Department of Health Polytechnic of Health Ministry of Health Surabaya, the result as shown in table 4.1 below:

Table 4.1: Observations Average Total Genesis Mice Experiencing Diarrhea S

TREATMENT	A	M	P	L	E
Treat ment 1 (50% conc)	Treat	Treat	ment 2	ment 3	
(75% (100% conc) conc)	Control	Positive	Negative	1	Positive Positive
	Negative	Positive	Negative	2	Positive Positive Negative
	Positive	Negative	4	Positive	Positive Negative
	Positive	Negative	5	Positive	Negative 6 Positive
	Positive	Negative	6	Positive	Negative

Information: Positive = Diarrhea
Negative = No diarrhea
A positive control = mice infected with the bacterium Escherichia coli
the negative control = mice untreated
To test the activity of turmeric white imunomodulator infusion in mice infected bacterium Escherichia coli, was observed on the incidence of diarrhea by counting the number of colonies of Escherichia coli, the TREATMENT Treat ment 1 (50% conc) Estimatited calculation Escherichia coli colony Infinity Treat ment 2 (75% conc) Infinity Treat Control Control Ment3 positive negative (100% conc) 102 CFU/ml Infinity 100 CFU/ml result as shown in table 4.2 below.

Table 4.2. Observations average Calculate the amount of Escherichia coli colonies Information: Negative if the number of bacterial colonies = <300

CFU / ml Positive if the number of bacterial colonies = Infinity From the results of field observation, to determine the type of bacteria that cause diarrhea do the identification and isolation of mouse feces. The results of the identification of bacterial cause of diarrhea in this experiment in getting the results as shown in Table 4.3 Table 4.3. Results identify the type of bacteria in the feces of mice. Stool samples Description Identify results from the Characteristic on media eosin Methylen treatment features Blue (EMB) group

Treatment 1 Liquid feces (50% conc) with an average of an infinite number of colonies Treatment 2 Liquid feces (75% conc) with an average of an infinite number of colonies There is a growth of Escherichia coli bacteria that is characterized by the presence of metallic sheen colonies There is a growth of Escherichia coli bacteria that is characterized by the presence of metallic sheen colonies Treatment 3 Stool solid with (100%conc) an average number of colonies of 102 CFU/ml There is a growth of Escherichia coli bacteria that is characterized by the presence of metallic sheen colonies Control Liquid feces positive with an average of an infinite number of colonies There is a growth of Escherichia colibacteria that is characterized by the presence of metallic sheen colonies Control negative Stool solid with an average number of colonies of 100 CFU/ml There is a growth of Escherichia coli bacteria that is characterized by the presence of metallic sheen colonies

DATA ANALYSIS From the observation of the body's response and stool cultures in experimental animals with diarrhea, after being given treatment that is giving turmeric white infuse for 21 days and then infected by the bacteria Escherichia coli obtained results as shown in Table 4.1; 4.2; and 4.3. In the treatment group the first and the second is the provision of a concentration of 50% and a concentration of 75% infusa turmeric white in mice that have been infected with the bacterium Escherichia coli showed that mice had diarrhea with the characteristics of the stool liquid amount of bacterial colonies infinity, the result of a culture at media EMB there is growth of bacteria Escherichia coli characterized by the colony metallic sheen, while the mice given 100% infusa turmeric white, the mice did not experience diarrhea with the characteristics of feces dense, found the average number of colonies of bacteria 102 CFU/ml, EMB media culture results in the growth of Escherichia coli bacteria are characterized by their metallic sheen colonies. In the positive control group is a group of mice infected bacteria Escherichia coli showed that mice had diarrhea with the characteristics of feces liquid, the number of bacterial colonies infinity, the result of a culture at the media EMB there is growth of bacteria Escherichia coli characterized by the colony metallic sheen, while the negative control group is the group of mice not given the treatment showed that the mice did not experience diarrhea with the characteristics of feces dense, found the average number of colonies of bacteria of 100 CFU/ml, the culture results in media EMB there is growth of bacteria Escherichia coli are characterized by their colony metallic sheen. In substance mice were said to diarrhea if the stool consistency of liquid, with a confirmatory test at the media EMB found the average number of bacterial colonies infinity, while saying no diarrhea if the stool consistency solid, with a confirmatory test at the media EMB found the average number of colonies of bacteria <300 CFU / ml, whereas in this study, administration of 100% infusa turmeric white showed mice no diarrhea, the media EMB found the average number of colonies of bacteria <300 CFU, so it can be concluded that infuse turmeric white activity as imunomodulator in mice infected bacteria Escherichia coli.

DISCUSSION Immunomodulator is a compound or substance that can stimulate, suppress, or restore immune system activity both specific immune system as well as non-specific immune system. In this case the compound found in turmeric white one is curcumin, curcumin has been shown to increase macrophage (Baratawidjaja, 2005), whereas in studies in vivo curcumin has the ability to be able to increase T cell proliferation, so that

curcumin had the outlook is quite good to boost the immune system (Lucia, 2015). Rahmawati, Et al., (2013) stated in a study in vitro infuse white turmeric can also be used as an antibacterial of the bacterium *Escherichia coli*. From the observation of the body's response and stool cultures in experimental animals, the results obtained in the first and second treatment group that is giving a concentration of 50% and 75% concentrations infuse white turmeric in mice that have been infected with the bacterium *Escherichia coli*, the mice had diarrhea with the features liquid stool characteristics, an infinite number of bacterial colonies, the culture results on EMB media that there is growing bacterium *Escherichia coli* which is characterized by the presence of metallic sheen colonies. It can be shown that curcumin and other active substances contained in turmeric white infusa yet to respond to the body to increase endurance. While the mice given 100% infusa turmeric white, the mice did not experience diarrhea with the characteristics of feces dense, found the average number of colonies of bacteria 102 CFU/ml, the culture results in media EMB there is growing bacterium *Escherichia coli* which is characterized by the existence of colonies metallic sheen. It can be shown that curcumin and other active substances contained in turmeric white infuse already provide a response to the body to increase endurance, in other words curcumin can function as an immunomodulator. Immunostimulatory is a substance that can improve the working komponen- immune system components. The immune system consists of nonspecific and specific immunity. Both the immune system cooperate in maintaining body balance. Healing the infection will be faster when the body's immune system function improved. Various materials of plant origin may stimulate the function of various immune system components nonspecific (phagocytes, NK cells) and specific immune system (the proliferation of T cells, B cells that produce antibodies) as well as the production of cytokines that can be used in the clinic as an adjuvant to enhance the healing of various infectious diseases (Baratawidjaya, 2005). The observation of the culture medium in each treatment EMB shows that there is growth of *Escherichia coli* bacteria that is characterized by the presence of metallic sheen colonies. This is caused by the bacterium *Escherichia coli* is a normal flora of the gastrointestinal tract bottom in warm-blooded animals and humans, and these bacteria become pathogenic if it is outside the network intestinal (Rahmawati et al, 2013). Normally the bacterium *Escherichia coli* were in the gastrointestinal tract neonates since the age of 40 days, which is obtained from the drinks, the food and the people who come into contact with the baby. Manifestations of *Escherichia coli* bacteria infection depends on the location of the infected area, and the symptoms that caused indistinguishable from symptoms caused by other bacterial infections (Roesli, 2014). According to Kusuma (2010), in *Escherichia coli* virulence can be classified into several groups, including the Enteropathogenic *Escherichia coli* (EPEC) is diarrhea usually strikes infants in developing countries. Manifestations that caused the infection EPEC is watery diarrhea that can generally be cured by itself but can also become chronic. The second group is the enterotoxigenic *Escherichia coli* colonization factor owned by specific ETEC to hold the attachment in the human intestine. Some ETEC produces two types of endotoxins that are not heat resistant namely LT while the heat resistance is ST. If the ETEC strains that produce the toxin, it will cause more severe diarrhea. The third group is *Escherichia coli* Enterohemorrhagic, where the group produces verotoxin. Serotype only produce verotoxin is serotype O157: H7. In EHEC infection causes severe diarrhea, hemolytic uremic syndrome and hemorrhagic colitis. The fourth group is enteroinvasive *Escherichia coli*, *Escherichia coli* from this group can infect a way to invade epithelial cells of the intestinal mucosa, and diarrhea that occur only found in humans. *Escherichia coli* latter group is Enteroagregatif *Escherichia coli*, *Escherichia coli* type can cause acute or

chronic diarrhea in the wider community in developing countries. How the infection of this group has a very distinctive pattern of attachment to human cells.

CONCLUSION AND RECOMMENDATION Conclusion Based on the results of this study concluded that there is the influence of immunomodulatory activity infuse white turmeric in mice infected bacterium *Escherichia coli*. Mice were given a drink infuse turmeric white 100% concentration did not experience diarrhea, found the average number of colonies of bacteria 102 CFU / ml, the culture results in media EMB there is growth of bacteria *Escherichia coli* characterized by the colony metallic sheen, this is because the bacteria *Escherichia coli* is a normal flora of the gastrointestinal tract bottom in warm-blooded animals and humans, and these bacteria become pathogenic if located outside the intestinal tissue.

Recommendation 1. For the Community Infusa white turmeric can be consumed daily to boost the immune system caused by bacterial infection, especially *Escherichia coli*. 2. For further research Further research is needed to determine the potential of turmeric and white using different bacteria.

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