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PROCEEDING

International Seminar

THE ROLE OF VETERINARY SCIENCE TO SUPPORT MILLENNIUM DEVELOPMENT GOALs *and* THE 12th ASIAN ASSOCIATION OF VETERINARY SCHOOLS CONGRESS

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Editors :
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Bambang Sektiari
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THE INFLUENCE OF *TEMU HITAM (CURCUMA AERUGINOSA* ROXB.) RHIZOMES ETHANOLIC EXTRACT AGAINST TOTAL INTRAEPITHELIAL LYMPHOCYTE SMALL INTESTINE ON LAYER CHICKEN WHICH INFECT BY *ASCARIDIA GALLI*

Handayu Untari[1)], Eka Pramyrtha Hestianah [2)]
[1)]Veterinary Medicine School, Brawijaya University, *corresponding author. email handayuntarivet@ub.ac.id, mobile +6281332254120 [2)] Anatomy Veteriner Departement, Airlangga University

ABSTRACT

Curcuma aeruginosa Roxb, is one of the plant which has an advantages herbal medicine. This plant has been used as a traditional herbal medicine since a long time ago and the part which used as herbal medicine is the rhizomes. Rhizomes of *Temu Hitam* have many advantages, more specific such as anthelmintics. This research has been done to determine the effect of ethanolic extract of *Curcuma aeruginosa* Roxb. rhizomes to increase the total lymphocyte in the small intestine that indicate the effectiveness of this extract to kill *Ascaridia galli*. In this study, 25 of layer chicken were infected by *Ascaridia galli*. After that, the ethanolic extract rhizome of *temu hitam* (*Curcuma aeruginosa* Roxb.) were orally given to layer chicken for seven days after they positively infected at various dose of 100, 200, 300, and 400 mg/chicken/day. This research demonstrated that the ethanolic extract rhizome of *temu hitam* (*Curcuma aeruginosa* Roxb.) in 200 mg/chicken/day give a significant difference compared with case control (P0) and P1 (100 mg/chicken/day). It showed that ethanolic extract rhizome of *temu hitam* can decrease the number of *Ascaridia galli* in small intestine which is indicated by the increasing amount of total lymphocyte in small intestine.
Key words : *Curcuma aeruginosa*, *Ascaridia galli*, lymphocyte, layer chicken.

INTRODUCTION

Ascaridiasis is one of poultry diseases that usually attacked poultry farm, especially layer farm. Ascaridiasis as helminthiasis disease is caused by *Ascaridia galli*. This disease eventually occured either in layer or broiler that caused the decreases of hen day and meat production (Kusumamihardja, 1993 and Subekti et all., 2005). Chicken which infected by *Ascaridia galli* worm may loose a lot of blood, hipoglicemia, increasing of uric acid level, thymus atrophy, growth disorder, and the increasing of mortality rate (Tabbu, 2002). According Subekti et al. (2005) ascaridiasis mortality rate in this case could reach 35%.

Infection by worms *Ascaridia galli* also cause an immune response in the intestinal mucosa through the activation of Th2 cells to release IL-4 and IL-5. This cytokine stimulate the proliferation of B cells to produce antibodies against antigens of the worm *Ascaridia galli* (Baratawidjaja, 2006; Hansen, 2003). Th2 cells is a subtype of T cells, which is one of the cells that live in intraepithelial lymphocytes are found in the intestinal epithelium of chicken (Soeparto, 1997).

The infection caused by worm *Ascaridia galli* is need to be treated by inhibiting the development of infecting eggs as the source of infection. The treatment can be conducted with synthetic anthelmintics or alternative medicine derived from plants and is commonly known as traditional medicine (Kuswinarti, 1993). *Temu ireng* rhizome (*Curcuma aeruginosa* Roxb.) is one of traditional medicinal plants which can be used as anthelmintics (Planthus, 2008). *Temu ireng* rhizome has an efficacy to cure worm disease, with a mechanism of essential oil contained in the plant that caused muscles paralysis of the worms (Setiawan, 1994). Due to the strong anthelmintic power, this study uses *temu ireng* rhizome extracts to figure out how much of the dose rhizome

extracts used which show significant changes in the number of intraepithelial lymphocytes. The alteration of the lymphocyte number assumed due to teh reduction number of worms in the gastrointestinal tract.

MATERIAL AND METHOD

Experimental animals used in this study were 25 ISA Brown laying hens 10 weeks aged with an average weight of 820 gram. Experimental animals that used in this research is in good health. The materials used in this study consist of *Temu ireng* rhizome (*Curcuma aeruginosa* Roxb.) processed with ethanol in order to obtain extracts of the *temu ireng*, *Ascaridia galli* worm eggs as an ingredient infections from chicken intestine, glucose saturated for examination materials of float method to count worm eggs, extraction of crude drug (ethanol 80%), materials for making histological sections such as: formaldehide 10 %, alcohol 70%, 80%, 95%, 96%, alcohol absolute, *xylol*, *Haematoxylin Eosin* dye, parafine, *canada balsam*, and fisiologic NaCl.

Treatment In Experimental Animals

After 2 weeks of adaptation period, experimental animals were infected with infectious *Ascaridia galli* eggs of 100 eggs/hen orally. Then the animals were kept for 100 days as the life cycle of Ascaridia galli worms (Subekti, 2005) and stool examination to ensure infected chickens. Then the animals were administered with the ethanol extract of *Temu ireng* rhizome (*Curcuma aeruginosa* Roxb.) per oral a day with various doses.

Experimental animals are divided into 4 groups, as follow : P0: Positive control, infected by worms *Ascaridia galli* eggs, tretment without extract (*Curcuma aeruginosa* Roxb.); P1: chickens are infected with worms and treatment with rhizome extract (*Curcuma aeruginosa* Roxb.) at 100 mg / day per oral; P2: chickens are infected with worms and treatment with rhizome extract (*Curcuma aeruginosa* Roxb.) at 200 mg / day per oral; P3: chickens are infected with worms and tretment with rhizome extract (*Curcuma aeruginosa* Roxb.) at 300 mg / day per oral; P4: chickens are infected with worms and treatment with rhizome extract (*Curcuma aeruginosa* Roxb.) at 400 mg / day per oral. Treatment given in 7 days consecutively. On the 8th day after treatment, the animals were dissected for ileum organs collection. Furthermore, histopathology of the ileum sections were prepared and calculated to analyse the increasing number of intraepithelial lymphocytes.

This research used Completely Randomized Design (CRD) with five treatments and five replications, were analyzed using analysis of variant (ANOVA) to determine the signification between the treatments, followed by a test at the level of trust with Duncan Method (Kusriningrum, 2008).

RESULT AND DISCUSSION

Table 1. The Amount of Total lymphocyte cells

Treatment	Mean ± SD (lymphocyte cells)
P0 (positive control)	67,00^{b} ± 15,281
P1 (100 mgs/kgs)	66,00^{b} ± 2,449
P2 (200 mgs/kgs)	82,00^{a} ± 8,062
P3 (300 mgs/kgs)	50,80^{c} ± 7,190
P4 (ekstrak 400 mg/kgBB)	27,00^{d} ± 4,359

Table 1 shows that the highest number of intraepithelial lymphocytes found in P2 treatment. It was the group with ethanolic extract rhizome of temu ireng (*Curcuma aeruginosa* Roxb.) given at 200 mg/day. In other hand, the P4 treatment, shows the lowest treatment group rhizome extracts of *temu ireng* with a dose of 200 mg / head / day. P4 group therapy or group *Ascaridia galli* infected chickens in addition also provided extracts of *temu ireng* at a dose of 400 mg/head/day treatment group with the lowest number of lymphocytes. Parasites that

enter into the lumen of the digestive tract will cause the activation of T lymphocytes, especially the increasing of CD4 T-cells (Baratawidjaja, 2006). Increasing of CD4 cells, will affect the number of intraepithelial lymphocytes. This is due, intraepithelial lymphocytes are major effector immune cells in the gastrointestinal tract and has an important role in the activities of protection against pathogens that enter the digestive tract (Kim *et al.,* 2008). Intraepithelial lymphocytes in the mucosa of the small intestine largely populated by T lymphocytes (Abbas and Andrew, 2003).

Increasing the number of lymphocyte cells in the treatment of P2, in this case, rhizome extracts of *temu ireng* with a dose of 200 mg / head / day is a combination of activities of the small intestine in response to entry of parasites (*self cure reaction*) and activity from rhizome extract of *temu ireng*. Setiawan (1994) stated that based on the symptoms caused by *Ascaris suum* worms in vitro after administration rhizome essential oils of *temu ireng* (*Curcuma aeruginosa* Roxb.), the workings of rhizome essential oils of *temu ireng* to inhibit neuromuscular electrical, with an action to depolarization the motor end plate accompanied with excitation of the worm, then prevents repolarization causing continuous depolarization, which ultimately occurred worm muscle paralysis.

Rhizome extracts of *temu ireng* in addition to containing essential oils, also contain other ingredients that have potential as immunostimulatory substances, called curcumin. Curcumin has immunostimulatory activity by increasing the synthesis of IgG antibodies, and increased cytotoxicity of Natural Killer cells (Bermawi, 2006). Other than that curcumin works by increasing the activity of macrophage phagocytosis (Anthony *et al.,* 1999). Phagocytosis is the process of absorption and elimination of microbes or other particles by a special cell called a phagocyte (Hargono, 1996). Activity Combination of curcumin and essential oils that promote the killing of worms by rhizome extracts of black meeting (*Curcuma aeruginosa* Roxb.) which looks at improving the immune response by increasing the number of lymphocytes intraepitelial (T cells limphocyte).

The number of lymphocytes in the treatment of P3 and P4 were infected with Ascaridia galli rhizome extracts of *temu ireng* (*Curcuma aeruginosa* Roxb.) with 300 and 400 tail-day mg dose and a significant reduction. Decrease in lymphocytes associated with the toxic effects of the rhizome of *temu ireng* of intestinal epithelial cells. Toxic effect of *temu ireng* (*Curcuma aeruginosa* Roxb.) associated with a high content of compounds monoterpenoidy equal to 59,26% (Srivastava *et al.,* 2006). The main monoterpenes contained in the rhizome of *temu ireng* (*Curcuma aeruginosa* Roxb.) is cineol and camphor compounds (Grayson, 2000). Cineol and camphor compounds can reduce cell division, leads to changes in organelle structure, and rupture of membranes and membrane organelles core (Purcaro, 2007). Mitochondria are cell organelles that have a membrane. Damaged of mitochondria cell membrane influence cell survival, because mitochondria are cell organelles ATP providers. The absence of ATP causes damage to the epithelial cells commonly known as necrosis. Epithelial cell damage caused by the compound camphor and cineol then will affect the number of intraepithelial lymphocytes.

CONCLUSION

The ethanol extract of *temu ireng* rhizome (*Curcuma aeruginosa* Roxb.) With a dose of 200 mg / head / day in an infected laying hens by Ascaridia galli worms to increase the number of intraepithelial lymphocytes on providing for seven days.

The ethanol extract of rhizome of black meeting with a dose of 300-400 mg / head / day to reduce the number of lymphocytes intraepitelial.

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