

Oral internasional 1

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DIFFERENTIATION OF LEUKOCYTE SHEEPS FED BY FRACTION KATUK LEAF EKSTRAKT (*SAUROPUS ANDROGYNUS L. MERR*)

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Blood leukocyte differentiation is one parameter to determine the health status of the animals, because of the differentiation of blood leukocytes is a component that has an important function as the body's first defense when disease or foreign substances into the body. Katuk is a medicinal plant that has several active substances that serve to health. This study aims to determine the leukocyte differentiation from sheep given the leaf extract fraction katuk. Complete randomized design (CRD) was used with 4 replications and 4 treatments fraction katuk leaf extract. The treatments include: control, Ethanol crude extract (1500 mg / day of extract ETOH), delipidasi fraction (1230 mg / day FDL) and hexane fraction (270mg / day FH). Fractions katuk leaf extract given to sheep in capsule form that is 2 times a day for 2 months. Leukocyte differentiation calculation performed after 2 months of treatment. The results show that the value of leukocyte differentiation sheep after administration leaf extract fraction katuk namely lymphocytes, neutrophils, monocytes, eosinophil and the sequence is (41.5-46)%, (47.7-54,7)%, (1.5-2.7)%, (2.2-6)%. Leukocyte differentiation value in this research is still in the normal range sheep. This shows that the treatment of fraction katuk leaf extract does not interfere with physiological health status of sheep.

Keywords: leukocytes, katuk leaves, sheep

Background

The health of the cattle is one of the factors that affect the productivity of livestock, and the one that is the effect on the health of leukocytes. Leukocytes picture of an animal can be used as an indicator of the deviation function of the organ or infection infectious agents and foreign bodies as well as to support the clinical diagnosis (Frandsen, 1992). Leukocyte function to protect the body against germs that attack the body by phagocytes, produce antibodies (Junguera, 1997). Leukocyte differentiation consisting of lymphocytes, monocytes, basophils, neutrophils and eosinophil are blood components that serve as the body's defense system (Nordenson, 2002). An increase or decrease in the number of leukocytes in the blood circulation can be interpreted as the presence of agents of diseases, inflammation, autoimmune diseases or allergic reactions, it is necessary to note the normal description of leukocytes in each individual (Nordenson, 2002).

Katuk (*Sauropus androgynus*) is a plant belonging to the family Euphorbiaceae. Katuk has several active compounds that may play a role in the body's metabolism is the metabolism of carbohydrates, fatty acids and proteins. Katuk plant has been used by the community as a medicine plant families (TOGA), facilitating breastfeeding, food and decorative plants. In addition, in some studies katuk leaves can also be used as additional feed for broiler chickens and sheep.

According Suprayogi (2000) katuk leaf content with hexane fraction contained the active compound Octadecanoic acid (C₁₈H₃₆O₂), 3, 4-Dimethyl-2-oxocyclopent-3-enylacetic acid (C₉H₁₂O₃). Bicyclo (2.2.2) octane-1-carboxylic acid 4-methyl (C₁₀H₁₆O₂). 8. Beta. H-Cedran-8-ol (C₁₅H₂₆O). Tricyclol (8.6.0.0 (2.9)) hexadeca-5, 13-dien, cis-2, 9-cisoid-9, 10-tr (C₁₆H₂₄). 2 H- Inden-2-one, 1, 4, 5, 6, 7,7a-hexahydro-7a-methyl (S) - (C₁₀H₁₄O). 9, 12, 15 Octadecatrienoic acid, ethyl ester (C₂₀H₃₄O₂). D1-2-4-Hydroxy-3-methyl-2-cyclopent-1-one ester or D1 cistran (C₁₀H₁₆O₂). The active compounds Octadecanoic acid serves as a precursor of eicosanoids and is involved in the biosynthesis of the compounds eicosanoids (prostaglandins, prostacyclin, thromboxane, lipoxines and leukotriene). The active compound may stimulate the production of erythropoietin so as to affect the formation of red blood cells (erythrocytes) (Guyton and Hall 1997).

This study uses a crude extract of ethanol (EtOH), the fraction delipidasi (FDL) and hexane fraction (FH) of the leaf katuk. Fractionation was conducted to separate grouping of active compounds, of compounds that are polar toward nonpolar compounds. EtOH crude extract and some fractions of the extract affects the body's metabolism possible. Based on this, the study aims to determine the effect of some fraction katuk leaf extract (*Sauropus androgynus*) to the picture of the health status of the sheep with the measurement parameters leukocyte differentiation sheep (*Ovis aries*).

Blood leukocyte differentiation picture is one parameter to determine the health status of the animals, because of the differentiation of blood leukocytes is a component which plays an important role in regulating the bodies physiological especially as a first defense when the disease is attacking (Dellman & Brown, 1989).

Research procedure

Katuk leaves freshly washed with clean water, then carried the sun to dry withered drying then grinding the ingredients until smooth. Extraction is done according to the procedures Suprayogi. Et al (2010), namely the dry milled katuk leaves as much as 2 kg mixed with ethanol (EtOH) as much as + 13 liters. The mixture was stirred for 30 minutes and then allowed to stand for 24 hours. Once macerated filtering using a flannel cloth and filter paper, to obtain a solution of ethanol extract of leaves katuk (filtrate). The filtrate from the filtering is evaporated using a rotary-evaporator temperature 40oC. Extraction is repeated several times until the color is relatively clear solvent and the obtained crude extract ethanol.

Separation of non-polar compounds by adding hexane crude extract is preceded by 20 g of ethanol dissolved in 500 ml of ethanol, then put into a glass separation (Separation flash) and add 500 ml of hexane. Both solvent mixtures are done shuffling resulting in the separation of solubility. After a few minutes there is a separation solvent again, and then made expenditures to the two solvents to hold them in a separate erlenmeyer glass. Mixing and shaking is repeated until the hexane was clear. Evaporation is done on both the solvent, thus obtained ethanol extract fraction which has a free non-polar compounds called delipidasi extract fraction (FDL) and hexane fraction (FH) or so-called lipid extract fractions.

Each fraction extraction katuk leaves used in powdered form by adding malt dextrin. Powders of each fraction katuk leaf extract included in a capsule (capacity 500 mg). Preparation for sheep feed formulations include: forage / grass field (55%) and concentrate (45%) consisting of 10% rice bran, oil cake 28%, 5% fish meal, salt 1%, and 1% premix. Sheep were used as research is Priangan sheep ram in the future to grow as much as 16 tails. Age sheep used 5 months and weighs 15.61 + 1.5 kg. Where food and drink beforehand cleaned. Feeding concentrates and grass done ad libitum.

The design used in this research is completely randomized design (CRD) with four kinds of treatments and four replications. Treatment capsule leaf extracts and fractions katuk in sheep given orally for 2 months. The leaf extract treatment katuk be rough EtOH extracts, fractions delipidasi (FDL) and hexane fraction (FH) as well as control. Based Suprayogi et al. 2009 size dose katuk leaf extract is obtained, namely: (1) the crude extract dose of ethanol (EtOH) was given 1500 mg / day. 1 sheep get 4 capsules per day is 375 mg / capsule. (2) Dose Fraction extract

delipidasi (FDL) was given 1230 mg / day. 1 sheep get 3 capsules per day is 410 mg / capsule. (3) Dose Fraction hexane extracts (FH) given 270 mg / day. 1 sheep get 3 capsules per day is 90 mg / capsule. (4) Control: placebo (empty capsule). EtOH extract dose is the total dose of the extract fractions delipidasi (FDL) and hexane fraction (FH). The capsules were administered orally two times a day ie morning and afternoon.

RESULTS AND DISCUSSION

RESULTS

Results showed that the leaf extract and the fractions extract katuk for 1 month showed no significant effect ($P > 0.05$) to the value of differentiation leucosis sheep. Leukocyte differentiation value in this research is still in the normal range sheep. According to Smith and Mangkoewidjojo (1988) leukocyte differentiation sheep namely lymphocytes, neutrophils, monocytes, eosinophil sequence is (50-75) %, (17.5 to 50)% (0-6)% (0-8)%. Based on previous research obtained by the total number of leukocytes sheep after administration katuk leaf extract is 8.45 ± 2.92 and 2.74 ± 10.09 thousand / mm^3 . This shows that such treatment does not interfere with physiological status (health) sheep.

Leukocyte Differentiation of sheep after being given the leaf extract fraction katuk

Treatment	Leukocyte Differentiation (%)			
	Lymphocytes	Neutrophils	Monocytes	Eosinophil
Control	40.50 \pm 16.94	47.00 \pm 17.78	3.00 \pm 1.41	9.50 \pm 9.75
E. EtOH	43.75 \pm 10.01	47.75 \pm 10.56	2.50 \pm 1.00	6.00 \pm 1.63
FDL	46.00 \pm 7.39	48.75 \pm 6.23	2.75 \pm 2.36	2.5 \pm 1.91
FH	41.50 \pm 14.20	54.75 \pm 14.97	1.50 \pm 1.00	2.25 \pm 2.22

Description: E. EtOH: Ethanol Extracts, FDL: Fraction Delipidasi, FH: Hexane fraction

DISCUSSION

Lymphocytes

Based on the observation table showed that the percentage of lymphocytes in the third treatment katuk leaf extracts and fractions higher than the control value. The percentage of lymphocytes in the range of normal values ranging sheep is (50-75) % (Smith and Mangkoewidjojo, 1988). Based on the statistical analysis that has been done, the result that the percentage of lymphocytes sheep on the third leaf fractions treatment katuk not significantly different from controls.

Moyle and Cech (1988) reported that the antibody-producing lymphocyte functions as to face the disease disorder or foreign objects. The process of formation of lymphocytes called lymphopoiesis, the formation of lymphocytes derived from maturation LSC (Lymphoid Stem Cell) or stem cells, LSC will develop into lymphocytes-T (thymus) and lymphocytes-B (bone marrow) and then enter the peripheral circulation with time intervals varying depends on the nature of the cells and assembled at lymph or lymphatic tissue organs.

The physiological condition of the body can affect the number of sheep lymphocytes, including genetic factors and environmental factors. Kusumawati (2003) states that the condition of the body's physiology is influenced by genetic factors and environmental factors, which include genetic factors and environmental factors is the nation is feeding. It is claimed that the leaf extract katuk toward sheep do not cause health problems in sheep.

Monocytes

The percentage of monocytes sheep on katuk leaf extract treatment was lower than controls, but the value is still within the normal range of monocytes sheep. Percentage range of normal values monocytes in sheep (0-6) %, (Smith and Mangkoewidjojo, 1988). Based on statistical analysis that has been done, the result that the percentage of monocytes sheep after administration leaf extract fraction katuk not significantly different from control. According Affandi & Tang (2002), Monocytes capable of penetrating the walls of capillaries, into the tissue and differentiate into macrophages. Monocytes can multiply rapidly in the area of inflammation as well as consuming the agent causing the infection. Condition in which increased monocyte called monocytosis. The main role of monocytes in the immune system, which responds to signs of inflammation by moving quickly (approximately 8-12 hours) to place infected, send

macrophages to stimulate an immune response, and remove substances that affect the occurrence of chronic inflammatory process (Swenson et al, 1993).

Neutrophils

Based on observations, showed that the percentage of neutrophils after administration of the extract ethanol (EtOH) and Fraction Delipidasi (FDL) katuk leaves still within the range of normal values sheep. The percentage of neutrophils in the range of normal values sheep ranged between (17.5 to 50) % and Mangkoewidjojo Smith (1988) ,, the percentage of neutrophils in hexane fraction has a value that is slightly higher when compared to control values and normal values neutrophil sheep .

The main role of neutrophils is a first line of defense against foreign substances, especially against infectious bacteria (gram-negative and gram-positive bacteria), in the event of acute bacterial infection, the bacteria will damage cells and cells to release chemotactic factor to the network. The chemotactic factors will attract neutrophils into the tissue through a process of diapedesis and neutrophils will be heading to the site of infection to phagocytosis (Duncan and Keith, 1977). After the neutrophils memfagositosis foreign bodies will digest foreign bodies will then undergo autolysis and release substances degradation results in lymphatic tissue. Lymph tissue will release histamine which stimulates the bone marrow neutrophil reserve releases that will increase production of neutrophils (Meyer et al. 1992).

Eosinophils

Percentage Eusinofil sheep on katuk leaf extract treatment was lower than controls, but the value is still within the range of normal eosinophil sheep. Percentage range of normal values eosinophil in sheep ((0-8)% and Mangkoewidjojo Smith (1988). Eosinophil play a role in parasitic infections and allergic response, which is associated with acute illness. Guyton (1997) suggests that the body's defense system, eosinophil responsible for fighting infections and parasites also control mechanisms associated with allergy. Moyes and Schute (2008) states that the function of eosinophil in the immune system against microorganisms and foreign matter in a way as chemical functions which are enzymatic. Type fraction katuk leaf extract did not affect the number of leukocytes such as lymphocyte differentiation of monocytes, neutrophils, and eosinophil this means there is no difference in conditions (difference against foreign bodies) on the sheep's body.

Tizard (1982) suggest that monocytes into macrophages that have both the blood flow and tissue called the mononuclear phagocytic system. The system functions are destroying and processing of foreign material into the body so it can provide immune response

CONCLUSION

Leukocyte differentiation sheep after administration leaf extract fraction katuk namely lymphocytes, neutrophils, monocytes, eosinophil sequence is (41.5-46)%, (47.7-54,7)% (1.5-2.7)%, (2.2-6)% . Leukocyte differentiation value in this research is still in the normal range lamb. This shows that the treatment fraction katuk leaf extract does not interfere with the physiological status of the health of sheep

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