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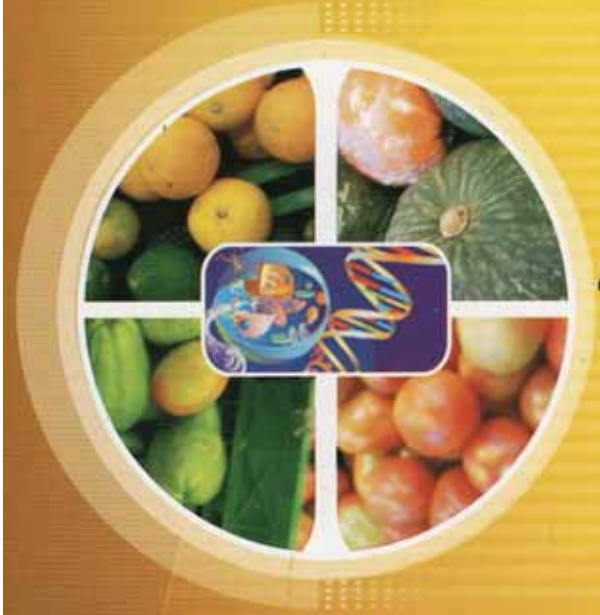
Fakultas Teknobiologi

PROCEEDING



1st International Seminar on
"Natural Resources Biotechnology: From Local to Global"

September 8th – 9th 2015
Faculty of Biotechnology
Universitas Atma Jaya Yogyakarta

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Welcome Speech
Chair of the Seminar Committee

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

It is a great pleasure to welcome all of you to the International Seminar "Natural Resources: From Local to Global". The Faculty of Biotechnology of Universitas Atma Jaya Yogyakarta runs this seminar to commemorate the 50th Anniversary of the Universitas Atma Jaya Anniversary and the 25th Anniversary of the Faculty of Biotechnology. Your presence is your present for the anniversary of our university and faculty as well.

The Anniversary is not the only reason to run this seminar. A greater reason is behind the seminar. Indonesia is rich in biodiversity. It is a challenge for us, as scientist, to maintain the biodiversity and to develop the potential of the biodiversity for the common good. Through this seminar, the scientific research on Indonesian biodiversity can be shared and probably the finding of the new research can inspire us for further exploration. Therefore, the seminars goal is to facilitate the spread of the research on local potential of biodiversity to the global level. Hopefully, it can attract more researchers to explore the wealth of local biodiversity.

The committee invites speakers who are expertise in the research concerning biodiversity. Our invited speakers are Assoc. Prof. Dr. Michael Murkovic from Graz University of Technology Austria (food scientist), Assoc.Prof. Worawidh Wajjwalku from Kasetsart University Bangkok Thailand (Veterinary disease biotechnology), Dr. Kathryn McMahon from Edith Cowan University Australia (Seagrass biotechnology), Prof. Marco Nemesio E. Montano, PhD from University of the Philippines (Seaweed biotechnology), Prof. Jun Kawabata from Hokkaido University Japan (food biochemist), Endang Semiarti, PhD from Universitas Gadjah Mada, Indonesia (Plant biotechnology), Ign. Pramana Yudha, PhD from Universitas Atma Jaya Yogyakarta (Conservation genetics), Dr Machmud Thohari from Technical Team for Environmental Biosafety, Ministry of Enviroment & Forestry Indonesia (Environmental Biosafety), Dr Harvey Glick from Asia Regulatory Policy & Scientific Affairs Monsanto Company (Regulatory Policy & Scientific Affairs Monsanto). It is a good opportunity to learn from the speakers to enhance and to update our knowledge. I hope this seminar is of benefit to all of us.

In conclusion, I wish you a successful seminar and a pleasant stay in Yogyakarta.

With kind regard
Coordinator of conference program

Dr. rer. nat. Yuliana Reni Swasti, S.TP., MP.

WELCOME SPEECH
DEAN
FACULTY OF BIOTECHNOLOGY
UNIVERSITAS ATMA JAYA YOGYAKARTA

Distinguished Guests,
Honorable Speakers,
Ladies and Gentlemen,

On behalf of the Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta and the Committee of the International Seminar, I would like to first of all to extend our heart-felt thanks for your presence at this Seminar. This seminar is so significant in a sense that it focuses on natural resources with local content but by utilizing biotechnology they will become global and worldwide products and services as well.

Biotechnology has been developed very rapidly and it is believed to be "a new wave in the economic world". Biotechnology has contributed in all aspects of humans' life, such as food production, health, industry, environment, etc. The role of biotechnology for the betterment of human beings, however, is still need to be improved. Indonesia, with its huge biodiversity, has a potency to develop and applied biotechnology nationwide.

The role of biotechnology has increased rapidly. Many are believed that biotechnology has become an integral part of modern industries with high economic values. On the other hand, it needs to be closely managed in order to avoid its negative impacts. The are some example of negative impacts with relate to biotechnology application, such as intellectual property rights, genetically modified organisms (GMOs), environmental degradations, biodiversity issues, indigenous people knowledge, biosafety, etc.

The Seminar covers topics such as: Functional Foods, Food Biotechnology, Biopharmacy, Health/Medical Biotechnology, Environmental Biotechnology, Legal Aspect of Biotechnology, Bioinformatics, and Social-Economic Aspects of Biotechnology. This Seminar will be presented nine (9) invited speakers with different topics and expertise. There will be some papers and posters to be presented also in this Seminar from some participants from the Philippines and Indonesia.

Henceforth, in commemorating its 50th anniversary Universitas Atma Jaya Yogyakarta (UAJY) and 25th anniversary of Faculty of Biotechnology, Universitas Atma Jaya Yogyakarta (UAJY) on September 2015, it is worthy and appropriate to explore the newest innovations in the field of research and development of biotechnology to be applied in many aspects for the betterment of human beings. The Seminar takes this opportunity to discuss and hopefully find ways to solve problems faced by human beings in the world.

I would like to take this opportunity to express my sincere thanks and gratitude to the Committee and in particular to the honorable speakers. Before closing this remarks, allow me to ask the Rector of Universitas Atma Jaya Yogyakarta to open this Seminar officially.

Finally, this is an opportune time for me to wish you all in the two (2) fruitful days of interesting and beneficial programs and hope you have a pleasant stay in Yogyakarta.

Thank you very much and may God bless us all. Amen.

Yogyakarta, 8 September 2015

Dean

Drs. B. Boy Rahardjo Sidharta, M.Sc

Brief Contents

Acute Lung Toxicity of Juice and Soup of Katuk (*Sauropus Androgynus*) Leaves as Breastmilkbooster Related to Bronchiolitis Obliterans

Amelia Lorensia[1], Oeke Yunita[2], Andreas Kharismawan[3], Cindy Edelweis[3]
[1]Departement of Clinical Pharmacy-Community, Pharmacy Faculty, Surabaya of University, Surabaya, Indonesia
[2]Departement of Pharmaceutical Botany, Pharmacy Faculty, Surabaya of University, Surabaya, Indonesia
[3] Student of Pharmacy Faculty, Surabaya of University, Surabaya, Indonesia
amelia.lorensia@gmail.com

Abstract

Sauropus androgynus (SA) (katuk) as a traditional medicine to supplement breast milk production, has been widely used by the people of Indonesia to improve and accelerate the production of breast milk (air susu ibu/ASI). However based on some research said katuk suspected to cause constrictive bronchiolitis obliterans because of toxin exposure. Some adverse events katuk leaves in some countries could reduce the sale value of katuk, either as food or food supplements in Indonesia, if not further investigated. Indonesian society can consume leaves katuk direct way in the form of salad or soup can be cooked in advance. Therefore in this study will be conducted in vivo toxicity tests on the leaves katuk originating from East Java, Indonesia, is given in the form of vegetable juice and stew katuk orally, in Wistar females.SA extract was separated into eight parts, namely 500, 1000, 3000, and 5000 mg/kg for jus and soup groups. After 14 days, each rat was observes in macroscopic and microscopic of the lung. Then, It analyzed with ANOVA. The administration of juice and soup of leaves SA don't cause changes in the physical condition of the rats. And katuk (Sauropus androgynus) leaf juice cause significant differences in the results of macroscopic observation that lung volume as well as the results of microscopic observation that the bronchial lumen ratio between treatment groups katuk leaf juice 5000 mg / kg and negative controls. Katuk leaf soup (Sauropus androgynus) causes significant variations in the macroscopic conditions (lung volume) and microscopic (the size of the bronchial lumen) in female Wistar rats. Our results indicate that the toxic necrosis of SA is dose-independent. More evidence is needed to clarify the incidence of necrosis in chronic used.

Keywords: Acute toxicity, *Sauropus androgenus*, lung histopathology

1. INTRODUCTION

Sauropus androgynus (SA) (katuk) has been widely used by the people of Indonesia to improve and accelerate the production of breast milk (air susu ibu/ ASI). Where ASI is useful for the growth and development of the baby, because it contains many growth factors and can boost the immune system because it contains many antibodies (Hapsari, 2000; Playford *et al.*, 2000). Katuk leaf infusion can increase milk production in mice, but the extract ether and ether-petroleum fractions did not

show an increase in milk production significantly. Supplement products containing katuk leaves have many in the market, which is intended to increase milk production (Sa'roni *et al.*, 2004; Azis and Muktiningsih, 2006).

But since 1994 has been raised as a result of side effects on the respiratory katuk consumption in Taiwan for a long time and without the rules of use, for the purpose of body slimming. The whole treatment kontroversional done, either steroids or bronchodilators does not give effective results to overcome these side effects. Even some of the patients died and the patients were still alive, there has been a chronic respiratory failure That is irreversible and require a transplant to overcome these side effects (Lin *et al.*, 1996; Chang *et al.*, 1998). A similar incident also raised in Japan, which was reported byOonakahara *et al.,* (2005).Katuk can cause constrictive bronchiolitis obliterans (CBO), the syndrome is fatal respiratory failure due to exposure to toxins. Lung biopsy of some patients who died showed that indicate obstruction of the bronchioles bronchiolitis obliterans (Lai *et al.*, 1997; Chang *et al.*, 1998). Bronchiolitis obliterans is a pulmonary obstructive disease caused by persistent inflammation in the bronchioles which lead to the proliferation of fibroblasts, resulting in obstruction of the lumen of bronchioles and can be demonstrated by several kinds of tests such as pulmonary function test (PFT) and high-resolution computed tomography (HRCT). Bronchiolitis obliterans is characterized by obstruction of the bronchioles, bronchiectasis, water-trapping and decrease in diffusion capacity(Laohaburanakit *et al.*, 2003). Histopathological changes in patients with bronchiolitis obliterans is the presence of inflammation, necrosis and fibrosis of the bronchioles (Wang *et al.*, 2000). Necrosis is cell death due to extreme stimuli from the environment that is characterized by swelling of the cytoplasm, decreased cell integrity and cell lysis. In the necrotic area are fibrosis and shrinkage of cartilage, fibroblasts and smooth muscle cells. Cell death can also be caused by programmed cell death (apoptosis) due to morphological and biochemical changes. In pathological conditions, apoptosis and necrosis often occur together (Yu *et al.*, 2007).

The severity of bronchiolitis obliterans due to consumption katuk leaves is influenced by several factors such as the amount, duration and manner of consumption leaves katuk (Oonakahara *et al.*, 2005; Yu *et al.*, 2007). On average, patients consumed more than 150 g leaf fresh katuk every day and breathing problems after 4-12 months (Oonakahara *et al.*, 2005; Yu *et al.*, 2007).Most patients taking katuk leaves in the form of juice. Only a small proportion of patients who consume the leaves by boiling prior katuk (Hsiue *et al.*, 1998; Yu *et al.*, 2007). Leaf consumption katuk way affect the prevalence of onset of bronchiolitis obliterans. Consumption katuk leaves with cooked first known to decrease the prevalence of bronchiolitis obliterans (Ger *et al.*, 1997). Katuk leaf juice consumption due to the long-term, at least 300 patients in Taiwan and eight patients in Japan experienced bronchiolitis obliterans (Sawahata *et al.*, 2010).

Some adverse events katuk leaves in some countries could reduce the sale value of katuk, either as food or food supplements in Indonesia, if not further investigated. Indonesian society can consume leaves katuk direct way in the form of salad or soup can be cooked in advance. In addition to salad and soups made, leaves katuk also been consumed in the form of dosage extract(Sa'roni *et al.*, 2004).Therefore in this study will be conducted in vivo toxicity tests on the leaves katuk originating from East

Java, Indonesia, is given in the form of vegetable juice and stew katuk orally, in Wistar females.

2. METHOD

This research is an experimental laboratory that tested the acute toxicity of juice and soup of the katuk leaves in female rats wistar strain. Observable toxic effects of changes in the physical condition of rats as well as changes in the macroscopic and microscopic lung conditions. Animals used in this study were female, because most consumers who consume katuk leaves is women, who are often used to facilitate breastfeeding. Wistar female rats were healthy and sexually mature weighing 150-200 g, aged 6-8 weeks, were obtained from Pusvetma (Pusat Veterinaria Farma, Surabaya).

2.2 Procedure

2.2.1 Preparation of Katuk Extract

Katuk leaves obtained from the TOGA garden, Faculty of Pharmacy, University of Surabaya, Surabaya, East Java, Indonesia. Katuk leaves used are katuk leaves dark green stalk and all the leaves (petiolus). Samples leaves cleared of impurity particles with water, dried at room temperature without heating so as not to damage the metabolites in the leaves.Then leaves katuk water content removed by freeze dry method. Samples katuk leaves dark green, further prepared into vegetable juice and vegetable stew (food processing).

2.2.2 Preparation of Animal experiments

Female rats chosen by reason of leaf consumption katuk leaves been done by women that is as facilitating breastfeeding. Also note that female rats are more sensitive to toxicity compared to male rats. Animal ages between 8-12 weeks with a weight variation of no more than 20% (OECD, 2001).Experimental animals are conditioned for at least seven days prior to treatment (DG POM, 1991). The whole mice were fed pelleted starter and drink ad libitum during the adaptation period. Maintenance space has an optimal temperature 22 ± 3□C and relative humidity 30-70% with 12-hour lighting cycle of light and 12 hours dark. The physical condition of the experimental animals include covering body weight, presence / absence of hair loss, eye clarity, presence / absence of mucus in the nose, presence / absence of diarrhea and motor activity was observed. After normal ascertained, rats were fasted overnight (18 hours) before administration of treatment (OECD, 2001).

2.2.3 Preparation of Experimental Animals for Katuk Leaves Juice dan Soup

Experimental animals were randomly divided into ten groups:

Negative control : negative control group
J_{500} : katuk leaves juice 500 mg/kg group
J_{1000} : katuk leaves juice 1000 mg/kg group
J_{3000} : katuk leaves juice 3000 mg/kg group
J_{5000} : katuk leaves juice 5000 mg/kg group
S_{500} : katuk leaves soup 500 mg/kg group
S_{1000} : katuk leaves soup 1000 mg/kg group
S_{3000} : katuk leaves soup 3000 mg/kg group
S_{5000} : katuk leaves soup 5000 mg/kg group

with each group consisting of three rats. Each animal in each group was given a dosage katuk leaf juice orally one time and then observed for 14 days (OECD, 2001). Administration of the test substance should correspond to the expected route of administration to humans. Test substances that can not be given once and must be divided into several times of administration, time of administration should not be more than 24 hours. Giving done orally using oral sonde. Test animals were fasted from food overnight (18 hours) before administration of the test material. Experimental animals were fed again after 3-4 hours of administration of katuk extract juice/soup (DirJen POM, 1991; OECD, 2001).

2.2.4 Physical observation

After 30 minutes of administration of the test preparation, test animals were observed carefully for the presence of toxic symptoms and death. Special attention is given during the first four hours after administration and periodically observed every hour on the first day. Furthermore, observations were made every day until day 14 to determine the beginning, intensity and duration of symptoms occurring toxic (DirJen POM, 1991; OECD 2001).

2.2.5 Macroscopic Observation

Macroscopic observation conducted to observe the presence/ absence of morphological changes in the organs. Pulmonary morphological observations include the presence/ absence of discoloration and lesions on the surface of the lung. Weighing lung organ is done in an indirect way by using the measuring cup, by weighing each organ in NaCl 0.9% solution. Immediately after macroscopic observation, the lungs were fixed in 10% buffered formalin. Furthermore, the procedure of making preparations and tissue staining using *Hematoksilin-Eosin*.

2.2.6 Microscopic Observation

Coloring process and making preparations for the lung tissue performed at the Faculty of Science and Technology University of Airlangga, Surabaya, East Java. Microscopic observation was done by measuring the ratio of the diameter of the bronchial lumen using a light microscope at 100x magnification and ocular micrometer. Diameter is the average diameter of the vertical and horizontal measurements. Observations were made on the field of view for each of the three preparations were then averaged. Bronchial lumen ratio is obtained by comparing the bronchial lumen diameter with diameter bronchioles and set in terms of percent (%).

2.2.7 Data Analysis

Analysis data used in this study is the method of one-way ANOVA was used for normally distributed data analysis and homogeneous while the data were not normally distributed and normally distributed data but not homogeneous analyzed by Kruskal-Wallis test. $p < 0.05$ indicates significant differences.

3. RESULT AND DISCUSSION

3.1. Observation results of Lung Macroscopic in Katuk Extrak Juice Intervention

Morphological observation of lung, lung weight, and lung volume throughout the experimental animals were observed. Did not reveal any morphological changes in

the lung. By Kruskal-Wallis test, known weights lung test group did not experience a significant difference (p (0.274)> 0.05) as compared to the negative control group. Lung volumes while the test group was significantly different (p (0.025) <0.05) with the negative control group. Results of lung volume measurements showed a downward trend with increasing dose proportional katuk leaf powder.

In this study found no lesions on the surface of the lung. Damage, discoloration or pleural surface morphological changes can indicate the presence of structural damage(Greaves, 2012). Determination of the presence / absence of lesions in the lungs can cause bias in the interpretation of research results as the observation presence/ absence of lesions is very subjective. Moreover, determination of lesion-related information must also be supported by the diagnosis, distribution, and severity of the pathological condition should therefore be made to ensure the histological observation of pathological changes that occur (Dua dan Jackson, 1988).

Lung weighing results showed no significant difference between the negative control group to the treatment group. The big difference in organ weights may be indicative of toxic effects on the organs involved in this case lung organ (Greaves, 2012). Generally, the proportion of lung weight is directly proportional to the size and weight of experimental animals (Suckow *et al.*, 2006; Greaves, 2012). Although it does not show significant differences, it can not be concluded that there are no toxic effects arising from treatment. Past studies have concluded that the weighing of organs is not recommended to be taken into consideration in the acute toxicity test because the weight of the new organ will be affected if it continues to be exposed to toxic substances for at least seven days (Sellers *et al.*, 2007).Based on the measurement results, there is any significant difference in lung volume between the animal test group and negative control group. This may be caused by trapping water in the lower respiratory tract so that outside air can not get into the lungs (Greaves, 2012).

3.2 Observation results of Lung Macroscopic in Katuk Extrak Soup Intervention

Normality test is done using the Shapiro-Wilk test to determine normality pulmonary organ weights. Normality test results that the data are normally distributed weight p> 0.05 (p = 0.095). Homogentitas test using Levene Test, obtained p> 0.05 (p = 0.135). Statistical parametric analysis was then performed using one-way ANOVA, showed no significant difference in lung organ weights, the value of p> 0.05 (p = 0.154). Test for normality using the Shapiro-Wilk test to determine the normality of the data volume of lung organ. Volume of data normality test results were not normally distributed p value <0.05 (p = 0.024). Non-parametric analysis performed using Kruskal-Wallis, obtained significant difference in lung volume, p <0.05 (p = 0.038).

In this study found no changes in lung morphology. Lung surface looks smooth, shiny and smooth. Changes in lung morphology showed toxic effects. Macroscopic observation was also made by weighing the lungs and lung pengkuruan organ volume, if there is a change may exhibit toxic effects which will affect respiration (Sellers *et al*., 2007). There is no significant difference between lung weights between negative control group with the test group, whereas lung volumes are significant changes between the negative control group to test group.

3.3. Observation results of Lung Microscopic in Katuk Extrak Juice Intervention

Histological observation of lung tissue was observed using a light microscope with a magnification of 400x. A layer of fibroblasts in the group of leaf juice katuk 5000 mg/kg thicker than the layer of fibroblasts in the other group.

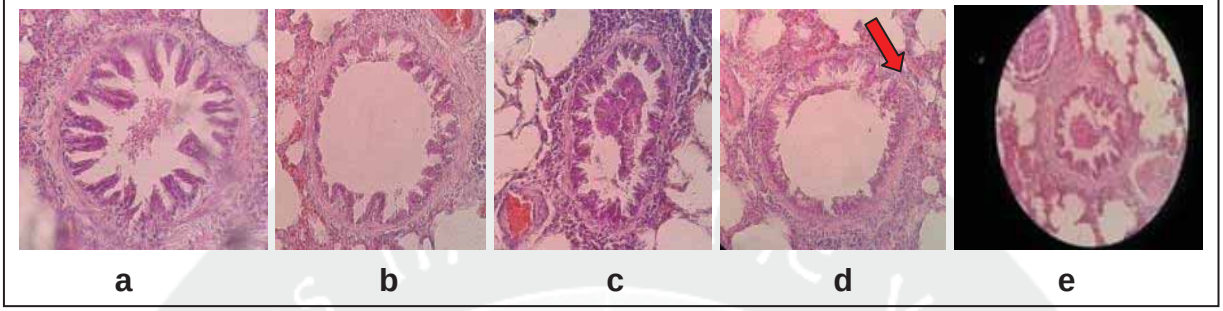


Figure 1. Lung Tissue Preparation: (a) Negative control group; (b) J_{500}; (c) J_{1000}; (d) J_{3000}; (e) J_{5000}

Description: ➡ :Fibrosis in the bronchial lumen

Diameter measurement is done using ocular micrometer on a microscope with a magnification of 10x power magnification 10x objective lens. Bronchial lumen ratio in the test group were significantly different (p (0.006) <0.05) compared to the negative control group. To determine the groups that provide meaningful difference Post Hoc analysis. From the analysis of Post Hoc test obtained the bronchial lumen ratio difference is significant between the negative control group with group leaf juice katuk 5000 mg / kg (p (0.022) <0.05) and the treatment group leaf juice katuk 1000 mg / kg with group leaf juice katuk dose of 5000 mg / kg (p (0.018) <0.05).

On microscopic observation found a decrease in bronchial lumen ratio were significant in animal groups that received leaf juice preparations katuk 5000 mg / kg. The results are consistent with bronchiolitis obiterans characteristic is the existence of fibrosis in the bronchial lumen which causes a narrowing of the bronchial lumen (Laohaburanakit *et al.*, 2003). Thickening layer of fibroblasts is due to the increase in cytokines including interleukin (IL-1, -2, -4, -6, -8, -10, -12, -13), tumor necrosis factor, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), insulin growth factor (IGF), transforming growth factor-β (TGF-β), endothelin-1 (ET-1) produced by leukocytes and cells that undergo necrosis (Cui *et al.*, 2003; Laohaburanakit *et al.*, 2003; Yu *et al.*, 2007; Pappas *et al.*, 2010). Increasing the number of cytokine that induces fibrogenesis process that led to the formation of excess collagen matrix that will narrow the lumen of bronchioles and thicken the layer of fibroblasts (Wang *et al.*, 2000; Greaves, 2012).

Compounds in the leaves that cause toxic effects katuk still unknown. Research conducted byYunita (2011) suspect phytol is a compound that has the potential to cause toxic effects. Phytol classified terpenoid compounds which are metabolized to a reactive epoxide group (Klaassen, 2008). The existence of these reactive compounds can cause cell damage resulting in necrosis of the cells that trigger an inflammatory reaction (Kumar and Robbins, 2007).

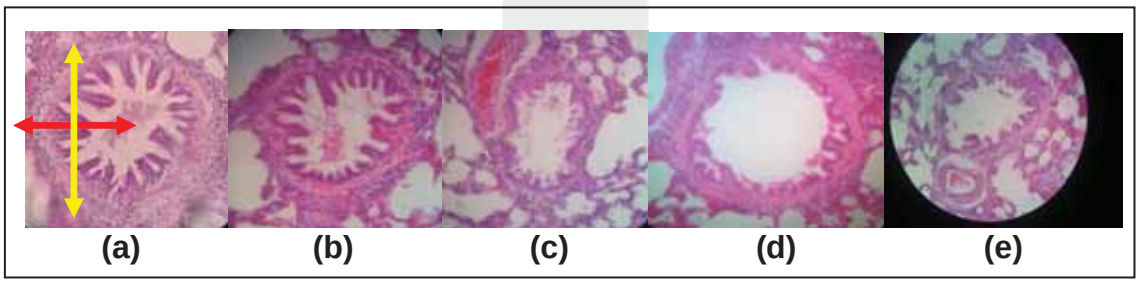
Not found any cell necrosis and leukocyte infiltration around the bronchial lumen. Cells undergo necrosis will difagosit by polymorphonuclear cells (PMN) such as

neutrophils, eosinophils, and basophils. Infiltration of PMN cells induced by cytokine and chemokine produced by cells of necrotic (Parslow *et al.*, 2001). Leukocytes in the necrotic area has undergone lysis or absorbed back into the lymph and so can not be observed during the end phase. Leukocyte infiltration can be found at the beginning of acute inflammation and the chronic inflammation which there are many chemokine produced by necrotic cell to attract leukocytes to areas of inflammation. In this study, the dosage is only given once and observations were made 14 days after the treatment so that the infiltration of leukocytes can not be observed at the end phase (Wang *et al.*, 2000).

Microscopic changes only occurred in experimental animals in the group receiving a dose of leaf juice katuk 5000 mg / kg, marked by a decrease in bronchial lumen ratio. This may be due to the lower dose of 5000 mg / kg is not enough to induce cell damage. Total exposure to toxic substances affect the presence / absence of damage to the cells because the cells have the capacity to adapt to exposure to toxic substances(Kumar dan Robbins, 2007).Bronchial lumen ratio dose group 1000 mg / kg dose group was significantly different to 5000 mg / kg body weight while those with larger doses of 3000 mg / kg body weight did not differ significantly with dose group of 5000 mg / kg. One of the factors that influence the genetic factors of each animal that can affect the immune system so the body's defense reactions expressing different. All the variables that can affect the results of the research have been equated and controlled. Genetic factors are one of the variables that can not be controlled so that anomalies in the results is most likely due to genetic factors of each animal.

3.4. Observation results of Lung Microscopic in Katuk Extrak Soup Intervention

Results of microscopic observation by measuring the diameter of the lumen of bronchioles. Microscopic observations were analyzed statistically using one-way ANOVA (p = 0.05) to detect significant differences bronchial lumen ratio between the control group and the test group. One-way ANOVA statistical results obtained significance value of p <0.05 (0.001) which means that there are significant differences between the groups. To find out which groups are having a significant difference, followed by statistical analysis using the Post Hoc Test. Post Hoc Test results obtained statistically significant difference between the control group and the dose of 500, 1000, 5000 mg/kg, whereas a dose of 3000 mg/kg found no significant difference against the control group.



Gambar 10. Lung Tissue Preparation: (a) Negative control group; (b) S_{500}; (c) S_{1000}; (d) S_{3000}; (e) S_{5000}

diameter of the lumen of bronchioles
diameter of bronchioles

Microscopic observations done by measuring the ratio of the bronchial lumen diamaeter on 3 field of view. Results of the bronchial lumen ratio for each animal the negative control group at 89.96. Lumen ratio for the group at a dose of 500 mg/kg (79,80); a dose of 1000 mg/kg (80,77); a dose of 3000 mg / kg is 84.94 and dose 5000 mg / kg is 82.14 µm. Bronchial lumen ratio were analyzed statistically using one-way ANOVA ($p < 0.05$) note that the significance value of $p < 0.05$ ($P = 0.006$), the results of statistical analysis showed that there were significant differences between negative control group and 4 test groups. Then proceed statistical analysis of data obtained Post Hoc test that between the negative control group at a dose of 500, 1000, and 5000 mg/kg had significant differences, whereas the dose of 3000 mg / kg there was no significant difference.

Observations lung tissue using light microscopy 400x magnification, the observation has not been the infiltration of leukocytes. Leukocyte infiltration will occur when there is inflammation. In this study does not happen dikarena leukocyte infiltration of leukocytes can undergo apoptosis (Parslow e*t al*., 2001).

On the network also has not been found necrosis, this may be caused by granting katuk leaves as much as one that has not been exposed to many toxic compounds. Increasing number of leaves katuk consume, the more doses of toxic compounds that are exposed. The toxic compound can lead to tissue damage that will trigger the inflammatory mediators such as cytokines and chemokines (Parslow *et a*l., 2001). Cytokines and chemokines will attract leukocyte cells such as T-lymphocyte cells and neutrofril who was instrumental in bronchiolitis, mediators also trigger inflammation that will cause the proliferation of fibroblasts (Laohaburanakit *et al*., 2003). Fibroblast proliferation is one of the causes of fibrosis that occurs in the bronchial lumen. Increasing number fibrosis, the fibroblasts will increasingly thicken. Thickening of fibroblasts causes a narrowing of the bronchial lumen and cause airway obstruction (Myong *et al*., 2000). In the lung tissue were observed preparations have not yet experienced the occurrence of fibrosis or narrowing of the lumen, but has been unable to demonstrate the bronchial lumen size changes. This could be due to the influence of the length of time consuming katuk (Ngatidjan, 2006).

4. CONCLUSION

Based on the research that has been done, it can be concluded: Katuk leaf juice (*Sauropus androgynus*) does not cause a change in the physical condition of female Wistar rats. But it cause significant differences are observed macroscopic lung volume as well as the results of microscopic observation that the bronchial lumen ratio between treatment groups katuk leaf juice 5000 mg/kg and negative controls. There isn't changes in the physical condition of female Wistar rats after administration katuk soup. However it can causes significant variations in the macroscopic conditions (lung volume) and microscopic (the size of the bronchial lumen) in female Wistar rats.

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Universitas Atma Jaya Yogyakarta
Jl. Babarsari No. 44, Yogyakarta 55281, Indonesia
Telp.: +62-274-487711 (hunting), Fax. +62-274-487748