

Ethanol Extract of *Annona Muricata* L. Leaves Reduced Goblet Cell in Bronchus of Animal Model of Smoking

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Abstract: *Dangerous effect of smoking like cancer, impotence, COPD and disorders of pregnancy and fetus are well known. COPD is well known as a diseases burden in developing country. Chronic inflammation in COPD leads to airways remodeling which is mark by Goblet cell hyperplasia and mucous overproduction. Annona muricata L leaves has been used for herbal for treating headache, diabetes, antiinflammation, dysnetry. It contains alkaloid and essential oil for antiinflammation. Based on our review there is no data about effect of Annona muricata L leaves with animal model of smoking. Twenty five mice were obtained from Animal Lab Facility, Faculty of Medicine, Udayana University. Mice with 8-10 weeks old and 25-30 gr weight were randomly chosen and adaptated for 7 days. Mice were divided into 5 groups and exposed with smoking by using small pump and container for 30 minutes once daily for 14 days. Group 1 (P1) was normal mice without smoking, grup 2 (P2) was mice with smoking, group 3 (P3) was mice with smoking and 25 mg/kg weight extract, group 4 (P4) was mice with smoking and 50 mg/kg weight extract, group 5 (P5) was mice with smoking and 75 mg/kg weight extract. After 14 days the mice was sacrificed. ANOVA analysis found significant difference in goblet cell number among the five groups (Figure 2) ($p < 0.05$). Post hoc analysis LSD found a significant difference of goblet cell number among P2 and P3, P4, and P5 ($p < 0.05$) and no significant difference ($p > 0.05$) among P1 and P5 which meaning Annona muricata L leaves ethanolic extract prevent goblet cell hyperplasia.*

Keywords: goblet cell, *Annona muricata* L leaves, smoking

1. Introduction

About third of human population is still smoking, although dangerous effect like cancer, impotence, COPD and disorders of pregnancy and fetus are well known. COPD is well known as a diseases burden in developing country. Chronic inflammation in COPD leads to airways remodeling which is mark by Goblet cell hyperplasia and mucous overproduction. This condition lead to functional decreased of lung and increase number of in way patient and increasing economic burden.[1,2,3]. Cigarette contains numerous chemical substances like oxidant and free radical. Exposure from smoking will stimulate EGFR signaling pathway to produce overproduction of mucous and hyperplasia of Goblet cell. Due to dangerous effect of smoking and only small portion of people which able to smoking cessation, another strategy is needed to aveliate Goblet hyperplasia. *Annona muricata* L leaves are abundant in Indonesia and traditionally *Annona muricata* L leaves has been used for herbal for treating headache, diabetes, antiinflammation, dysnetry. It contains alkaloid and essential oil for antiinflammation.[1,2,4] Based on our review there is no data about effect of *Annona muricata* L leaves with animal model of smoking.

2. Material and Methods

2.1 *Annona muricata* L leaves extract.

Annona muricata L leaves were obtained from Penebel, Regency Tabanan, Bali, Indonesia. As much of 2 kg of *Annona muricata* L leaves was dried in room temperature, the homogenized and filtered. Maseration (96% ethanol) for 2 x 24 hours at 4⁰C. Next, the mixture was filtered through

Whatman Paper no. 1. After that, the filtrate was evaporated in Rotary Evaporator then the extract was kept at 4⁰C and protected from direct sunlight.



Figure 1: *Annona muricata* L. Leaves

2.2 Animals

Twenty five mice were obtained from Animal Lab Facility, Faculty of Medicine, Udayana University. The research protocol was approved by Ethics Committee of Udayana University of Medical Faculty/ RSUP Sanglah (No. 299/UN.14.2/KEP/2016). Mice with 8-10 weeks old and 25-30 gr weight were randomly chosen and adaptated for 7 days. Mice were divided into 5 groups and exposed with smoking by using small pump and container for 30 minutes once daily for 14 days. Group 1 (P1) was normal mice without smoking, grup 2 (P2) was mice with smoking, group 3 (P3) was mice with smoking and 25 mg/kg weight extract, group 4 (P4) was mice with smoking and 50 mg/kg weight extract, group 5 (P5) was mice with smoking and 75 mg/kg weight extract. After 14 days the mice was euthanized by using Ketamine and Xylazine injection.

2.3 Lung Examination

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Briefly after euthanized, the lung was obtained and immersed into formaline buffer, until 24 hours. Then the lung was dehydrated by using grading ethanol, clearing by using xylene and embedding into paraffin. The paraffin was cutted as thick as 5 μm and placed on object glass. Then stained by using Hematoxylin and Eosin staining. Photomicrograph was obtained by using 400x magnification using Olympus microscope CX-41 (Olympus, Japan) and camera OptilabPro (Miconos, Indonesia). Picture of primary and secondary bronchus was obtained and analyzed to count the Goblet cell. Each sample was captured 3 fields for 400x magnification.

2.4 Statistic

All statistical analysis was performed using SPSS v. 16.0 (IBM Corp., USA). The data analyzed for distribution and normality, followed by ANOVA and LSD.

3. Results

ANOVA analysis found significant difference in goblet cell number among the five groups (Figure 2) (p <0.05). Post hoc analysis LSD found a significant difference of goblet cell number among P2 and P3, P4, and P5 (p <0.05) and no significant difference (p>0.05) among P1 and P5 which meaning *Annona muricata* L leaves ethanolic extract prevent goblet cell hyperplasia. Figure 3, 4, 5, 6, 7 showed number of Goblet cell in normal mice, mice with smoking, mice with smoking and 25 mg/kg weight, mice with smoking and 50 mg/kg weight, mice with smoking and 75 mg/kg weight respectively. The P1 vs P5 (p=0.082) showed Goblet cell number quite similar hence highest dose prevent Goblet cell hyperplasia.

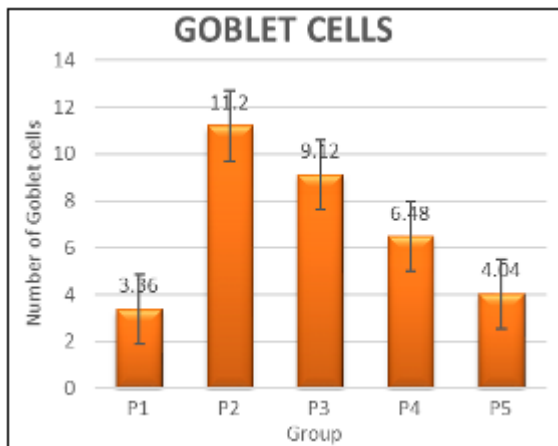


Figure 2: Number of Goblet cells of P1, P2, P3, P4 and P5

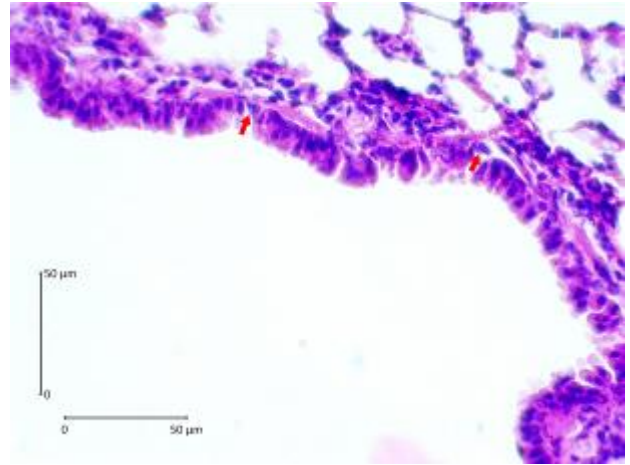


Figure 3: Goblet Cell of P1 (red arrow)

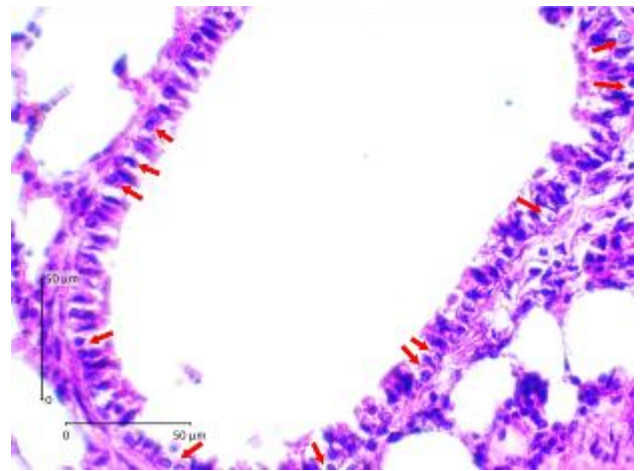


Figure 4: Goblet Cell of P2 (red arrow)

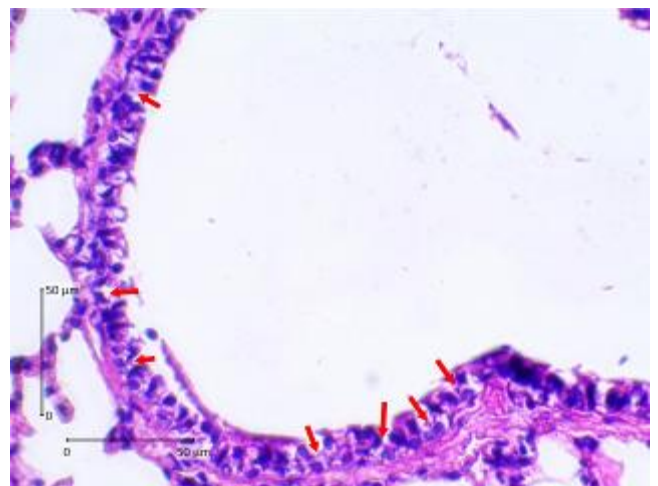


Figure 5: Goblet Cell of P3 (red arrow)

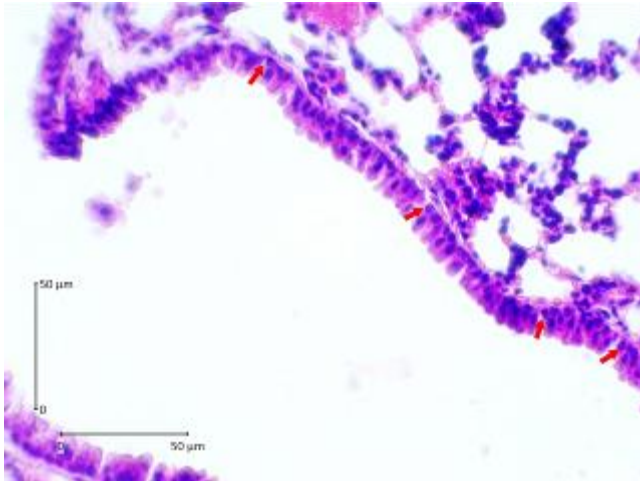


Figure 6: Goblet Cell of P4 (red arrow)

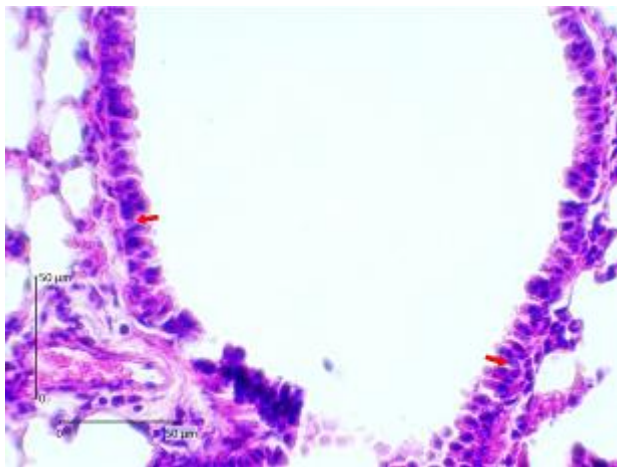


Figure 7: Goblet Cell of P5 (red arrow)

4. Discussion

This research reveals antioxidant properties of *Annona muricata* L leaves which contains alkaloid and essential oil for antiinflammation. Based on our review there is no data about effect of *Annona muricata* L leaves with smoking mice animal model. Chronic inflammation of COPD leads to remodeling of airway which mark by Goblet cell hyperplasia and mucous overproduction. Smoking exposure stimulate various signaling pathway involved in mucous overproduction like epidermal growth factor receptor (EGFR) and extracellular signal-regulated kinase (ERK). [2,5,6,7]. Based on our study the possibility of alkaloid and essential oil inhibit this pathway. Further study is required to confirm.

5. Conclusion

Soursup leaves ethanolic extract effectively prevent Goblet cell hyperplasia in animal model of smoking through its antioxidant properties.

References

[1] Xiao, J., Wang, K., Feng, Y.L., Chen, X.R., Xu, D., and Zhang, M.K. Role of extracellular signal-regulated kinase 1/2 in cigarette smoke-induced mucus hypersecretion in a

rat model. Chinese medical journal 2011. 124, 3327-3333.
[2] de Sousa, O.V., Vieira, G.D., de Jesus, R.G.d.P.J., Yamamoto, C.H., and Alves, M.S. Antinociceptive and Anti-Inflammatory Activities of the Ethanol Extract of *Annona muricata* L. Leaves in Animal Models. International journal of molecular sciences 11, 2010. 2067-2078.
[3] Gonzales, D., Rennard, S.I., Nides, M., Oncken, C., Azoulay, S., Billing, C.B., Watsky, E.J., Gong, J., Williams, K.E., and Reeves, K.R. Varenicline, an alpha4beta2 nicotinic acetylcholine receptor partial agonist, vs sustained-release bupropion and placebo for smoking cessation: a randomized controlled trial. JAMA 2006. 296, 47-55.
[4] White W.B. Smoking-related morbidity and mortality in the cardiovascular setting. Prev. Cardiol. 2007. 10:1-4.
[5] Adewole, S dan Ojewole J.A.O.. Protective Effects of *Annona Muricata* Linn. (Annonaceae) Leaf Aqueous Extract on Serum Lipid Profiles and Oxidative Stress in Hepatocytes of Streptozotocin-Treated Diabetic Rats. Afr J Tradit Complement Altern Med. 2009 6(1): 30-41.
[6] Maas PJM: Studies on Annonaceae. XXI. Index to species and infraspecific taxa of neotropical Annonaceae, Candollea 1994, 49:389-481.
[7] Muthu S, Durairaj aB: Evaluation of antioxidant and free radical scavenging activity of *Annona muricata*, European Journal of Experimental Biology 2015, 5(2):39-45.

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