

Effectiveness of Malang Apple Cider (*Malus sylvestris*) against the Decreased Number of Mast Cells in Allergy-induced Rat (*Rattus novergicus*) Lung

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Abstract: Allergy is a state of hypersensitivity induced by exposure to certain antigens that cause reactions immunology. Mast cells have a role in triggering allergies. There is a new innovation as an anti-allergy, one of them is apple because it contains quercetin. Quercetin are believed to have an influential effect on human health, that of anti-allergy and anti-inflammatory activity. This study to determine the effectiveness of Malang Apple Cider (*Malus sylvestris*) on decreasing the number of mast cells in the lung of an allergic induced rat (*Rattus novergicus*). This study was experimental research using Post Test Control Group Design. Samples were white male Wistar Strain Rats which were divided into 5 groups, each group contains 5 rats. The positive control group was induced with ovalbumin through intraperitoneal and inhalation. Besides ovalbumin induction, treatment groups were also given apple cider with doses of 15%, 20%, and 25%. Lung tissue was taken and then paraffin blocks were created and stained using Methylene blue staining and evaluated with the microscope in 40 x magnification. Data analyzed using One Way ANOVA. Statistical results showed there was no significant ($p = 0,079 > p 0.05$). This decline is due to the levels of Quercetin in apple cider can prevent mast cell degranulation. However, the decrease was not significant occurs due to several factors, including the strength of apple cider, the absorption in the intestine, stress factors, and environmental circumstances.

1 INTRODUCTION

Every year, the prevalence of allergies shows a significant increase in almost all countries. It began to become a serious health problem. In recent years, the incidence of allergies has continued to increase significantly both domestically and abroad. The World Allergy Organization (WAO) said that 22% of the world's population suffer from allergies and continues to increase every year (Candra et al, 2011).

Allergy is a complex chronic inflammatory process that is influenced by genetic, environmental and internal control factors. Activation of CD4 T cells with predominance of T helper 2 (Th2) will induce an inflammatory process, with one of the cytokines involved is interleukin-4 (IL-4). Interleukin 4 plays a role in the differentiation of CD4 T cells into Th2 cells, switching B-lymphocytes to produce immunoglobulin (Ig) E and increasing the production of eosinophils. IgE produced will be attached to the

surface of mast cells and will bind to allergens. The bonds between mast cell, IgE and allergen can cause mast cell rupture, and secrete chemical mediators. The effect of this chemical mediators cause vasodilatation, edema, and spasm of smooth muscle (Abbas et al, 2016).

Mast cells is one part of connective tissue that has pseudopodia for mobilization. Basically, mast cells are found in all organs, especially in the lung mucosal tissue. Based on Merijanti research (2011), quercetin can inhibit histamine production and release from mast cells and basophil cells. This is because quercetin has a strong affinity for mast cells and basophils. Quercetin is one of the flavonoids that has an antioxidant effect. That is why these antioxidants can reduce the chance of someone infected with various allergens and also help cure allergies (Merijanti, 2011).

Quercetin can be found in apples. The quercetin content in 100-gram apples is 4.4 mg. Malang and

Batu are the main apple producing regions in Indonesia (Yuwono, 2015). About 80% of apple production in East Java is concentrated in Malang and Batu. Therefore, author want to prove the efficacy of Malang apple juice (*Malus sylvestris*) ad an anti-allergic, especially to mast cell responses.

2 METHODS

This research is an experimental study using posttest control group design approach. The samples used were male *Rattus novergicus strain wistar* aged 2-3-month, body weight 150-250 grams, in good condition, characterized by active movements, thick fur, and clear eyes. The sampling technique used simple random sampling. In this study, there were 25 samples of rats divided into 5 groups namely positive control group, negative control group, and 3 treatment group with different dosage.

2.1 Preparation Ovalbumin Solution

Preparation of ovalbumin solution as an allergen can be done by using 50 ml of purebred chicken egg whites then stirred until there are no lumps (Barlianto, 2009).

2.2 Cider Apple Preparation

Apple cider has been made through a juicing process. The apple juice than dissolved in distilled water. The apple cider dose used in this study was: 15% (15 ml apple juice and 85 ml distilled water), 20% (20 ml apple juice and 80 ml distilled water), and 25% (25 ml apple juice and 75 ml distilled water) (Widyaningtyas, 2014).

2.3 Administration of Ovalbumin and Apple Cider

Rats used as many as 25, were divided into 5 groups and each group consisted of 5 rats. The negative control group was given standard BR-1 feed of 40 gr/day/rat and drinking distilled water for 30 days. The positive control group and treatment group besides being given standard food, it given the administration of 70 mg ovalbumin and 14 mg Al(OH) 3 in 1.4 cc of normal saline intraperitoneally on the first day, 7th day and 14th day. Then it given 7% ovalbumin in 10 ml of normal saline by inhalation using nebulizer for 30 minutes in the 19th day and 22nd

day (Laesa, 2010). The treatment group was also given apple cider orally based on the dose determined (15%, 20% and 25%) on days 15th – 22nd. At the end of the treatment, all rats were killed to observe its mast cell in the lungs.

2.4 Mast Cell Observation

Rats that have been killed, pulmonary organs were taken and made paraffin block preparation. To observe mast cell, methylene blue has been stained. Observation were made by counting mast cells using a microscope with 400x magnification in 5 visual fields.

2.5 Data Analysis

The data obtained in this study were analyzed using the normality test, homogeneity test, and One-Way ANOVA test that processing using SPSS 23 for windows with 95% confidence level.

3 RESULTS AND DISCUSSION

Table 1 shows the number of mast cells in male white rats in the negative control (C-), positive control (C+) and treatment groups (T1, T2, T3) using methylene blue staining. During the study, 3 rats were dropped out for various reasons, so the data used only 3 samples per group.

Histopathology examination in each study group can be seen in Figure 1 - 5. Mast cells were identified as dark blue using methylene blue staining.

Homogeneity test was performed with $p > 0.05$ and ANOVA test with $p = 0.079$. Based on the One-Way ANOVA result, there was no significant difference between each group.

Table 1: The Number of Mast Cells in Each Group.

Group	The Number of Mast Cell in Rats					Average	Saphiro-Wilk*
	1	2	3	4	5		
C-	23	22	21	17	Drop out	20.91	-
C+	31	33	45	35	29	34.80	0.346
T1	30	22	30	27	Drop out	27.58	0.147
T2	22	36	27	24	Drop out	27.66	0.283
T3	26	30	22	25	29	26.80	0.833

Note :

C- : negative control group

C+ : Positive control group

T1 : 1st Treatment group, with 15% apple squeeze

T2 : 2nd Treatment group, with 20% apple squeeze

T3 : 3rd Treatment group, with 25% apple squeeze

*Significant if >0.005

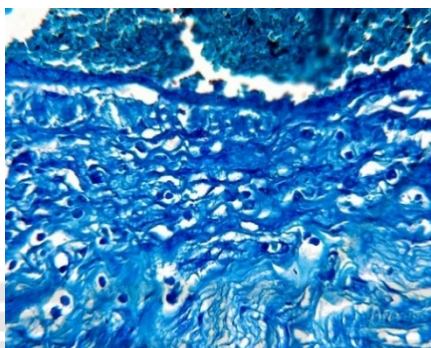


Figure 1: Mast cell in negative control group.

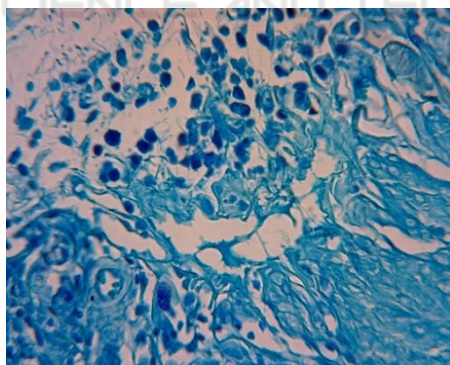


Figure 2: Mast cell in positive control group.

Histocompatibility Complex (MHC) to T helper (Th0) lymphocyte cells. Th0 cells will release Interleukin (IL-4) which converts Th into Th2. So Th2 cells induce B lymphocytes to produce immunoglobulin E (IgE). This causes an increase in production of IgE. IgE in the circulation will be attached to receptors with high affinity on the surface of mast cells. The bond between IgE and allergens will reduce the stability of mast cell membranes, resulting in mast cell degranulation. Degranulation of these mast cells results in the release of chemical mediators into the circulation, and gives rise to clastic manifestation of allergies (Busse and Lemanske, 2001).

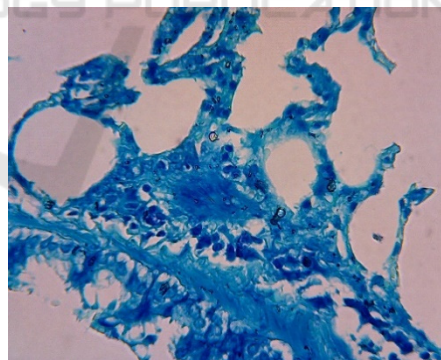


Figure 3: Mast cell in treatment group 1 (15%).

This study uses ovalbumin as an allergen. The results showed that exposure to ovalbumin for 29 days could increase the average mast cell count in rats. This increase occurs because ovalbumin which acts as an allergen will spread to the digestive tract, skin and respiratory tract through the blood circulation. When allergens enter, they will be processed by Antigen Presenting Cells (APCs) in endosomes. Then APC will present Class II Major

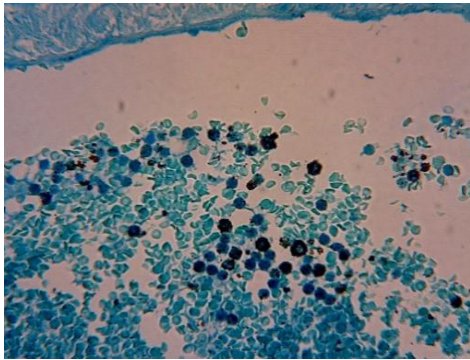


Figure 4: Mast cell in treatment group 2 (20%).

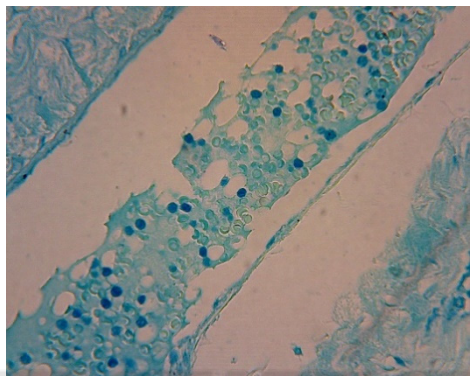


Figure 5: Mast cell in treatment group 3 (25%).

Ovalbumin will also activate mast cells and CD4+Th2 cells in reaction to allergies. Activated CD+Th2 cells will produce inflammatory mediators such as histamine, leukotriene, and cytokines such as IL-4, IL-5, IL-10 and IL-13 (Abbas et al, 2016). IL-4 and IL-10 can stimulate mast cell growth. Cytokines produced by CD4+Th2 cells play a role in mast cell hyperplasia and are very influential in allergic responses (Li Weber and Krammer, 2003).

The result of the study between the positive control group and treatment group showed a decrease in the number of mast cells in the treatment group (Table 1). This is caused by the treatment group that is given apple cider which contains flavonoids, especially quercetin, has an anti-inflammatory effect by affecting Th2 production and by maintaining stability of the mast cell wall membrane thereby inhibiting mast cell degranulation and inhibiting the release of inflammatory mediators like histamine (Chirumbolo, 2010).

Statistical test results using One Way Anova obtained $p=0.079$, which means there is no significant difference between the control group and the treatment group. Many factors can influence this,

including the duration of poor apple cider which is only 1 week, so that the flavonoid effect is optimal. Another thing that can be a factor also is the extract content and the cider content, will have more effect when using extracts because the extract compound content is strengthened so that the effects that arise will be more optimal (Rachmadian, 2011).

Genetic factors can affect the genetic predisposition to hypersensitivity reactions (atopic). Atopic individuals have an innate tendency to produce IgE antibodies to specific allergens and excessive allergen responses (Ningrum et al, 2016).

In this study, suggest that there are several factors that influence insignificant results where quercetin which is given orally is not absorbed optimally in the intestine because quercetin contained in apple cider will bind cholesterol carriers when it passes through the brush border membrane. It will reduce the effect of quercetin as an anti-inflammation (Witosari, 2014).

Other studies said that quercetin is an aglycone where the bond is very weak by sugar. However, this study did not examine how much sugar levels in the sample (Kelly, 2011).

Another factor that can influence in this study but not examined in this study is the rat's level stress during the research process. Stress experienced by rats can cause increases suppressive effects on eosinophils and other pro-inflammatory cytokines (Chang, 2010).

4 CONCLUSIONS

There was a mast cell levels decrease in the treatment group given apple cider (*Malus sylvestris*) in ovalbumin-induced wistar male rats. However, statistically the result of the reduction was not significant.

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REFERENCES

- Abbas, AK, Lichtman, AH, Pillai, S. (2016). 'Cellular and Molecular Immunology'. 5th Ed., Canada: W.B, Saunders Company
- Barlianto, Wisnu (2009). 'Pengembangan Model Mencit Alergi dengan Paparan Kronik Ovalbumin'. (25)1, 1-5.
- Busse and Lemanske (2001). 'Change in Adenosine Metabolism in Astma. A Study of Adenosine, 5'-NT, Adenosine Deaminase and Its Isoenzyme Levels in Serum, Lymphocytes and Eritrocytes. (5)2.
- Candra, Yolanda, Setiawati, Asih, Rengganis, Iris (2011). 'Gambaran Sensitivitas Terhadap Alergen Makanan', (15)1, 44-50.
- Chang, Wen-Hsin, Shene-Pin, Hu, Ying-Fen, Huang, Tzu-Shao, Yen, Jeng Fang, Lie. (2010). 'Effect of Purple Sweet Potato Leaves Consumption on Exercise-Induced Oxidative Stress and IL-6 and HSP72 Levels'. *Jappt Physiol* (109):1710-1715.
- Chirumbolo, Salvatore. (2010). 'The Role of Quercetin, Flavonols and Flavones in Modulating Inflammatory'. Department of Pathology and Diagnostics, University of Verona, Italy, *Inflammation & Allergy - Drug Targets*, 9, pp. 263-285
- Kelly GS., (2011), 'Quercetin, Alternative Medicine Review', 16, pp. 172-194
- Leasa, Bianca Natania, (2010), 'Pemberian Ovalbumin Sebagai Penyebab Alergi Pada Marmot'. Departemen Biokimia, Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor.
- Li-Weber dan Krammer, (2003). 'Function and regulation of the CD95 (APO-1/Fas) ligand in the immune system'. 145-57
- Merijanti S, Lie T, (2011). 'Peran sel mast dalam reaksi hipersensitivitas Tipe-I'. *Histologi Fakultas Kedokteran Universitas Trisakti*
- Ningrum TS, Suprihati, Santosa YI, (2016). 'Pengaruh Pemberian Ekstrak Kunyit (Curcuma longa) Terhadap Jumlah Eosinofil Di Jaringan Paru Pada Penyakit Alergi: Studi Eksperimental Pada Mencit Balb/C Yang Diinduksi Ovalbumin'. *Jurnal Kedokteran Diponegoro*. (5) 4.
- Rachmadian, Okky Dita, (2011), 'Pengaruh Ekstrak Etanol Propolis Terhadap Hitung Sel Mast Intestinal pada Tikus Putih (Rattus norvegicus) Model Asma Alergi', Fakultas Kedokteran, Universitas Sebelas Maret, Surakarta.
- Widyaningtyas, Rr. T., et al., (2014). 'Efek Perasan Buah Apel (Malus Domestica) Varietas Red Delicious Sebagai Anti Alergi Terhadap Respon Anafilaksis Pada Tikus Jantan Galur Wistar Yang Diinduksi Ovalbumin'. Program Studi Farmasi STIKES Ngudi Waluyo Ungaran.
- Witosari, Nidya, Nurmasari Widyastuti. (2014). 'Pengaruh Pemberian Jus Daun Ubi Jalar (Ipomoea batatas) Terhadap Kadar kolesterol Total Tikus Wistar Jantan Rattus norvegicus yang diberi pakan tinggi lemak'. *Journal of Nutrition College*. Vol 3. Pp. 638-646.
- Yuwono, Sudarminto Setyo, (2015). 'Apel Mallus Sylvestris Mill'. Universitas Brawijaya Malang.