

The Effect of Red Onion Extract (*Allium cepa* L.) Against Number of Mast Cells in Bronchus Induced By Ovalbumin

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ABSTRACT

Background: Allergy is one type of hypersensitivity type 1; the condition is influenced by the presence of specific IgE and mast cells. The degranulation process of mast cells can release inflammatory mediators, such as histamine. But can be lowered by stabilizing the mast cells' wall. Onion extract (*Allium cepa* L.) can inhibit the process of mast cell degranulation because of the high levels of quercetin which acts as a mast cell stabilizer. **Objective:** To determine the effect of onion extract (*Allium cepa* L.) on the number of granulated bronchial mast cells in the reaction induced by ovalbumin. **Method:** True experiment with post-test only control group design. The sample used 20 rats which were divided into 5 groups; normal group, positive group that is induced by ovalbumin route intraperitoneal and inhalation, and the other three groups induced by ovalbumin route intraperitoneal and inhalation plus onion extract at dose 35mg/kg BW, 70mg/kg BW and 140mg/kg BW. Then mast cell observation was carried out by a light microscope with a 400x magnification and confirmation of mast cell with 1000x magnification. **Results and Discussion:** One-way Anova test result showed a significant effect (p: 0.000). The result of Posthoc showed a significant (p; 0.000) in the group P1, P2, and P3. The result of linear regression obtained Adjusted R2: 0.574 (Onion extract affects 57.4%). The decrease in the number of mast cells is due to the high level of quercetin in the onion extract so that it can stabilize the wall and reduce the number of mast cells. **Conclusion:** There is an effect of giving onion extract (*Allium cepa* L.) on granulated mast cells in rat bronchi induced by ovalbumin

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1. INTRODUCTION

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The increase in allergies from year to year are experiencing a significant inclined not only in Indonesia but also in many countries around the world as well, so that allergies are a serious health problem. In the last few decades, the incidence of allergies has increased quite sharply in all countries. (WAO) states that 22% of the population suffer from allergies and this is increasing [1]. The incidence of allergic rhinitis in Indonesia is also a percentage between 1.5-12.5% and has increased every year [2]. In addition, Asthma is a disease that is closely related to allergy, the prevalence of recurrence of asthma in every province in Indonesia is quite high, in East Java the prevalence recurrence asthma is 60 %, higher than national average 57,5% [3].

Type 1 hypersensitivity is a hypersensitivity caused by antibody IgE and make anaphylaxis. Allergic reaction is systemic or local due to induction by IgE. Type I hypersensitivity response is the product of cross-linking of antigens with membrane-bound IgE antibodies from basophil or mast cells [4].

Ovalbumin (OVA) is substance that causes allergy, this substance have a spesific allergen derived from white egg protein. Injection OVA in intraperitoneal to experiment animal then continue route inhalation cause activation and domination TH2 then the last product is specific IgE [5].

Quercetin has an important role due to allergic reaction, the mechanism is inhibitor realese histamine, cytokin pro inflammatory and leukotrien, as well as inhibition of the release of Interleukin 4 or commonly called IL-4 [6]. Red onion is a kitchen ingridient that easy to find, which are usually used for cooking for daily needs. Red onion are very popular among Indonesian because of they taste delicious and are also used for traditional medicine. [7]. Red Onion have a lot of function, one of which there is highest quercetin level compared by the other fruit and vegetable 739-1000mg/kg [8].

2. METHOD

Experiment use True Experimental with Post Test Only Control Group Design Method, with random sampling 20 rat divided into 5 groups, normal group, control positive and three treatment groups with different red onion extract dose Sample use healthy *Rattus norvegicus strain wistar* with 150-200g body weight and characterized by thick fur, have a clear red eye and active movement.

2.1 Ovalbumin Preparation

The preparation of ovalbumin solution which is used as an allergen can be done by using 50 ml of chicken egg white and then stirring until there are no lumps, then use the clear part [9].

2.2 Red Onion Preparation

Extraction Method use Etanol, 20gr red onion sample pour into 200cc etanol 70% and then heat into 60°C in water. The extract residue filtered twice time. After that the solvent is evaporated using an evaporation tool. This extract will then be stored in a drying tube. The dry extract is then stored at a temperature of 20 °C [10]

2.3 Ovalbumin And Extract Route

The rats used were divided into five groups in each group there were 4 rats. In the negative group, only feed and drink was given until day 22. The positive group and treatment for induction were given 70 µg added with Al (OH)₃ dissolved in 1.4 ml normal saline, given intraperitoneal on days 0, 7 and 14 Then continued to be given inhalation using 7% OVA dissolved in 10 ml of normal saline for 30 minutes on days 19 and 22 [11,12], treatment group have added 3 dose extract in each group, 35mg/kgBW, 70mg/kgBW and 140mg/kgBW, then in the last day the rat were terminated and take the sample of bronchi.

2.4 Mast Cell Preparation

The bronchial sample organs that had been taken were made of paraffin blocks and then stained using *Methylene Blue*, after that the mast cells were calculated using a microscope with a magnification of 400x, counted in 3 fields of view.

3. RESULTS AND DISCUSSION

The research lasted a total of 30 days, with details of 7 days of adaptation period and 22 days of treatment period and 1 day of determination.

The study was divided into 5 groups of rats, namely a negative group, a positive control group and a total of 3 groups as the treatment group, each group consisting of 4 rats obtained from the middle between maximum and minimum samples. In the first group, namely, the negative group can be called normal group as a

comparison. The next group was a positive control group and three treatment groups, these four groups were given ovalbumin induction via intraperitoneal on day 0, 7 and 14 and continued induction route via inhalation using a nebulizer on day 19 to day 22. In the treatment group, red onion extract was added from day 15 to day 22 with three different doses, namely, the first treatment group (P1) was given a dose of 35mg / kgBW / day, the second treatment group

(P2) was given a dose of 70mg / kgBB / day and the third treatment group (P3) at a dose of 140mg / kgBW / day.

After the determination process, the process of making rat bronchial preparations was carried out and stained using *Methylene Blue* to see the number of mast cells in each group.

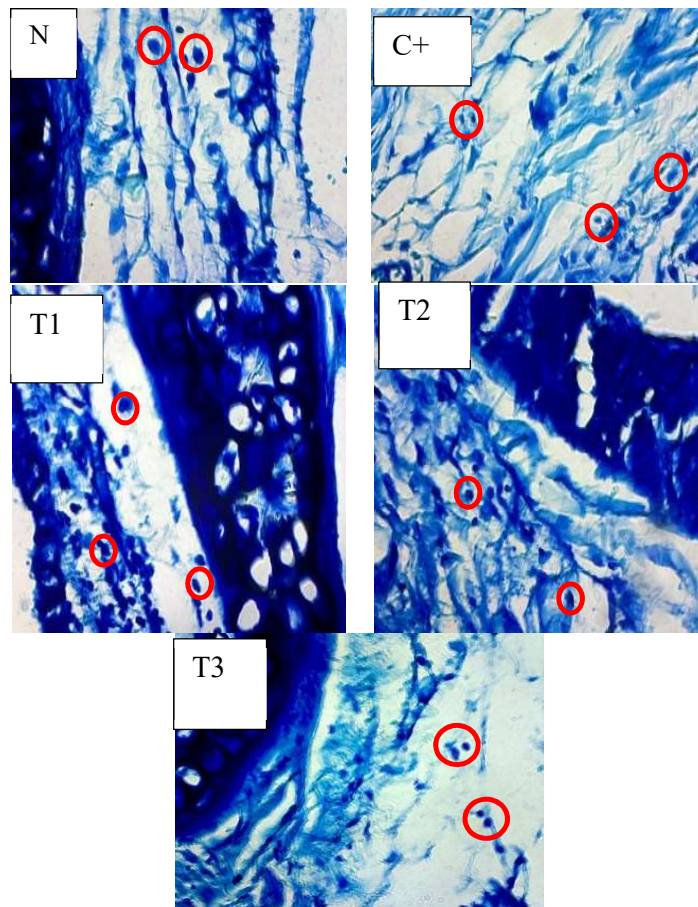


Figure 1. Mast Cells in 400x Magnification

Table 1. Number of Mast Cell Counts 3 Field of Views

Group	Mast Cells Count				Average
	1	2	3	4	
N	50	62	42	45	49,75±8,80
C+	92	77	87	91	86,75±6,84
T1	62	49	62	38	52,75±11,58
T2	51	59	44	44	49,5±7,14
T3	38	44	44	47	43,25±3,77

Note

N	: Normal Group (Healthy Group)
C+	: Induced by Ovalbumin
T1	: Induced by Ovalbumin+35mg/kgBW/day <i>Allium cepa L.</i> extract
T2	: Induced by Ovalbumin+70mg/kgBW/day <i>Allium cepa L.</i> extract
T3	: Induced by Ovalbumin+140 mg/kgBW/day <i>Allium cepa L.</i> extract

Based on the data that has been obtained (Table 1) N is a reference for determining the normal value of mast cells, which is an average of 49.75 cells. Other data in the C + group showed a number of 86.75. It can be concluded that there is an increase in the number of mast cells of 37 cells compared to N. Group T1 have an increase in the number of mast cells compared to N but decreased compared to group C+, Group P1 had an average of 52.75 cells, a decrease compared to the average for C+ group, which was 34 cells. The T2 group has a decrease in the K + group too with a decrease of 37.5 cells. Meanwhile, the T3 group compared to C + experienced a decrease of 43.5 cells. In the T2 and T3 groups the average number of cells decreased compared to the normal values, besides that it can be concluded that the P3 group experienced the largest decrease in C + compared to the average decrease of the P1 or P2 groups.

Tabel 2 *Oneway Anova Test*

Variable	Sig.	Note
Mast Cell	0,000	Significant

Tabel 3 *Poshoc Bonferroni Test*

Group	Treatment	Sig.
C+	T1	0.000
	T2	0.000
	T3	0.000

After that, use *Poshoc Bonferroni*, it shows that T1,T2 or T3 compared with C+ have a significant value 0.000, So it can be concluded.

Tabel 4 *Linier Regresion Test*

Model	Coefficients	
	B	Sig.
(Constant)	74.400	0.000
Dose	-1.334	0.001

Based on the linear regression test, it can be concluded that 1 mg of onion extract (*Allium cepa L.*) can reduce the number of mast cells by 1.334.

Studies have shown an increase in the number of mast cells in the bronchi, allergens that arise as a result of OVA induction. The results of the study were the exposure of three days of experimental animals by intraperitoneal. on days 0, 7 and 14 aims because giving OVA route via Intraperitoneal has an absorption rate of quarter faster than intravenous [13]. After that continue induction route via inhalation can increase the number of cell mast in bronchii. OVA is a white protein egg substance, when it start enter the body will be recognized as allergen and stimulated immune response, especially type 1 hipersensitivity [14]. In another study explained that addition of Al(OH)₃ can increase allergy reaction, it marked by increase of eosinophill into lungs compared without addition [15]. Early stage sensitisation, induction via intraperitoneal can stimulate TH2 to increase allergy mechanism, TH2 will stimulate B cells to form plasma cell, then after that it will change into IgE specific for allergy. Another effect TH2 will secretion IL-4 and IL-13. IgE have specific receptor on mast cells and basophill FcεRI. When re-exposure occurs, the incoming allergens will be immediately responded by active mast cells and basophils, causing three biological phases, namely, granule release, synthesis and secretion of cytokines that cause symptoms of allergy. (Owen, 2013;Daubeuf and Frossard, 2013). Giving OVA which induces TH2 to finally form IgE also increases the growth and addition of mast cells which also affects the remodeling process of the respiratory tract airway. (Daubeuf and Frossard, 2013).

The results of the experiment, it showed that the Group that was only given ovalbumin induction without being given red onion extract (Control +) had higher mast cell levels than the treatment group. The data

displayed from the One Way Anova test shows a significant difference of 0.000. The decrease in the number of mast cells was due to the provision of red onion extract (*Allium cepa L.*) which contains high flavonoids, especially quercetin, with the best extract processing method using 70% ethanol, a sample of 20 grams will produce a value of 62, 39 mg / g [16]

Quercetin that contained in red onion extract has a many effects, which is on mast cell by affecting the production MCP-1 and IL-8. This mechanism similar with dexametasone, by inhibiting MCP-1 and IL-8 to binding in mast cell can reduce Ca influx [16] thus when mast cells are not degranulated there will be no allergic response. In addition, the reduction in the number of mast cells in the treatment group is because quercetin can reduce and stabilized of Th2, which is another function of TH2 to stimulate the growth of mast [15,17].

Furthermore, to determine the differences in each group, the *Posthoc Bonferroni* test was carried out. It shows that mast cell levels in the treatment group with a dose of 35 mg/kgBW, 70 mg/kgBW, and 140 mg/kgBW have a significance value of 0.000, so it can be concluded that when the dose has the same ability to reduce the number of mast cells significantly. So, it can be concluded that the smallest dose of (35mg / kg) has had a significant effect.

Previous research that was used as a reference for the dose of extract administration carried out showed that the second dose (70mg / kgBW) with the extract process without ethanol can have the same effect as giving dexamethasone [18]. The extract by processing using ethanol 70% has proven to be very effective to maximizing the potency of quercetin, with the same dosage but with different processing makes quercetin extracted by ethanol 70% at a low dose are the most effective.

Research on decreasing the number of mast cells has also been carried out using the juice of malang apples (*Malus sylvestris*), with the results showing that the juice of malang apples (*Malus sylvestris*) has quercetin levels that can reduce mast cell levels although with less significant results, due to research. in the control group using juice that does not contain high quercetin which is stronger than the extract.

Another study using red onion extract using several measurements, one of which is by using the bronchial diameter parameter, it shows the result of a decrease in the diameter of the bronchi with each increase of the dose. This is because the measurement of eosinophil levels in the control group compared to treatment experienced a decrease as well. This is due to the decreased levels of IL-5, which are responsible for modulating eosinophils and degranulating mast cells so that the bronchi show inflammation.

In this study, there are several other factors apart from extracts that affect the results of the study, however, they are not studied into two, namely endogenous and exogenous factors. Endogenous factors, one of which is the tendency of the rat themselves to respond to the incoming allergens, in some samples there is a tendency for the number of mast cells to be quite high compared to the others, this can be due to the tendency to produce excessive IgE in plasma cells, occurs in individuals with atopic tendencies. Another endogen factor is stress, because of the high level of cortisol, which resulted domination of TH2, then can worsening of the allergy condition.

Exogenous factors are all factors outside of the rat, but in this study to minimize the differences between mice using the same rats from the same breeder and placed and treated the same, starting from food, conditions of drink cages and lighting.

4. CONCLUSION

There is an effect of red onion extract (*Allium cepa L.*) on undegranulated mast cells in rat bronchi induced by ovalbumin. The dose of red onion extract (*Allium cepa L.*) which has a significant effect on reducing the number of bronchial mast cells is 35mg / kgBW (T1).

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